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Complete List of Authors:	Chen, Qingsong; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Chen, Guiping; Guangdong Province Hospital for Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China Xiao, Bin; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Lin, Hansheng; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Qu, Hongying; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Zhang, Danying; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Shi, Maogong; Yantaishan Hospital, Yantai, Shandong264000, China Lang, Li; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Yang, Bei; Yantaishan Hospital, Yantai, Shandong264000, China Yan, Maosheng; Guangdong Province Hospital for Occupational Disease Prevention and Treatment
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Danying Zhang[1], Maogong Shi[2], Li Lang[1], Bei Yang[2], Maosheng Yan[1]

[1] *Guangdong Province Hospital for Occupational Disease Prevention and Treatment；And Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China;*

[2] *Yantaishan Hospital, Yantai, Shandong264000, China.*

[†] *These authors contributed equally to this work.*

* *Author to whom correspondence should be addressed; E-Mail: qschen@gdoh.org;*

Add.: No.68, Xingang Xi Road, Haikang Street, Haizhu District, Guangzhou, China.

Tel.: +86-20-8926-6013 (Q.C.); Fax: +86-20-8902-1031 (Q.C.).

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***Data sharing**: Extra data is available by emailing to qschen@gdoh.org*

***Ethics approval:** This study was approved by Ethical Committee of Guangdong Province Hospital for Occupational Disease Prevention and Treatment.*

***Contributors**: Qingsong Chen designed the project and Guiping Chen developed and executed the nailfold capillaroscopy performance. Guiping Chen and Danying Zhang discussed and decided the nailfold capillaroscopy characteristics which should be taken into consideration, then carried out the nailfold capillary measurements. Bin Xiao and Hansheng Lin devised and executed the vibration measurement. Maogong Shi and Bei Yang visited the collaborating gold mine industry administration and confirmed the mine workers eligibility. Maosheng Yan, Li Lang and Hongying Qu carried out the data analysis. Qingsong Chen and Guiping Chen wrote the final document together.*

Abstract

Background: Raynaud phenomenon has been observed in patients who suffer from enduring exposure to high-frequency hand-arm vibration. The skin, arteries, and nerves of the upper extremities can be modified by such vibration exposure. In this study, we focus on the impact of vibration exposure on the arteries. Vibration-induced white finger (VWF) is the most typical clinical manifestation of vascular injuries in hand-arm vibration syndrome (HAVS). The pathology of VWF is not well understood. However, the vascular disorders resulting from HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities. Capillaroscopy is widely accepted and utilized in the clinical diagnosis of Raynaud's syndrome. It has also been introduced for the diagnosis of HAVS in recent years, due to its advantages of non-invasive vivo examination, ability to follow disease progression overtime, uncomplicated and non-time-consuming performance, provision of various characteristics for analysis, and inexpensive costs.

Objective: The purpose of this study was to investigate the effectiveness of nailfold capillaroscopy on the diagnosis of HAVS in the gold industry.

Methods: In total, 113 male gold miners were recruited by cluster sampling; 35 workers who were chronically exposed vibration then developed VWF were defined as the HAVS group, 39 workers who were exposed to vibration but did not have HAVS were classified as the vibration-exposed controls group (VEC), and 39 workers without vibration exposure were categorized as the non-vibration-exposed controls group (NVEC). Video capillaroscopy was used to capture images of the 2nd, 3rd, and 4th fingers of both hands of all subjects at heart level after 10 minutes of rest in a 20±2°C room. The following nailfold capillary characteristics were included: number of capillaries/mm, avascular areas, hemorrhages, and enlarged capillaries. The experiments was carried out in the same winter. All characteristics were evaluated under blinded conditions.

Results: Significant differences in all morphological characteristics existed between the groups (P<0.05). Avascular areas in the HAVS, VEC, and NVEC groups appeared in 74.3%, 43.6%, and 25.0% of subjects, respectively. A higher percentage of subjects had hemorrhages in the HAVS group (65.7%) compared with the other groups (VEC: 7.7% and NVEC: 7.5%). The number of capillaries/mm (cut-off: 8.75, sensitivity: 76.9%, specificity: 77.1%), input loop width (cut-off: 16.72, sensitivity: 80.0%, specificity: 74.4%), output loop width (cut-off: 13.73, sensitivity: 77.1%, specificity: 74.7%), apical width (cut-off: 18.51, sensitivity: 80.0%, specificity: 79.5%), and ratio of output loop and input loop (cut-off: 1.29, sensitivity: 74.0%, specificity: 71.8%) had more than 70% sensitivity or specificity of its cut-off value while the capillary loops length (cut-off: 165.37, sensitivity: 65.7%, specificity: 56.4%) and capillary total width (cut-off: 44.46, sensitivity: 62.9%, specificity: 53.8%) had low sensitivity and specificity of their cut-off values.

Conclusion: Nailfold capillary characteristics, especially the number of capillaries/mm, avascular areas, hemorrhages, output loop width, input loop width, and apical width have high value in the diagnosis of HAVS.

Keywords: Nailfold capillaroscopy; HAVS; Vibration-induced white finger; vascular injuries;

Strengths and limitations of this study

1. Nailfold capillaroscopy is widely accepted and utilized in the clinical diagnosis of Raynaud's syndrome. Although there were some studies on nailfold capillaroscopy have been published, detailed morphological characteristics values of nailfold capillaroscopy on HAVS have not been fully established yet.
2. Results in our study provide a comprehensive list of nailfold capillaroscopic morphological characteristics reference values which may contribute to the HAVS artery diagnosis procedure.
3. The pathology of VWF is not well understood. In this paper, we also tried to explore the vibration-induced vessel injuries patterns development on HAVS.
4. Demographic characteristics of people in the North and South China may vary, the measurement results in this study may be suitable for subjects from North China with a HAVS diagnosis and further studies are required for South China subjects with HAVS.

Introduction

Hand-arm vibration syndrome (HAVS) is characterized by asymmetrical vasospasms of the digital arteries. Vibration-induced white finger (VWF), the most dominant symptom and the most typical clinical manifestation of vascular injuries of HAVS, is a secondary form of Raynaud's phenomenon (RP), resulting from enduring occupational exposure to hand-held vibrating tools (eg, drills, buffs, and riveters). VWF was first prescribed as an industrial disease in 1985 [1, 2]. Prior to this, HAVS had been recognized legally as and occupational disease since 1957 in China [3]. The pathogenesis of HAVS is not fully understood, but it is clear that vibration can result in the damage of vascular, neurological, and musculoskeletal systems of the upper limbs [4, 5]. Workers who suffer from HAVS may undergo neurological tingling, numbness in the fingers, sensory perception reductions, tactile discrimination and manipulative dexterity, musculoskeletal swelling, and stiffness in hands or loss of grip strength. HAVS may also result in deterioration of manipulative dexterity [6] or operation performance impairment.

In this study, we focus on the impact of vibration exposure on arteries. Raynaud's phenomenon has been observed in patients who suffer from enduring exposure to high frequency hand-arm vibration [7-9]. A positive relationship between high level hand-arm vibration exposure and the appearance of HAVS vascular symptoms has been reported [10]. Exposure to high intensity vibration may be related to vascular changes in the digits, which include vasospasms, fixed narrowing of the vessel lumen, and ischemia [11]. The pathophysiology of RP in HAVS is complicated, but it is presumed that chronic vibration exposure may trigger an exaggerated central sympathetic vasoconstrictor reflex and local changes in the digital vessels, vasoactive substances, including endothelin and immunologic factors, and alterations in blood viscosity. Furthermore, vascular disorders of HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities [2, 12].

Nailfold capillaries, which are typically characterized by their hair pin shape, are mostly parallel to the skin surface (Supplemental Figure 1a). Their structure can be easily seen in vivo by means of nailfold capillaroscopy. Nailfold capillary abnormalities associated with HAVS can be recognized by structural alterations, such as degeneration of capillary density, avascular areas (Supplemental Figure 1b), appearance of enlarged capillaries (Supplemental Figure 1c), local hemorrhages (Supplemental Figure 1d), and angiogenesis. To assess the condition of capillaries, both the morphological characteristic changes and measurements are able to provide disease identification at every stage. The history of capillaroscopy dates back to approximately 200 years ago, and was extensively used after the advent of the work of Maricq and Leroy [13]. Cutolo et al. began their studies on capillaries [14], eventually detailing a capillaroscopy procedure [15] and suggesting a set of parameters which should be taken into consideration, including presence of enlarged and giant capillaries, hemorrhages, loss of capillaries, disorganization of the vascular array, and ramified/bushy capillaries. These researchers also defined the major sclerodermic nailfold capillaroscopy patterns in the "early", "active" and "late" stages. Despite the general description and measurement of nailfold capillaries, the categorization of nailfold capillaries is also worthwhile. Sakaguchi et al. classified workers' nailfold capillary morphology into 5 types [16]. Based on the previous studies, their capillaroscopy studies involved other Raynaud diseases [14, 15, 17], such as connective autoimmune rheumatic diseases [18, 19], systemic lupus erythematosus [20], dermatomyositis [21], and Sjögren syndrome [22]. This method was later introduced in the scanning of VWF. In 2011, Mahbub and Harada reported that few capillaroscopic studies on VWF existed, and the ones that did commonly focused on capillary abnormalities on loop dropouts and/or morphology alterations [23].

Compared with other HAVS diagnostic methods, capillaroscopy provides a relatively objective and feasible way to diagnose HAVS. Vascular Doppler and duplex scanning, cold provocation tests, finger skin temperature (FST), finger systolic blood pressure (FSBP), and plethysmography are extensively used in HAVS diagnosis. However, these methods have been questioned for several reasons. For example, the cold provocation test performance conditions are quite unpleasant which might be unbearable for the subjects, and its test outcome may leads to unreproducible measurement results [24]. Some studies have indicated that use of FSBP without pre- or post-cold stress digit plethysmography or cold provocation might underestimate the severity of a patient's condition. Even though a demonstrable decrease in FSBP occurs upon cooling, it is not specific to HAVS, which means other disease or factors may contribute to the FSBP drop [10]. The diagnostic ability of FSBP in the assessment of HAVS remains a controversial topic [10, 23, 25, 26]. Plethysmography requires expensive equipment and is complicated to perform [23]. The effectiveness of FST on HAVS diagnosis has been doubted for its accuracy of readings, which heavily depend on the thermistor probe position. The specificity and sensitivity of the results were found not to be consistent and reproducible between studies [24, 25]. VWF is the "combination of sympathetic hyperactivity and lesion to structures and functions in the walls of blood vessels in the skin" [5]. The capillary alterations reveal the vascular injuries of HAVS. Thus, the morphology of injured vessels can be visually observed through capillaroscopy, which cannot be achieved using other methods. Capillaroscopy provides an accurate way to measure and analysis data objectively. Also capillaroscopy has advantages for its low-cost, non-invasive and simple manipulation [27, 28].

Thus, the purpose of this study was to investigate the effectiveness of nailfold capillaroscopy on the diagnosis of HAVS.

Methods

Subjects

Subjects with any of the following conditions were not recruited in this study: history of or current hypertension, diabetes, hepatitis, nephritis, trauma (muscular, neural, and osseous). Subjects who were smoked or had alcohol intake within 24 hours or with fingers affected by recent local trauma did not recruit in the experiment, neither. All male participants were recruited by cluster sampling from one gold industry of Shandong province of China.

In total, 35 patients with HAVS were defined as the HAVS group. In addition, 39 age-matched participants, without symptoms of HAVS but occupying the same positions as the HAVS group, were categorized as the vibration-exposed controls (VEC) group. Finally, 39 participants who were employed in positions other than a vibrating tool operation in the same gold industry were classified as the non-vibration-exposed controls (NVEC) group. Subjects were also performed cold provocation after the nailfold capillaroscopy examination, the criterion judged one subject as HAVS group subjects was the observation of cyanosis finger or white finger after cold provocation as well as subjects themselves description of VWF history with evidence (photo or witnesses of other people).

Mineworker information was obtained by face to face interview. The questionnaire, which is based on the questionnaire by SWS, included 4 parts: basic personal information, employment status, habits, and medical history and clinical characteristics. Basic personal information involved name, nationality, age, height, weight, degree of education, and dominant hand. Employment status covered detailed history of past and current work ability, job titles, types of applied vibrating tool exposure, and duration of vibration exposure. Habits consisted of smoking, alcohol intake, and daily transportation. Medical histories and clinical characteristics were documented in detailed with hypertension, diabetes, immunological diseases, hepatitis, nephritis, primary Raynaud's diseases, medicine intake, and digit alteration (appearance of VWF or cyanosis, tingling, numbness, etc.). Informed consent was obtained from each respondent, ethics approval was received from the Ethical Committee of Guangdong Province Hospital for Occupational Disease Prevention and Treatment.

Vibration measurement

Vibration produced by rock drill operation was measured with a VI-400 Pro 3-axial human vibration monitor (QUEST Technologies, Oconomowoc, Wisconsin, USA) by a qualified technician. Daily 8 hours' time-weighted average vibration exposure level (A (8)) was calculated according to ISO 5439-2:2001. Total hand-transmitted vibration exposure dose was calculated as cumulative exposure index (*CEI*) under the following formula:

$$CEI = \sum A(8)^2{}_i t_{di} t_{yi}$$

Where t_{di} is the working days per year of each tool, t_{yi} is the years of using vibrating machine. Napierian logarithm *CEI* (LN (*CEI*)) was used as a final index to calculate the vibration exposure dose.

Nailfold capillaroscopy

Vibration exposure should have occurred at least 24 hours prior to the nailfold capillaroscopy examination. At the beginning of the examination procedure, all subjects were adapted to the room temperature (20-22℃) with their hands placed on the table at heart level for at least 10 minutes. Meanwhile, measurements of the 2nd knuckle of finger skin temperature were also taken. The experiments was carried out in the same winter.

Nailfold capillaroscopy was performed by capillaroscopy (QY-990 video-capillaroscopy, Shenzhen, Guangdong Province, China), which was equipped with a ×400 optical probe, on the 2nd, 3rd, and 4th fingers of both hands prone to developing representative morphological patterns, including enlarged capillaries, capillary density, angiogenesis, local hemorrhages, and avascular areas. A drop of immersion oil was applied to the nailfold bed in order to increase the translucency of the keratin layer. Images of capillaries located in the middle areas of the nailfold within 2 mm were captured for a continuous 4 pictures per digit, 24 pictures per subject. Capillaroscopy was performed by a trained technician.

For every image, the following parameters were taken into analysis: the number of capillaries/mm, avascular areas, hemorrhages, and enlarged capillaries, input loop width, output loop width, apical width, capillary loops length, capillary total width, and ratio of output loop and input loop. Only the distal row of capillaries was used for imagery and data counting. Capillaries with a limb larger than 20μm were defined as "enlarged capillaries", and the lack of at least 2 successive capillaries was defined as an avascular area [29].

The measurements of input loop width, output loop width, apical width, capillary loops length, and capillary total width were calculated by the Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA). Before the calculation the images were modified; the edges were sharpened and brightness and contrast were adjusted properly.

Statistical analysis

The measurement results (the number of capillaries/mm, input loop width, output loop width, apical width, capillary loops length, and capillary total width) are expressed as mean ± standard deviation (with 95% CI); categorical information (avascular areas, hemorrhages, and enlarged capillaries) is presented as proportion (with percentage). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyze the normally distributed quantitative values, and the Mann-Whitney U test was used to analyze the non-normally distribution ones. The Chi-square test was used to analyze categorical information. The measurement results were also evaluated via ROC curves, and then the optimal specificity and sensitivity were selected as cut-off points determined by Youden index for each variable for discriminating between the HAVS group and NVEC group. Logistic regression was applied to observe the association of habits and nailfold capillary characteristics. A probability value of $P<0.05$ (two-sided) was considered statistically significant. All analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics of participants

In total, 113 male gold miners were recruited in this study. As shown in Table 1, the average ages of the HAVS, VEC, and NVEC groups were 42.8, 40.72, and 41.37 years, respectively. The HAVS group had longer seniority than the VEC group; the average seniority of the HAVS and VEC groups was 127.60 and 70.77 months, respectively. The BMIs of the HAVS group (22.52), VEC group (23.92), and NVEC group (24.59) were slightly different. Approximately 80% of participants were smokers and more than 50% of participants were drank alcohol. The LN*CEI* of the HAVS group was 10.52, which is marginally higher than that of the VEC group at 9.91.

Table 1: Demographic characteristics and nailfold capillary characteristics of participants

	HAVS (n=35)	VEC (n=39)	NVEC (n=39)	*P*
Demographic characteristics				
Age (years), mean ± SD	42.80±5.96	40.72±7.35	41.37±8.74	>0.05
Seniority (months), mean ± SD	127.60±63.68[a]	70.77±50.27	—	<0.001†
BMI, mean ± SD	22.52±2.59[a, c]	23.92±2.75	24.59±3.18	0.008
Smoking, n (%)	28 (80)	34 (87.2)	33 (82.5)	>0.05
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	29 (72.5)	>0.05
LN*CEI*, mean ± SD	10.52±1.07	9.91±1.12	—	<0.05†
Nailfold capillary characteristics				
Hemorrhages, n (%)	23 (65.7)[a,c]	3 (7.7)	3 (7.5)	<0.001
Enlarged capillaries, n (%)	21 (60.0)[c]	9.98 (25.6)[b]	9.75 (25.0)	0.04
Avascular areas, n (%)	26 (74.3)[c]	17 (43.6)[b]	10 (25.0)	<0.001
Number of capillaries/mm, mean±SD	7.41±1.79[a,c]	8.87±1.80[b]	9.69±1.22	<0.001
Output loop width (μm), mean±SD	21.09±4.44[a,c]	16.06±2.05	15.74±2.21	<0.001
Input loop width (μm), mean±SD	15.77±3.26[a,c]	13.05±1.75	12.84±1.60	<0.001
Apical width (μm), mean±SD	21.40±4.34[a,c]	16.65±3.41	16.92±2.53	<0.001
Capillary loops length (μm), mean±SD	191.77±52.88[a,c]	180.21±64.99	159.83±29.40	<0.05
Capillary total width (μm), mean±SD	50.71±10.92[c]	42.33±6.25	43.16±5.32	0.001
Ratio of output loop and input loop (μm), mean±SD	1.36±0.13[a,c]	1.25±0.08	1.24±0.10	<0.001

[a]HAVS vs. VEC: $P<0.05$;[b]VEC vs. NVEC: $P<0.05$;[c]HAVS vs. NVEC: $P<0.05$.

†Comparison between HAVS vs. VEC.

Results of Nailfold capillary characteristics

Nailfold capillary characteristics in this study are shown in Table 1 as well. Hemorrhages existed to a greater extent in the HAVS group (65.7%) compared with the VEC and NVEC groups (7.7% and 7.5%, respectively). Enlarged capillaries were also observed more frequently in the HAVS group (60.0%) compared with the VEC (25.6%) and NVEC (25.0%) groups. Avascular areas were evident in 74.3% of HAVS participants, but these areas were only apparent in 43.6% and 25.0% of participants in the VEC and NVEC groups, respectively.

The results of the ROC curve analysis are presented in Table 2. The average number of capillaries/mm in the HAVS group was 7.41±1.79 loops, compared with 8.87±1.80 loops in the VEC group and 9.69±1.22 loops in the NVEC group; the cut-off value was 8.75 capillaries/mm with a sensitivity of 76.9% and a specificity of 77.1%, the area under the ROC curve was 0.846 with a 95% confidence interval ranging from 0.753 to 0.939. The average output loop width (μm) in the HAVS group was 21.09±4.44 compared with 16.06±2.05 and 15.74±2.21 in the VEC and NVEC groups, respectively. The cut-off value of this characteristic was 16.72, with 80.0% sensitivity and 74.4% specificity; the area under the ROC curve was 0.866 and the 95% confidence interval ranged from 0.781 to 0.950. Input loop width (μm) had an average of 15.77±3.26 in the HAVS group compared with 13.05±1.75 and 12.84±1.60 in the VEC and NVEC groups, respectively. The cut-off value was 13.73, with similar sensitivity (77.1%) and specificity (74.7%), and the area under the ROC curve was 0.782 with a 95% confidence interval ranging from 0.669 to 0.895. The mean value of apical width (μm) was 21.40±4.34, 16.65±3.41, and 16.92±2.53 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value of this parameter was 18.51, with a sensitivity of 80.0% and a specificity of 79.5%; the area under the ROC curve was 0.823 with a 95% confidence interval ranging from 0.714 to 0.932. The average of capillary loops lengths (μm) was 191.77±52.88 in the HAVS group compared with 180.21±64.99 and 159.83±29.40 in the VEC and NVEC groups, respectively. The cut-off value of capillary loops length was 165.37, while the sensitivity (65.7%) and specificity (56.4%) were relatively lower than the other characteristics. The trend in the capillary total widths (μm) was similar to that of the capillary loops length, the averages of the HAVS, VEC, and NVEC groups were 50.71±10.92, 42.33±6.25, and 43.16±5.32, respectively; the cut-off value was 44.46 with a low sensitivity (62.9%) and specificity (53.8%). The mean values of the output and input loop ratio were 1.36±0.13, 1.25±0.08, and 1.24±0.10 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value was 1.29 with a sensitivity of 74.0% and specificity of 71.8%; the area under the ROC curve was 0.775, and the 95% confidence interval ranged from 0.669 to 0.881.

We suggest that the characteristics of output loop width, input loop width and apical width had quite good sensitivity (80.0%, 77.1% and 80.0%), while the number of capillaries/mm and apical width had the greatest specificity. Apical width was graded the best sensitivity (80.0%) and specificity (79.5) at the same time among the 4 characteristics. Thus, number of capillaries/mm, output loop width, input loop width and apical width are considered to be the optimum parameters to the HAVS diagnosis.

Significant differences in all nailfold capillary characteristics existed between all groups (*P*<0.05).

Table 2: ROC curves of various nailfold capillary parameters

	Cut-Off Value	Sensitivity (%)	Specificity (%)	Area Under the ROC Curve	95%CI
Number of capillaries/mm	8.75	76.9	77.1	0.846	0.753-0.939
Output loop width, μm	16.72	80.0	74.4	0.866	0.781-0.950

Input loop width, µm	13.73	77.1	74.7	0.782	0.669-0.895
Apical width, µm	18.51	80.0	79.5	0.823	0.714-0.932
Capillary loops length, µm	165.37	65.7	56.4	0.686	0.561-0.812
Capillary total width, µm	44.46	62.9	53.8	0.704	0.580-0.829
Ratio of output loop and input loop	1.29	74.0	71.8	0.775	0.669-0.881

Results of association of nailfold capillaroscopy with habits

Of the 74 subjects in the HAVS and NVEC groups who had a nailfold capillaroscopy, there was an even distribution (*P*>0.05) of participants who smoke or drank alcohol, see Table 3. In total, 80% and 87.2% of subjects in the HAVS and NVEC groups were smokers and 54.3% and 53.8% of subjects in drank alcohol.

Table 3: Distribution of smoking and alcohol intake in the HAVS and NVEC groups

	HAVS (n=35)	NVEC (n=39)	*P*
Smoking, n (%)	28 (80.0)	34 (87.2)	0.403
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	0.970

Results of Logistic regression results

For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (*P*>0.05). Some studies have revealed that there is a relationship between vessel wall injuries, which may contribute to the development of enlarged capillaries, and alcohol intake; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed (Table 4).

Table 4: Logistic regression results of several nailfold capillary characteristics associated with the habits of smoking and alcohol intake in the HAVS and NVEC groups

	Control factor(s)	N (n=74)	Wald	*P*	OR (95%CI)
Hemorrhages, n (%)	Smoking	62 (83.8)	0.286	>0.05	1.412 (0.398,5.007)
	Alcohol drinking	40 (54.1)	0.232	>0.05	0.789 (0.301,2.071)
Enlarged capillaries, n (%)	Smoking	62 (83.8)	1.485	>0.05	2.192 (0.621,7.740)
	Alcohol drinking	40 (54.1)	0.629	>0.05	1.462 (0.572,3.739)
Avascular areas, n (%)	Smoking	62 (83.8)	0.017	>0.05	1.085 (0.313,3.755)
	Alcohol drinking	40 (54.1)	0.522	>0.05	0.713 (0.284,1.787)
VWF, n (%)	Smoking	62 (83.8)	0.693	>0.05	1.703 (0.486,5.960)
	Alcohol drinking	40 (54.1)	0.005	>0.05	0.968 (0.386,2.432)

Results of bivariate correlations

We assumed that the capillary characteristics (number of capillaries/mm, hemorrhages, enlarged capillary, and avascular areas) might be correlated with each other and with the symptoms of VWF, thus we explored the bivariate correlations of the variables mentioned above (Table 5).

Hemorrhages, enlarged capillaries, and avascular areas were positively correlated with VWF (r=0.607, *P* <0.001; r=0.348, *P*<0.05; and r=0.486, *P*<0.001, respectively), while the number of capillaries/mm (r=-0.601, *P*<0.001) was negatively correlated with VWF. Avascular areas were negatively correlated with number of capillaries/mm (r=-0.568, *P*<0.001) and positively correlated with hemorrhages (r=0.416, *P*<0.001). Hemorrhages were negatively correlated with number of capillaries/mm (r=-0.458, *P*<0.001). Avascular areas were not correlated with enlarged capillaries, and enlarged capillaries were not correlated either number of capillaries/mm or hemorrhages.

Table 5: Bivariate correlation of several nailfold capillaroscopy characteristics between the HAVS and NVEC groups

	Number of Capillaries/mm	Hemorrhages	Enlarged Capillaries	Avascular Areas	VWF
Number of capillaries/mm	1	r=-0.458**	r=-0.157	r=-0.568**	r=-0.601**
Hemorrhages	—	1	r=0.178	r=0.416**	r=0.607**
Enlarged capillaries	—	—	1	r=-0.004	r=0.348*
Avascular areas	—	—	—	1	r=0.486**
VWF	—	—	—	—	1

N=74, * *P*< 0.05, ** *P*< 0.001.

Discussion

The nailfold capillaroscopy diagnostic method has been used for RP for years. Abnormal vascular specific patterns are observed differently from one pathological stage to the next. Therefore, diagnostic methods should include a number of parameters, and the proper medical range of each parameter must be set up in order to provide a more complete diagnosis.

Hand-arm vibration syndrome is a secondary form of RP. Although some studies have explored the effectiveness of nailfold capillaroscopy on HAVS diagnosis, its diagnostic parameters were either not comprehensive or were mainly focused on the classification of variables for levels of disease categorization. Few studies have put forward parameters for clinical cut-off values, and most of the nailfold capillaroscopy studies have focused on the semi-quantitative scale methods of nailfold capillary patterns, rather than giving exact values of nailfold capillary measurements. The capillary alterations in immunological diseases with respect to RP are quite apparent and different in every pathological stage. However, the situation is different for HAVS, and the prevention of the development of HAVS needs medical reference values as thresholds for judging vibration exposure to workers. This will ultimately help to decide if workers are still suitable for their position.

In this study, we attempted to obtain a set of reliable medical reference values of vascular measurements, and we also attempted to find out the relationships between each vascular pattern and VWF pathological changes.

As the bivariate correlation analysis results showed, the appearance of hemorrhages was negatively correlated with the number of capillaries/mm, which meant that the more hemorrhages that appeared, the less capillaries were present within 1mm. The number of capillaries/mm (r=-0.568, P<0.001) and hemorrhages (r=0.416, P<0.001) were, correspondingly, negatively and positively correlated with avascular areas, which indicated that the avascular areas arose with the appearance of hemorrhages and the degeneration of capillary number. The manifestation of VWF was positively correlated with hemorrhages (r=0.607, P<0.001), enlarged capillaries (0.348, P<0.05), and avascular areas (r=0.486, P<0.001), but was negatively correlated with number of capillaries/mm (r=-0.601, P<0.001). This revealed that more hemorrhages, enlarged capillaries, and avascular areas were associated with more severe VWF (and vice versa). In addition, fewer capillaries appearing within 1 mm was associated with more serious VWF.

Thus, we infer that: operating hand-held vibrating tools up to a certain degree, might lead to neurological and vascular damage of the hands, characterized by vasoconstriction caused by endothelium damage followed by morphological alterations of the capillaries. Pathological changes of capillary alterations manifest as enlarged or tortuous capillaries occur as blood hypoxia, which arise from vascular compensation. Moreover, as a consequence of chronical vessel wall impairment and rupture, erythrocytes are able to cross over the vessel wall then form hemorrhages, which gives rise to degeneration of capillaries. Finally, capillaries begin to drop off, which causes vascular areas.

Shandong province, located in the northeast of China, has extreme cold weather in the winter. Gold mine workers who are exposed to hand-arm vibration under such weather and working conditions may develop hand capillary impairment, characterized as VWF, which is seen as vasoconstriction.

Morphological alterations were seen on nailfold capillaroscopy. A previous study indicated that hemorrhages are the bridge between enlarged capillaries and avascular areas [18]. This is consistent with our study, where, in the HAVS group, the ratio of hemorrhages was similar to that of enlarged capillaries, while avascular areas increased up to 74.3%. We suggest that the appearance of enlarged capillaries and hemorrhages triggered vascular degeneration forming the avascular areas. Thus, the proportion of hemorrhages and enlarged capillaries was greater than the presence of avascular areas. However, it is possible that, as many of the HAVS subjects were still undergoing the process of developing HAVS, the existence of enlarged capillaries and hemorrhages but not avascular areas could be observed. In the VEC and NVEC groups, the avascular areas were greater than or equal to the existence of hemorrhages and enlarged capillaries, which was in accordance with our previous hypothesis mentioned in paragraph one.

HAVS subjects had more hemorrhages and enlarged capillaries than those in the VEC and NVEC groups. Generally, enlarged capillaries are much more likely than hemorrhages to be observed in subjects who are chronically exposed to hand arm vibration and those subjects tend to develop HAVS. Once participants are exposed to hand-arm vibration for a certain duration, for example those in the HAVS group, hemorrhages are more likely to be observed. We propose that hemorrhages form after the appearance of enlarged capillaries, as previous study mentioned. Thus, it is feasible that hemorrhages and enlarged capillaries should be included as diagnostic characteristics.

Avascular areas are likely related to capillary density ($r=-0.568$, $P<0.001$). The inhomogeneous distribution of capillaries results in drop off, which decreases the amount of capillaries, creating avascular areas.

The effectiveness in counting the number of capillaries per millimeter in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects [30, 31]. Ingegnoli et al. indicated that the number of capillaries per millimeter in healthy subjects ranged from 7 to 10. Our results, 7.41±1.79/mm in the HAVS group, 8.87±1.80/mm in the VEC group, and 9.69±1.22/mm in the NVEC group, are consistent with the previous data, and we also believe that counting the number of capillaries per millimeter is the proper way to judge some capillary abnormalities. The results of number of capillaries per millimeter published here have reference significance for HAVS clinical parameters, with a cut-off value of 8.75 capillaries in 1mm, of which sensitivity and specificity are 76.9% and 77.1%, respectively.

In the process of setting reliable parameters of HAVS medical reference values, the ROC curves in our study showed several parameters with high sensitivity or specificity. Parameters with high sensitivity (more than 77%) were output loop width, input loop width, and apical width, while the parameters with high specificity were number of capillaries/mm and apical width. It is worth mentioning that apical width shares a high sensitivity and a high specificity. Here, we recommend that the parameters of number of capillaries/mm, output loop width, input loop width, and apical width could be taken into consideration as HAVS medical diagnosis parameters, especially apical width. What makes the sensitivity and specificity of these parameters so different is the loop shape itself. Generally, the output or input loop is uneven, which means that the measurement point varies, thus resulting in diverse measurements. However, the capillary apex is fixed in a narrow range, thus the measurement of apical width would be relatively more objective than measurement of the loops.

Results in this study are generally in accordance with previous RP studies. We believe it is because HAVS is a secondary form of RP that shares a familiar pathology with other Raynaud diseases [32, 33]. Therefore, we suppose our results are reliable in the diagnosis of HAVS.

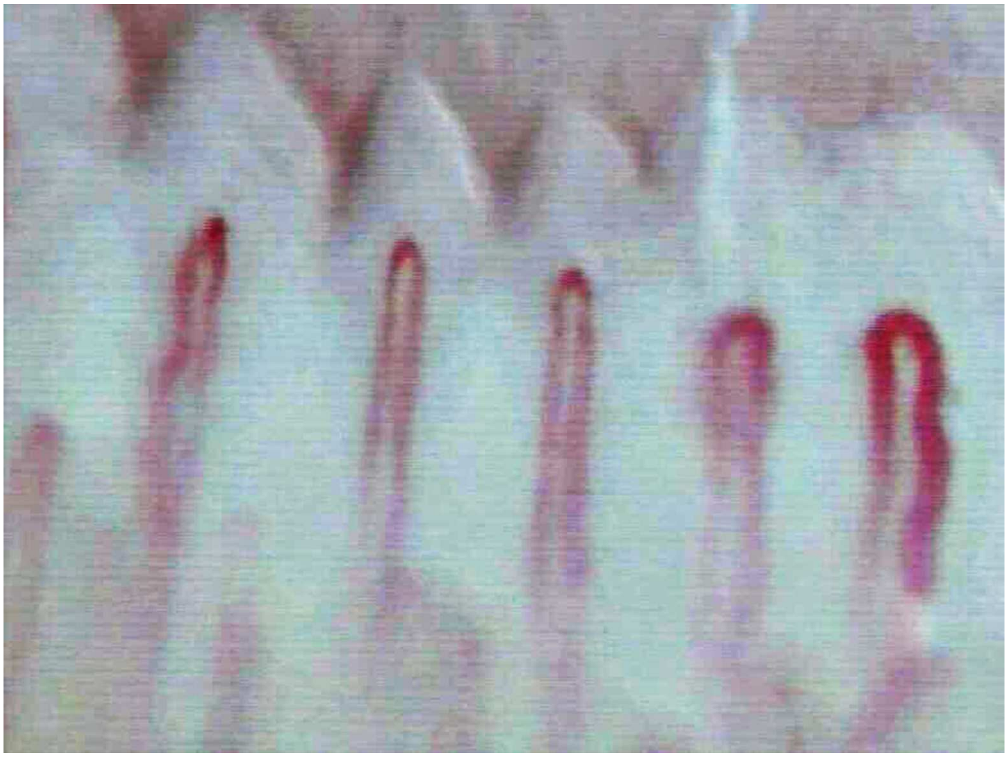
The intent of this study was to propose and support an objective and feasible HAVS diagnosis method. Our study supports both on-the-job and pre-job screening examinations. To this end, workers exposed to hand-arm vibration could be protected by changing positions once they are diagnosed by capillaroscopy. Reserve workers could be assessed to see if they are suitable for a hand-arm vibration position or if they are susceptible populations who should be settled in non-hand-arm vibration position by pre job capillaroscopy examination.

This study has some limitations. Demographic characteristics of people in the North and South China may vary. As an example, the subjects in this study were from North China, which must adapt to the extreme cold winters. Persons in the South of China, who are not adapted to cold winters, may have different vascular measurement results. Hence, the measurement results in this study may be suitable for subjects from North China with a HAVS diagnosis and further studies are required for South China subjects with HAVS. Nevertheless, in future studies, we highly recommend that a weighted index be considered as one of the diagnostic rules, which may enhance the procedure in clinical practice with more accuracy and raise the diagnosis procedure efficiency.

References

1. Cutolo, M., et al., *Capillaroscopy.* Best Pract Res Clin Rheumatol, 2008. **22**(6): p. 1093-108.
2. Noel, B., *Pathophysiology and classification of the vibration white finger.* Journal of the Peripheral Nervous System, 2000. **5**(4): p. 242-242.
3. Lin, W., et al., *The study on hand-arm vibration syndrome in China.* Industrial health, 2005. **43**(3): p. 480-483.
4. Griffin, M.J., *Handbook of human vibration*. 2012: Academic press.
5. Gemne, G., *Diagnostics of hand-arm system disorders in workers who use vibrating tools.* Occupational and Environmental Medicine, 1997. **54**(2): p. 90-95.
6. Sauni, R., et al., *Work disability after diagnosis of hand-arm vibration syndrome.* Int Arch Occup Environ Health, 2015. **88**(8): p. 1061-8.
7. Nyantumbu, B., et al., *Hand-arm vibration syndrome in South African gold miners.* Occup Med (Lond), 2007. **57**(1): p. 25-9.
8. Harada, N. and M.H. Mahbub, *Diagnosis of vascular injuries caused by hand-transmitted vibration.* Int Arch Occup Environ Health, 2008. **81**(5): p. 507-18.
9. Hatron, P., P. Frimat, and E. Hachulla, *[Raynaud's phenomena of occupational origin].* La Revue du praticien, 1998. **48**(15): p. 1653-1658.
10. Heaver, C., et al., *Hand–arm vibration syndrome: a common occupational hazard in industrialized countries.* Journal of Hand Surgery (European Volume), 2011. **36**(5): p. 354-363.
11. Maundrell, A. and S.M. Proudman, *Epidemiology of Raynaud's Phenomenon*, in *Raynaud's Phenomenon*. 2015, Springer. p. 21-35.
12. Olsen, N., *Centrally and locally mediated vasomotor activities in Raynaud's phenomenon.* Scandinavian journal of work, environment & health, 1987: p. 309-312.
13. Maricq, H.R. and E. Carwile LeRoy, *Patterns of finger capillary abnormalities in connective tissue disease by "wide - field" microscopy.* Arthritis & Rheumatism, 1973. **16**(5): p. 619-628.
14. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis & rheumatism, 2003. **48**(11): p. 3023-3030.
15. Cortes, S. and M. Cutolo, *Capillarosecopic patterns in rheumatic diseases.* Acta reumatológica

portuguesa, 2006. **32**(1): p. 29-36.
16. Sakaguchi, S., et al., *Morphologic classification of nailfold capillary microscopy in workers exposed to hand-arm vibration.* Industrial health, 2011. **49**(5): p. 614-618.
17. Smith, V., et al., *Nailfold capillaroscopy for day-to-day clinical use: construction of a simple scoring modality as a clinical prognostic index for digital trophic lesions.* Annals of the rheumatic diseases, 2010: p. annrheumdis132431.
18. Cutolo, M., et al., *The contribution of capillaroscopy to the differential diagnosis of connective autoimmune diseases.* Best Practice & Research Clinical Rheumatology, 2007. **21**(6): p. 1093-1108.
19. Cutolo, M., et al., *Nailfold capillaroscopy is useful for the diagnosis and follow-up of autoimmune rheumatic diseases. A future tool for the analysis of microvascular heart involvement?* Rheumatology, 2006. **45**(suppl 4): p. iv43-iv46.
20. Bongard, O., et al., *Association of anticardiolipin antibodies and abnormal nailfold capillaroscopy in patients with systemic lupus erythematosus.* Lupus, 1995. **4**(2): p. 142-144.
21. Marie, I., et al., *Polymyositis and dermatomyositis: short term and longterm outcome, and predictive factors of prognosis.* The Journal of rheumatology, 2001. **28**(10): p. 2230-2237.
22. Tektonidou, M., et al., *Microvascular abnormalities in Sjögren's syndrome: nailfold capillaroscopy.* Rheumatology, 1999. **38**(9): p. 826-830.
23. Mahbub, M. and N. Harada, *Review of different quantification methods for the diagnosis of digital vascular abnormalities in hand-arm vibration syndrome.* Journal of occupational health, 2011. **53**(4): p. 241-249.
24. Harada, N., *Cold-stress tests involving finger skin temperature measurement for evaluation of vascular disorders in hand-arm vibration syndrome: review of the literature.* International archives of occupational and environmental health, 2002. **75**(1-2): p. 14-19.
25. Poole, K., J. Elms, and H. Mason, *The diagnostic value of finger systolic blood pressure and cold-provocation testing for the vascular component of hand–arm vibration syndrome in health surveillance.* Occupational Medicine, 2004. **54**(8): p. 520-527.
26. Thompson, A., R. House, and M. Manno, *The sensitivity and specificity of thermometry and plethysmography in the assessment of hand–arm vibration syndrome.* Occupational medicine, 2008. **58**(3): p. 181-186.
27. Etehad Tavakol, M., et al., *Nailfold Capillaroscopy in Rheumatic Diseases: Which Parameters Should Be Evaluated?* Biomed Res Int, 2015. **2015**: p. 974530.
28. Filosa, G. and R. De Angelis, *Capillaroscopy.* 2015: p. 1107-1114.
29. Ingegnoli, F., et al. *Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations*. in *Seminars in arthritis and rheumatism*. 2009. Elsevier.
30. Ingegnoli, F., et al., *Nailfold capillary patterns in healthy subjects: a real issue in capillaroscopy.* Microvascular research, 2013. **90**: p. 90-95.
31. Hoerth, C., et al., *Qualitative and quantitative assessment of nailfold capillaries by capillaroscopy in healthy volunteers.* Vasa, 2012. **41**(1): p. 19-26.
32. Herrick, A.L., *Pathogenesis of Raynaud's phenomenon.* Rheumatology (Oxford), 2005. **44**(5): p. 587-96.
33. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis Rheum, 2003. **48**(11): p. 3023-30.



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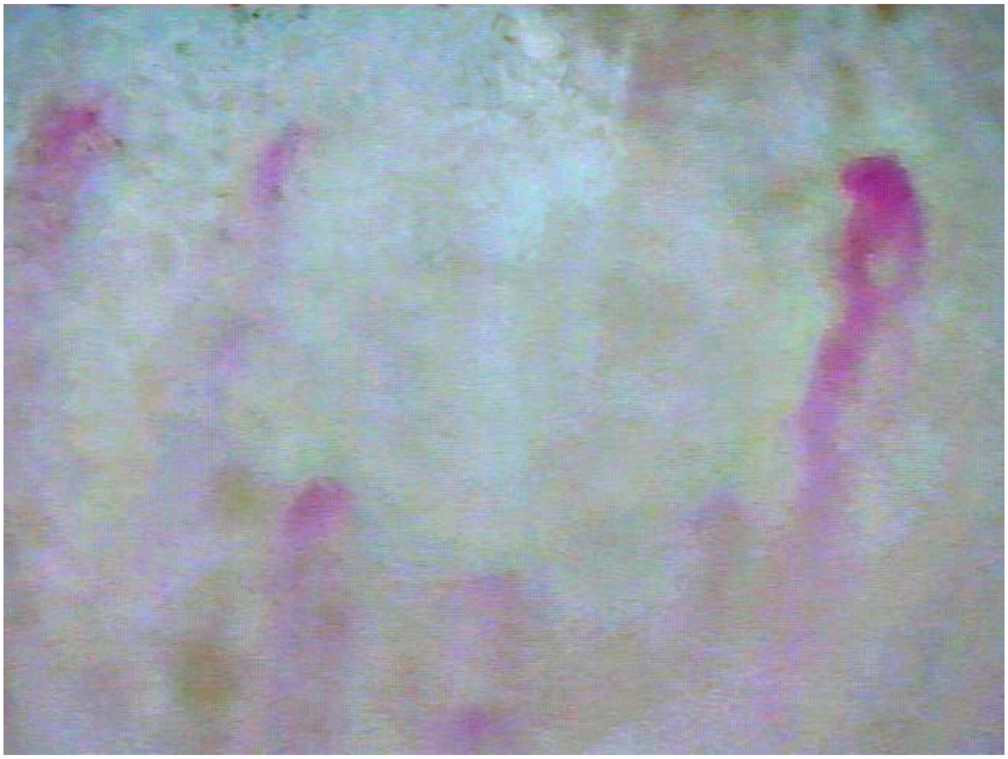

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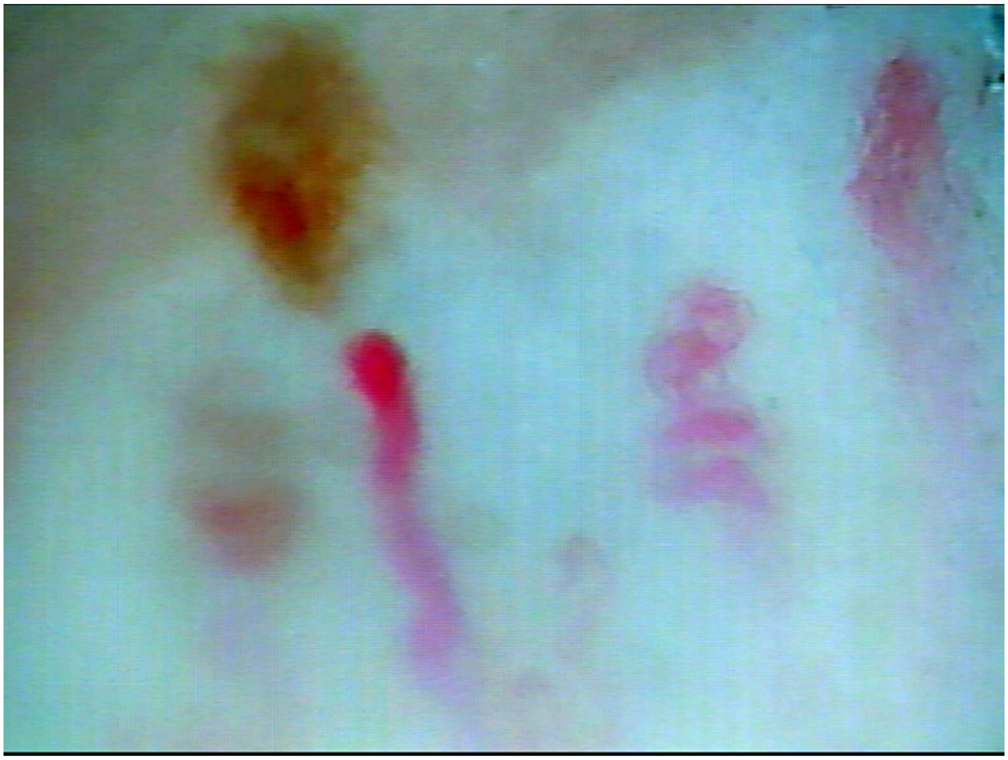

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Nailfold capillary morphological characteristics of hand-arm vibration syndrome: a cross-sectional study

Qingsong Chen [†,*,1], **Guiping Chen**[†,1], **Bin Xiao**[1], **Hansheng Lin**[1], **Hongying Qu**[1],

Danying Zhang[1], **Maogong Shi**[2], **Li Lang**[1], **Bei Yang**[2], **Maosheng Yan**[1]

[1] *Guangdong Province Hospital for Occupational Disease Prevention and Treatment；And Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China;*

[2] *Yantaishan Hospital, Yantai, Shandong264000, China.*

† *These authors contributed equally to this work.*

* *Author to whom correspondence should be addressed; E-Mail: qschen@gdoh.org;*

Add.: No.68, Xingang Xi Road, Haikang Street, Haizhu District, Guangzhou, China.

Tel.: +86-20-8926-6013 (Q.C.); Fax: +86-20-8902-1031 (Q.C.).

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[1] *Guangdong Province Hospital for Occupational Disease Prevention and Treatment；And Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China;*

[2] *Yantaishan Hospital, Yantai, Shandong 264000, China.*

† *These authors contributed equally to this work.*

* *Author to whom correspondence should be addressed; E-Mail: qschen@gdoh.org;*

Add.: No.68, Xingang Xi Road, Haikang Street, Haizhu District, Guangzhou, China.
Tel.: +86-20-8926-6013 (Q.C.); Fax: +86-20-8902-1031 (Q.C.).

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Competing interests: No potential competing interests relevant to this article were reported, and all authors have declared no competing interests.

***Contributors**: Qingsong Chen designed the project, Guiping Chen developed and executed the nailfold capillaroscopy performance. Guiping Chen, Danying Zhang and Bei Yang discussed and decided the nailfold capillaroscopy characteristics which should be taken into consideration, then carried out the nailfold capillary measurements. Bin Xiao and Hansheng Lin devised and executed the vibration measurement. Maogong Shi and Bei Yang visited the collaborating gold mine company administration and confirmed the mine workers eligibility. Maosheng Yan, Li Lang and Hongying Qu* *carried out the data analysis. Qingsong Chen and Guiping Chen wrote the final document together.*

Abstract

Objective: The purpose of this study was to investigate the characteristics of nailfold capillaroscopy associate to hand-arm vibration syndrome.

Methods: In total, 113 male goldminers were recruited: 35 workers who were chronically exposed vibration then developed VWF were defined as the HAVS group, 39 workers who were exposed to vibration but did not have HAVS were classified as the vibration-exposed controls group (VEC), and 39 workers without vibration exposure were categorized as the non-vibration-exposed controls group (NVEC). Video capillaroscopy was used to capture images of the 2nd, 3rd, and 4th fingers of both hands. The following nailfold capillary characteristics were included: number of capillaries/mm, avascular areas, haemorrhages, and enlarged capillaries. The experiments was carried out in the same winter. All characteristics were evaluated under blinded conditions.

Results: Significant differences in all morphological characteristics existed between the groups (P<0.05). Avascular areas in the HAVS, VEC, and NVEC groups appeared in 74.3%, 43.6%, and 25.0% of subjects, respectively. A higher percentage of subjects had haemorrhages in the HAVS group (65.7%) compared with the other groups (VEC: 7.7% and NVEC: 7.5%). The number of capillaries/mm, input limb width, output limb width, apical width, and ratio of output limb and input limb had more than 70% sensitivity or specificity of its cut-off value.

Conclusion: Nailfold capillary characteristics, especially the number of capillaries/mm, avascular areas, haemorrhages, output limb width, input limb width, and apical width alterations revealed significant relation with hand-arm vibration syndrome.

Keywords: Nailfold capillaroscopy; Hand-arm vibration syndrome; Vibration-induced white finger; vascular injuries;

Strengths and limitations of this study

1. Nailfold capillaroscopy is widely accepted and utilized in the clinical diagnosis of Raynaud's syndrome. Although there were some studies on nailfold capillaroscopy have been published, detailed morphological characteristics of nailfold capillaroscopy on hand-arm vibration syndrome have not been fully established yet.
2. The pathology of VWF is not well understood. In this article, we also tried to explore the vibration-induced vessel injuries patterns development on hand-arm vibration syndrome.
3. Demographic characteristics of people may vary from the northern to the southern China, the measurement results in this study are probably suitable for the northern population, and further studies in southern population are required in the future.

Introduction

Hand-arm vibration syndrome (HAVS) is characterized by asymmetrical vasospasms of the digital arteries. Vibration-induced white finger (VWF), the most dominant symptom and the most typical clinical manifestation of vascular injuries of HAVS, is a secondary form of Raynaud's phenomenon (RP), resulting from enduring occupational exposure to hand-held vibrating tools (e.g. drills, buffs, and riveters). VWF was first prescribed as an industrial disease in 1985 [1, 2]. Prior to this, HAVS had been recognized legally as and occupational disease since 1957 in China [3]. The pathogenesis of HAVS is not fully understood, but it is clear that vibration can results in the damage of vascular, neurological, and musculoskeletal systems of the upper limbs [4, 5]. Workers who suffer from HAVS may undergo neurological tingling, numbness in the fingers, sensory perception reductions, tactile discrimination and manipulative dexterity, musculoskeletal swelling, and stiffness in hands or loss of grip strength. HAVS may also results in deterioration of manipulative dexterity [6] or operation performance impairment.

In this study, we focus on the impact of vibration exposure on arteries. Raynaud's phenomenon has been observed in patients who suffer from enduring exposure to high frequency hand-arm vibration [7-9]. A positive relation between high level hand-arm vibration exposure and the appearance of HAVS vascular symptoms has been reported [10]. Exposure to high intensity vibration may be related to vascular changes in the digits, which include vasospasms, fixed narrowing of the vessel lumen, and ischemia [11]. The pathophysiology of RP in HAVS is complicated, but it is presumed that chronic vibration exposure may trigger an exaggerated central sympathetic vasoconstrictor reflex and local changes in the digital vessels, vasoactive substances, including endothelin and immunologic factors, and alterations in blood viscosity. Furthermore, vascular disorders of HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities [2, 12].

Nailfold capillaries, which are typically characterized by their hair pin shape, are mostly parallel to the skin surface (Figure 1). Their structure can be easily seen in vivo by means of nailfold capillaroscopy. Nailfold capillary abnormalities associated with HAVS can be recognized by structural alterations, such as degeneration of capillary density, avascular areas (Figure 2), appearance of enlarged capillaries (Figure 3), local haemorrhages (Figure 4), and angiogenesis. To assess the condition of capillaries, both the morphological characteristic changes and measurements are able to provide disease identification at every stage. The history of capillaroscopy dates back to approximately 200 years ago, and was extensively used after the advent of the work of Maricq and Leroy [13]. Cutolo et al. began their studies on capillaries [14], eventually detailing a capillaroscopy procedure [15] and suggesting a set of parameters which should be taken into consideration, including presence of enlarged and giant capillaries, haemorrhages, loss of capillaries, disorganization of the vascular array, and ramified/bushy capillaries. These researchers also defined the major sclerodermic nailfold capillaroscopy patterns in the "early", "active" and "late" stages. Despite the general description and measurement of nailfold capillaries, the categorization of nailfold capillaries is also worthwhile. Sakaguchi et al. classified workers' nailfold capillary morphology into 5 types [16]. Based on the previous studies, their capillaroscopy studies involved other Raynaud diseases [14, 15, 17], such as connective autoimmune rheumatic diseases [18, 19], systemic lupus erythematosus [20], dermatomyositis [21], and Sjögren syndrome [22]. This method was later introduced in the scanning of VWF. In 2011, Mahbub and Harada reported that few capillaroscopic studies on VWF existed, and the ones that did commonly focused on capillary abnormalities on limb dropouts and/or morphology alterations [23]. Vanessa Smith et al. even are standardized the method of interpreting individual capillaries reliably and introduce a more practical way for novice to distinguish from normal capillary to abnormal capillary individually after having a quick training course[24].

Compared with existing hand-arm vibration syndrome diagnostic methods, capillaroscopy provides a relatively objective and feasible way to assess HAVS. Vascular Doppler and duplex scanning, cold provocation tests, finger skin temperature (FST), finger systolic blood pressure (FSBP), and plethysmography are extensively applied in HAVS diagnosis. However, some of these methods have been questioned for several reasons. For example, the performance conditions of cold provocation test are quite unpleasant which might be unbearable for the subjects, and its test outcomes may lead to unreproducible measurement results [25]. Some studies have indicated that use of FSBP without pre- or post-cold stress digit plethysmography or cold provocation might underestimate the severity of a patient's condition. Even though a demonstrable decrease in FSBP occurs upon cooling, it is not specific to HAVS, which means other disease or factors may contribute to the FSBP drop [10]. The diagnostic ability of FSBP in the assessment of HAVS remains a controversial topic [10, 23, 26, 27]. Plethysmography requires expensive equipment and it is complicated to perform[23]. The effectiveness of FST on HAVS diagnosis has been doubted for its accuracy of readings, which heavily depend on the thermistor probe position. The specificity and sensitivity of the results were found not to be consistent and reproducible between studies [25, 26]. VWF is the “combination of sympathetic hyperactivity and lesion to structures and functions in the walls of blood vessels in the skin” [5]. The capillary alterations reveal the vascular injuries of HAVS. Thus, the morphology of injured vessels can be visually observed through capillaroscopy, which cannot be achieved by using other methods. Capillaroscopy provides an accurate way to measure and analysis data objectively. Also, capillaroscopy has advantages for its low-cost, non-invasive and simple manipulation [28, 29].

Thus, this study aimed to investigate the characteristics of nailfold capillaroscopy associate to hand-arm vibration syndrome.

Methods

Subjects

All participants were males, workers exposed to hand-arm vibration with at least 12 continuous months seniorities. Subjects with any of the following conditions were not recruited in this study: history of or current hypertension, diabetes, hepatitis, nephritis, immunological diseases and trauma (muscular, neural, and osseous); who smoked or had alcohol intake within 24 hours or with fingers affected by recent local trauma did not recruit in the experiment, neither. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covered 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited by a particular health exam. Cold provocation test was performed on all subjects after taking nailfold capillaroscopy examination.

In total, 113 subjects were recruited and participated in this study: 35 patients, defined by the revealed vibration white finger symptom after the cold provocation as well as the workers themselves description of VWF history with evidence (photo or third party witnesses), were categorized as the HAVS group. In addition, 39 age-matched participants, occupied the same hand-arm vibration exposed position but without symptoms of HAVS, were categorized as the vibration-exposed controls (VEC) group. Finally, 39 age-matched participants who were employed in positions without hand-arm vibration exposure were classified as the non-vibration-exposed controls (NVEC) group.

Goldminer information was obtained by face to face interview. The questionnaire, based on the questionnaire by SWS, included 4 parts: basic personal information, employment status, habits, and medical history and clinical characteristics. Basic personal information involved name, nationality, age, height, weight, degree of education, and dominant hand. Employment status covered detailed history of past and current work ability, job titles, types of applied vibrating tool exposure, and duration of vibration exposure. Habits consisted of smoking, alcohol intake, and daily transportation. Medical histories and clinical characteristics were documented in detailed with hypertension, diabetes, immunological diseases, hepatitis, nephritis, primary Raynaud’s diseases, medicine intake, and digit alteration (appearance of VWF or cyanosis, tingling, numbness, etc.).

Informed consent was obtained from each respondent, ethics approval was received from the Ethical Committee of Guangdong Province Hospital for Occupational Disease Prevention and Treatment.

Vibration measurement

Vibration produced by rock drill operation was measured with a VI-400 Pro 3-axial human vibration monitor (QUEST Technologies, Oconomowoc, Wisconsin, USA) by a qualified technician. Daily 8 hours’ time-weighted average vibration exposure level (A (8)) was calculated according to ISO 5439-

2:2001. Total hand-transmitted vibration exposure dose was calculated as cumulative exposure index (*CEI*) under the following formula:

$$CEI = \sum A(8)^2{}_i t_{di} t_{yi}$$

Where t_{di} is the working days per year of each tool, t_{yi} is the years of using vibrating machine. Napierian logarithm *CEI* (LN (*CEI*)) was used as a final index to calculate the vibration exposure dose.

Nailfold capillaroscopy

Vibration exposure should have occurred at least 24 hours prior to the nailfold capillaroscopy examination. At the beginning of the examination procedure, all subjects adapted to the room temperature (20-22℃) with their hands placed on the table at heart level for at least 10 minutes. Meanwhile, measurements of the 2nd knuckle of finger skin temperature were also taken. The experiments were carried out in the same winter.

Nailfold capillaroscopy was performed by capillaroscopy (CSW-XW2000 video-capillaroscopy, Shenzhen, Guangdong Province, China), which was equipped with a ×400 optical probe, on the 2nd, 3rd, and 4th fingers of both hands which prone to developing representative morphological patterns, including enlarged capillaries, capillary density, angiogenesis, local haemorrhages, and avascular areas. A drop of immersion oil was applied to the nailfold bed in order to increase the translucency of the keratin layer. Images of capillaries in the middle areas of the nailfold within 2 mm were captured for a continuous 4 pictures per digit, 24 pictures per subject. Capillaroscopy was performed by a trained technician.

For every image, the following parameters were taken into analysis: the number of capillaries/mm, avascular areas, haemorrhages, enlarged capillaries, input limb width, output limb width, apical width, capillary limbs length, capillary total width, and ratio of output limb and input limb. Only the distal row of capillaries was used for imagery and data counting. Capillary with a limb larger than 20μm was defined as "enlarged capillaries", and the lack of at least 2 successive capillaries was defined as an avascular area [30]. All characteristics were evaluated under blinded conditions.

The measurements of input limb width, output limb width, apical width, capillary limbs length, and capillary total width were calculated by the Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA). Before calculation the images were modified: the edges were sharpened, brightness and contrast were adjusted properly.

Statistical analysis

The measurement results (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are expressed as mean ± standard deviation (with 95% CI); categorical information (avascular areas, haemorrhages, and enlarged capillaries) is presented as proportion (with percentage). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distribution ones. The Chi-square test was used to analyse categorical information. The measurement results were also evaluated via sensitivity analysis, and then the optimal specificity and sensitivity were selected as cut-off points determined by Youden index for each variable for discriminating between the HAVS group and NVEC group. Logistic regression was applied to observe the association of habits and nailfold capillary characteristics. A probability value of $P<0.05$ (two-sided) was considered statistically significant. All analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics of participants

In total, 113 male goldminers were recruited in this study. As shown in Table 1, the average ages of the HAVS, VEC, and NVEC groups were 42.8, 40.72, and 41.37 years, respectively. The HAVS group had longer seniority than the VEC group; the average seniority of the HAVS and VEC groups was 127.60 and 70.77 months, respectively. The BMIs of the HAVS group (22.52), VEC group (23.92), and NVEC group (24.59) were slightly different. Approximately 80% of participants were smokers and more than 50% of participants drank alcohol. The LN*CEI* of the HAVS group was 10.52, which is marginally higher than that of the VEC group at 9.91.

Table 1: Demographic characteristics and nailfold capillary characteristics of participants

	HAVS (n=35)	VEC (n=39)	NVEC (n=39)	*P*
Demographic characteristics				
Age (years), mean ± SD	42.80±5.96	40.72±7.35	41.37±8.74	>0.05
Seniority (months), mean ± SD	127.60±63.68[a]	70.77±50.27	—	<0.001†
BMI, mean ± SD	22.52±2.59[a, c]	23.92±2.75	24.59±3.18	0.008
Smoking, n (%)	28 (80)	34 (87.2)	33 (82.5)	>0.05
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	29 (72.5)	>0.05
LN*CEI*, mean ± SD	10.52±1.07	9.91±1.12	—	<0.05†
Nailfold capillary characteristics				
Haemorrhages, n (%)	23 (65.7)[a,c]	3 (7.7)	3 (7.5)	<0.001
Enlarged capillaries, n (%)	21 (60.0)[c]	9.98 (25.6)[b]	9.75 (25.0)	0.04
Avascular areas, n (%)	26 (74.3)[c]	17 (43.6) [b]	10 (25.0)	<0.001
Number of capillaries/mm, mean±SD	7.41±1.79[a,c]	8.87±1.80[b]	9.69±1.22	<0.001
Output limb width (μm), mean±SD	21.09±4.44[a,c]	16.06±2.05	15.74±2.21	<0.001
Input limb width (μm), mean±SD	15.77±3.26[a,c]	13.05±1.75	12.84±1.60	<0.001
Apical width (μm), mean±SD	21.40±4.34[a,c]	16.65±3.41	16.92±2.53	<0.001
Capillary limbs length (μm), mean±SD	191.77±52.88[a,c]	180.21±64.99	159.83±29.40	<0.05
Capillary total width (μm), mean±SD	50.71±10.92[c]	42.33±6.25	43.16±5.32	0.001
Ratio of output limb and input limb (μm), mean±SD	1.36±0.13[a,c]	1.25±0.08	1.24±0.10	<0.001

[a]HAVS vs. VEC: $P<0.05$;[b]VEC vs. NVEC: $P<0.05$;[c]HAVS vs. NVEC: $P<0.05$.

†Comparison between HAVS vs. VEC.

Note: Napierian logarithm CEI (LN (CEI)) was used as a final index to calculate the vibration exposure dose.

Results of Nailfold capillary characteristics

Nailfold capillary characteristics in this study are shown in Table 1 as well. Haemorrhages existed to a greater extent in the HAVS group (65.7%) compared with the VEC and NVEC groups (7.7% and 7.5%, respectively). Enlarged capillaries were also observed more frequently in the HAVS group (60.0%) compared with the VEC (25.6%) and NVEC (25.0%) groups. Avascular areas were evident in 74.3% of HAVS participants, but these areas were only apparent in 43.6% and 25.0% of participants in the VEC and NVEC groups, respectively.

The results of the sensitivity analysis are presented in Table 2. The average number of capillaries/mm in the HAVS group was 7.41±1.79 limbs, compared with 8.87±1.80 limbs in the VEC group and 9.69±1.22 limbs in the NVEC group; the cut-off value was 8.75 capillaries/mm with a sensitivity of 76.9% and a specificity of 77.1%, the area under the ROC curve was 0.846 with a 95% confidence interval ranging from 0.753 to 0.939. The average output limb width (μm) in the HAVS group was 21.09±4.44 compared with 16.06±2.05 and 15.74±2.21 in the VEC and NVEC groups, respectively. The cut-off value of this characteristic was 16.72, with 80.0% sensitivity and 74.4% specificity; the area under the ROC curve was 0.866 and the 95% confidence interval ranged from 0.781 to 0.950. Input limb width (μm) had an average of 15.77±3.26 in the HAVS group compared with 13.05±1.75 and 12.84±1.60 in the VEC and NVEC groups, respectively. The cut-off value was 13.73, with similar sensitivity (77.1%) and specificity (74.7%), and the area under the ROC curve was 0.782 with a 95% confidence interval ranging from 0.669 to 0.895. The mean values of apical width (μm) were 21.40±4.34, 16.65±3.41, and 16.92±2.53 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value of this parameter was 18.51, with a sensitivity of 80.0% and a specificity of 79.5%; the area under the ROC curve was 0.823 with a 95% confidence interval ranging from 0.714 to 0.932. The average of capillary limbs lengths (μm) was 191.77±52.88 in the HAVS group compared with 180.21±64.99 and 159.83±29.40 in the VEC and NVEC groups, respectively. The cut-off value of capillary limbs length was 165.37, while the sensitivity (65.7%) and specificity (56.4%) were relatively lower than the other characteristics. The trend in the capillary total widths (μm) was similar to that of the capillary limbs length, the averages of the HAVS, VEC, and NVEC groups were 50.71±10.92, 42.33±6.25, and 43.16±5.32, respectively; the cut-off value was 44.46 with a low sensitivity (62.9%) and specificity (53.8%). The mean values of the output and input limb ratio were 1.36±0.13, 1.25±0.08, and 1.24±0.10 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value was 1.29 with a sensitivity of 74.0% and specificity of 71.8%; the area under the ROC curve was 0.775, and the 95% confidence interval ranged from 0.669 to 0.881.

It can be seen that the characteristics of output limb width, input limb width and apical width had quite good sensitivity (80.0%, 77.1% and 80.0%), while the number of capillaries/mm and apical width had the greatest specificity. Apical width was graded the best sensitivity (80.0%) and specificity (79.5) at the same time among the 4 characteristics. Thus, number of capillaries/mm, output limb width, input limb width and apical width are considered to be the optimum parameters to the HAVS diagnosis. Significant differences in all nailfold capillary characteristics existed between all groups (P<0.05).

Table 2: Sensitivity analysis of various nailfold capillary parameters

	Cut-Off Value	Sensitivity (%)	Specificity (%)	Area Under the ROC Curve	95%CI
Number of capillaries/mm	8.75	76.9	77.1	0.846	0.753-0.939
Output limb width, μm	16.72	80.0	74.4	0.866	0.781-0.950
Input limb width, μm	13.73	77.1	74.7	0.782	0.669-0.895
Apical width, μm	18.51	80.0	79.5	0.823	0.714-0.932
Capillary limbs length, μm	165.37	65.7	56.4	0.686	0.561-0.812
Capillary total width, μm	44.46	62.9	53.8	0.704	0.580-0.829
Ratio of output limb and input limb	1.29	74.0	71.8	0.775	0.669-0.881

Results of association of nailfold capillaroscopy with habits

Of the 74 subjects in the HAVS and NVEC groups who had a nailfold capillaroscopy, there was an even distribution (*P*>0.05) of participants who smoke or drank alcohol, see Table 3. In total, 80% and 87.2% of subjects in the HAVS and NVEC groups were smokers and 54.3% and 53.8% of subjects in drank alcohol.

Table 3: Distribution of smoking and alcohol intake in the HAVS and NVEC groups

	HAVS (n=35)	NVEC (n=39)	*P*
Smoking, n (%)	28 (80.0)	34 (87.2)	0.403
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	0.970

Results of Logistic regression results

For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (*P*>0.05). Some studies have revealed that there is a relation with vessel wall injuries and alcohol intake, which may contributes to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed (Table 4).

Table 4: Logistic regression results of several nailfold capillary characteristics associated with the habits of smoking and alcohol intake in the HAVS and NVEC groups

	Control factor(s)	N (n=74)	Wald	*P*	OR (95%CI)
Haemorrhages, n (%)	Smoking	62 (83.8)	0.286	>0.05	1.412 (0.398,5.007)
	Alcohol drinking	40 (54.1)	0.232	>0.05	0.789 (0.301,2.071)
Enlarged capillaries, n (%)	Smoking	62 (83.8)	1.485	>0.05	2.192 (0.621,7.740)
	Alcohol drinking	40 (54.1)	0.629	>0.05	1.462 (0.572,3.739)
Avascular areas, n (%)	Smoking	62 (83.8)	0.017	>0.05	1.085 (0.313,3.755)
	Alcohol drinking	40 (54.1)	0.522	>0.05	0.713 (0.284,1.787)
VWF, n (%)	Smoking	62 (83.8)	0.693	>0.05	1.703 (0.486,5.960)
	Alcohol drinking	40 (54.1)	0.005	>0.05	0.968 (0.386,2.432)

Results of bivariate correlations

We assumed that the capillary characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, and avascular areas) might correlated with each other and with the symptoms of VWF. Therefore we explored the bivariate correlations of the variables mentioned above (Table 5).

Haemorrhages, enlarged capillaries, and avascular areas positively correlated with VWF (r=0.607, *P* <0.001; r=0.348, *P*<0.05; and r=0.486, *P*<0.001, respectively), while the number of capillaries/mm (r=-0.601, *P*<0.001) negatively correlated with VWF. Avascular areas negatively correlated with number of capillaries/mm (r=-0.568, *P*<0.001) and positively correlated with haemorrhages (r=0.416, *P*<0.001). Haemorrhages negatively correlated with number of capillaries/mm (r=-0.458, *P*<0.001). Avascular areas did not correlate with enlarged capillaries, and enlarged capillaries did not correlate either number of capillaries/mm or haemorrhages, neither.

Table 5: Bivariate correlation of several nailfold capillaroscopy characteristics between the HAVS and NVEC groups

	Number of Capillaries/mm	Haemorrhages	Enlarged Capillaries	Avascular Areas	VWF
Number of capillaries/mm	1	r=-0.458**	r=-0.157	r=-0.568**	r=-0.601**
Haemorrhages	—	1	r=0.178	r=0.416**	r=0.607**
Enlarged capillaries	—	—	1	r=-0.004	r=0.348*
Avascular areas	—	—	—	1	r=0.486**
VWF	—	—	—	—	1

N=74, * *P*< 0.05, ** *P*< 0.001.

Discussion

The nailfold capillaroscopy examination has been applied for Raynaud's phenomenon (RP) for years. The observed abnormal specific vascular patterns are distinctive from one pathological stage to the other. Therefore, this method should include a number of parameters, and the proper value range of each parameter needs to be set up in order to provide a more complete assessment.

Hand-arm vibration syndrome (HAVS) is a secondary form of Raynaud's phenomenon (RP). Although some studies have explored nailfold capillaroscopy on HAVS, its parameters were either not comprehensive or were mainly focused on the classification of variables for levels of disease categorization. Few studies suggested parameters for clinical cut-off values, and most of the nailfold capillaroscopy studies focused on the semi-quantitative scale methods of nailfold capillary patterns, rather than giving exact values of nailfold capillary measurements. The capillary alterations in immunological diseases with respect to RP are quite apparent and contrast in every pathological stage. However, it is different in HAVS condition, and the prevention of the HAVS development needs reference values as thresholds for judging vibration exposure to workers. This will ultimately help to decide if a worker is still suitable for his position.

In this study, we attempted to obtain a set of reliable reference values of vascular measurements, and also to find out the relations between each vascular pattern and vibration white finger pathological change.

As the bivariate correlation analysis results showed, the appearance of haemorrhages negatively correlated with the number of capillaries/mm, which meant that the more haemorrhages appeared, the less capillaries presented within 1mm. The number of capillaries/mm (r=-0.568, P<0.001) and haemorrhages (r=0.416, P<0.001), correspondingly, negatively and positively correlated with avascular areas, which indicated that the avascular areas arose with the appearance of haemorrhages and the degeneration of capillary number. The manifestation of VWF positively correlated with haemorrhages (r=0.607, P<0.001), enlarged capillaries (0.348, P<0.05), and avascular areas (r=0.486, P<0.001), but negatively correlated with number of capillaries/mm (r=-0.601, P<0.001). This revealed that more haemorrhages, enlarged capillaries, and avascular areas associated with more severe VWF (and vice versa). In addition, fewer capillaries appearing within 1 mm associated with more serious VWF.

Thus, we infer that: operating hand-held vibrating tools up to a certain degree, might leads to neurological and vascular damage of the hands, characterized by vasoconstriction caused by endothelium damage, followed by morphological alterations of the capillaries. Pathological changes of capillary alterations manifest as enlarged or tortuous capillaries occurring as blood hypoxia, which arise from vascular compensation. Moreover, as a consequence of chronical vessel wall impairment and rupture, erythrocytes are able to cross over the vessel wall then form haemorrhages, which give rise to degeneration of capillaries. Finally, capillaries begin to drop off, which cause vascular areas.

Shandong province, located in the northeast of China, has extreme cold weather in the winter.

Goldminers who are exposed to hand-arm vibration under such weather and working conditions may develop capillary impairment, characterized as VWF, which is seen as vasoconstriction.

Morphological alterations can be observed on nailfold capillaroscopy. A previous study indicated that haemorrhages are the bridge between enlarged capillaries and avascular areas [18]. Our study consists with this, where, in the HAVS group, ratio of haemorrhages was similar to that of enlarged capillaries, while avascular areas increased up to 74.3%. We suggest that the appearance of enlarged capillaries and haemorrhages triggered vascular degeneration, forming the avascular areas. Thus, the proportions of haemorrhages and enlarged capillaries were greater than the presence of avascular areas. However, it is possible that, as many of the HAVS subjects were still undergoing the process of developing HAVS, the existence of enlarged capillaries and haemorrhages but not avascular areas could be observed. In the VEC and NVEC groups, the avascular areas were greater than or equal to the existence of haemorrhages and enlarged capillaries, which were in accordance with our previous hypothesis mentioned in paragraph one.

Hand-arm vibration syndrome (HAVS) subjects had more haemorrhages and enlarged capillaries than those in the VEC and NVEC groups. Generally, enlarged capillaries are much more likely to be observed than haemorrhages in subjects who are chronically exposed to hand arm vibration and those subjects tend to develop HAVS. Once participants are exposed to hand-arm vibration for a certain duration, for example those in the HAVS group, haemorrhages are more likely to be observed. We propose that haemorrhages form after enlarged capillaries appearance, as previous study mentioned. Thus, it is feasible that haemorrhages and enlarged capillaries should be included as reference characteristics.

Avascular areas likely related to capillary density (r=-0.568, $P<0.001$). The inhomogeneous distribution of capillaries results in drop off, which decreases the amount of capillaries, creating avascular areas.

The effectiveness in counting the number of capillaries per millimeter in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects [31, 32]. Ingegnoli et al. indicated that the number of capillaries per millimeter in healthy subjects ranged from 7 to 10. Our results, 7.41±1.79/mm in the HAVS group, 8.87±1.80/mm in the VEC group, and 9.69±1.22/mm in the NVEC group, are consistent with the previous data, and we also believe that counting the number of capillaries per millimeter is a proper way to judge capillary abnormalities. The results of number of capillaries per millimeter published here have reference significance for HAVS, with a cut-off value of 8.75 capillaries within 1mm, of which sensitivity and specificity are 76.9% and 77.1%, respectively.

In the process of setting reliable parameters of HAVS reference values, the sensitivity analysis in our study showed several parameters with considerable sensitivity or specificity. Parameters with considerable sensitivity (more than 77%) were output limb width, input limb width, and apical width, while the parameters with considerable specificity were number of capillaries/mm and apical width. It is worth mentioning that apical width shares a considerable sensitivity and a considerable specificity. Here, we recommend that the parameters of number of capillaries/mm, output limb width, input limb width, and apical width can be taken into consideration as HAVS examination parameters, especially the apical width. What makes the sensitivity and specificity of these parameters so different is the limb shape itself. Generally, the output or input limb is uneven, which means that the measurement point varies, thus results in diverse measurements. However, the capillary apex is fixed in a narrow range, thus the measurement of apical width would be relatively more objective than measurement of the limbs.

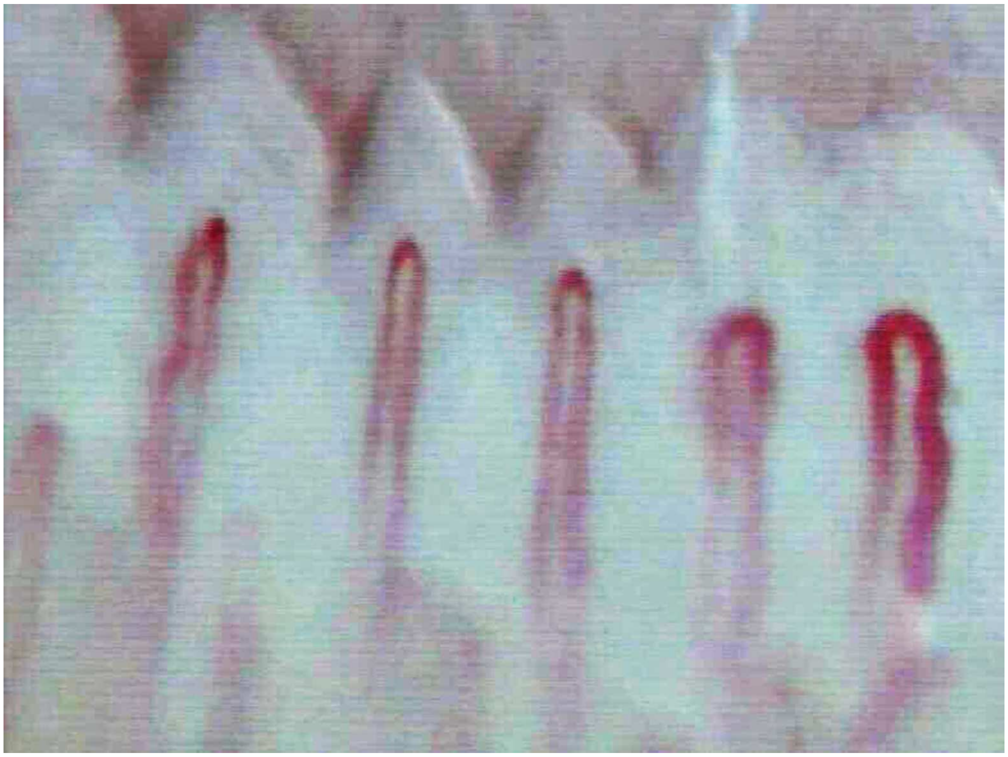
Results in this study are generally in accordance with previous Raynaud's phenomenon (RP) studies. We believe it is because hand-arm vibration syndrome is a secondary form of RP that shares a familiar pathology with other Raynaud diseases [33, 34]. Therefore, we suppose our results are reliable in the assessment of HAVS.

However, there are some limitations in this study: demographic characteristics of populations may vary from the northern to the southern China. As an example, the subjects in this study were from northern China, who adapt to the extreme cold winters while the population in the southern China, who do not adapt to cold temperature, this will lead to different vascular measurement results. Hence, the measurement results in this study probably represent for population in northern place, and further studies are required for the southern place population. Nevertheless, in future studies, we highly recommend that a weighted index can be considered as one of the assessment rules, which may enhance the nailfold capillaroscopy analysis with more accuracy, and also raise the HAVS assessment procedure efficiency.

References

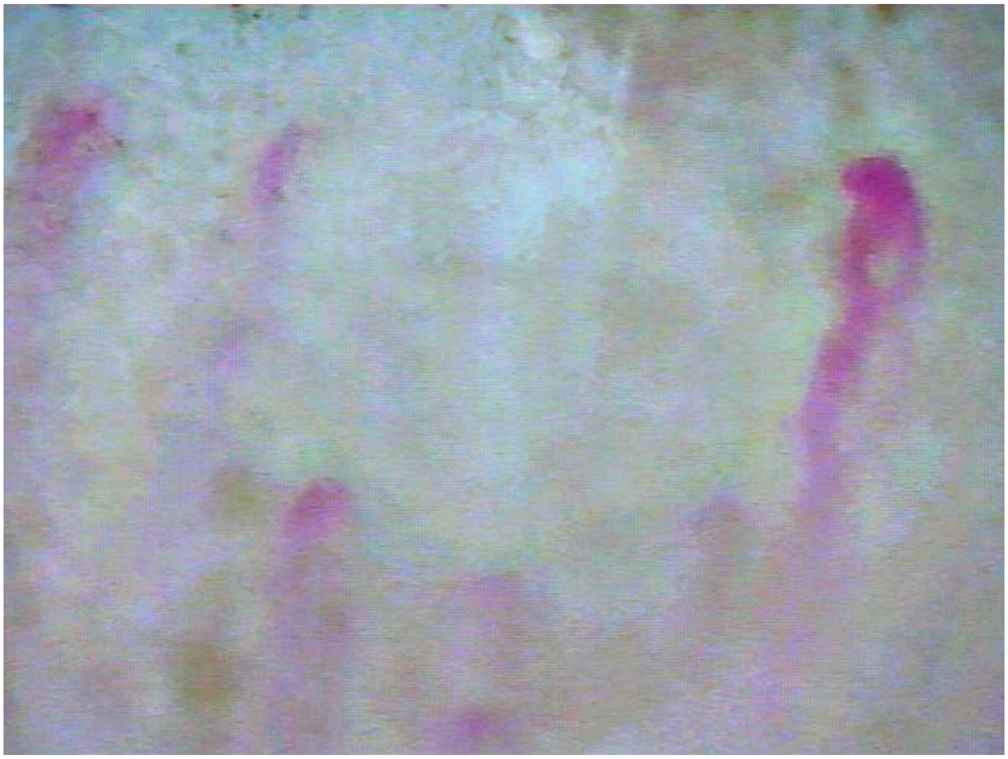
1. Cutolo, M., et al., *Capillaroscopy.* Best Pract Res Clin Rheumatol, 2008. **22**(6): p. 1093-108.
2. Noel, B., *Pathophysiology and classification of the vibration white finger.* Journal of the Peripheral Nervous System, 2000. **5**(4): p. 242-242.
3. Lin, W., et al., *The study on hand-arm vibration syndrome in China.* Industrial health, 2005. **43**(3): p. 480-483.
4. Griffin, M.J., *Handbook of human vibration*. 2012: Academic press.
5. Gemne, G., *Diagnostics of hand-arm system disorders in workers who use vibrating tools.* Occupational and Environmental Medicine, 1997. **54**(2): p. 90-95.
6. Sauni, R., et al., *Work disability after diagnosis of hand-arm vibration syndrome.* Int Arch Occup Environ Health, 2015. **88**(8): p. 1061-8.
7. Nyantumbu, B., et al., *Hand-arm vibration syndrome in South African gold miners.* Occup Med (Lond), 2007. **57**(1): p. 25-9.
8. Harada, N. and M.H. Mahbub, *Diagnosis of vascular injuries caused by hand-transmitted vibration.* Int Arch Occup Environ Health, 2008. **81**(5): p. 507-18.
9. Hatron, P., P. Frimat, and E. Hachulla, *[Raynaud's phenomena of occupational origin].* La Revue du praticien, 1998. **48**(15): p. 1653-1658.
10. Heaver, C., et al., *Hand–arm vibration syndrome: a common occupational hazard in industrialized countries.* Journal of Hand Surgery (European Volume), 2011. **36**(5): p. 354-363.
11. Maundrell, A. and S.M. Proudman, *Epidemiology of Raynaud's Phenomenon*, in *Raynaud's Phenomenon*. 2015, Springer. p. 21-35.
12. Olsen, N., *Centrally and locally mediated vasomotor activities in Raynaud's phenomenon.* Scandinavian journal of work, environment & health, 1987: p. 309-312.
13. Maricq, H.R. and E. Carwile LeRoy, *Patterns of finger capillary abnormalities in connective tissue disease by "wide - field" microscopy.* Arthritis & Rheumatism, 1973. **16**(5): p. 619-628.
14. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis & rheumatism, 2003. **48**(11): p. 3023-3030.
15. Cortes, S. and M. Cutolo, *Capillarosecopic patterns in rheumatic diseases.* Acta reumatológica portuguesa, 2006. **32**(1): p. 29-36.
16. Sakaguchi, S., et al., *Morphologic classification of nailfold capillary microscopy in workers exposed to hand-arm vibration.* Industrial health, 2011. **49**(5): p. 614-618.
17. Smith, V., et al., *Nailfold capillaroscopy for day-to-day clinical use: construction of a simple scoring modality as a clinical prognostic index for digital trophic lesions.* Annals of the rheumatic diseases, 2010: p. annrheumdis132431.
18. Cutolo, M., et al., *The contribution of capillaroscopy to the differential diagnosis of connective autoimmune diseases.* Best Practice & Research Clinical Rheumatology, 2007. **21**(6): p. 1093-1108.
19. Cutolo, M., et al., *Nailfold capillaroscopy is useful for the diagnosis and follow-up of autoimmune rheumatic diseases. A future tool for the analysis of microvascular heart involvement?* Rheumatology, 2006. **45**(suppl 4): p. iv43-iv46.
20. Bongard, O., et al., *Association of anticardiolipin antibodies and abnormal nailfold capillaroscopy in patients with systemic lupus erythematosus.* Lupus, 1995. **4**(2): p. 142-144.

21. Marie, I., et al., *Polymyositis and dermatomyositis: short term and longterm outcome, and predictive factors of prognosis.* The Journal of rheumatology, 2001. **28**(10): p. 2230-2237.
22. Tektonidou, M., et al., *Microvascular abnormalities in Sjögren's syndrome: nailfold capillaroscopy.* Rheumatology, 1999. **38**(9): p. 826-830.
23. Mahbub, M. and N. Harada, *Review of different quantification methods for the diagnosis of digital vascular abnormalities in hand-arm vibration syndrome.* Journal of occupational health, 2011. **53**(4): p. 241-249.
24. Smith, V., et al., *An EULAR study group pilot study on reliability of simple capillaroscopic definitions to describe capillary morphology in rheumatic diseases.* Rheumatology, 2016. **55**(5): p. 883-890.
25. Harada, N., *Cold-stress tests involving finger skin temperature measurement for evaluation of vascular disorders in hand-arm vibration syndrome: review of the literature.* International archives of occupational and environmental health, 2002. **75**(1-2): p. 14-19.
26. Poole, K., J. Elms, and H. Mason, *The diagnostic value of finger systolic blood pressure and cold-provocation testing for the vascular component of hand–arm vibration syndrome in health surveillance.* Occupational Medicine, 2004. **54**(8): p. 520-527.
27. Thompson, A., R. House, and M. Manno, *The sensitivity and specificity of thermometry and plethysmography in the assessment of hand–arm vibration syndrome.* Occupational medicine, 2008. **58**(3): p. 181-186.
28. Etehad Tavakol, M., et al., *Nailfold Capillaroscopy in Rheumatic Diseases: Which Parameters Should Be Evaluated?* Biomed Res Int, 2015. **2015**: p. 974530.
29. Filosa, G. and R. De Angelis, *Capillaroscopy.* 2015: p. 1107-1114.
30. Ingegnoli, F., et al. *Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations*. in *Seminars in arthritis and rheumatism*. 2009. Elsevier.
31. Ingegnoli, F., et al., *Nailfold capillary patterns in healthy subjects: a real issue in capillaroscopy.* Microvascular research, 2013. **90**: p. 90-95.
32. Hoerth, C., et al., *Qualitative and quantitative assessment of nailfold capillaries by capillaroscopy in healthy volunteers.* Vasa, 2012. **41**(1): p. 19-26.
33. Herrick, A.L., *Pathogenesis of Raynaud's phenomenon.* Rheumatology (Oxford), 2005. **44**(5): p. 587-96.
34. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis Rheum, 2003. **48**(11): p. 3023-30.



Normal capillaries

232x174mm (300 x 300 DPI)

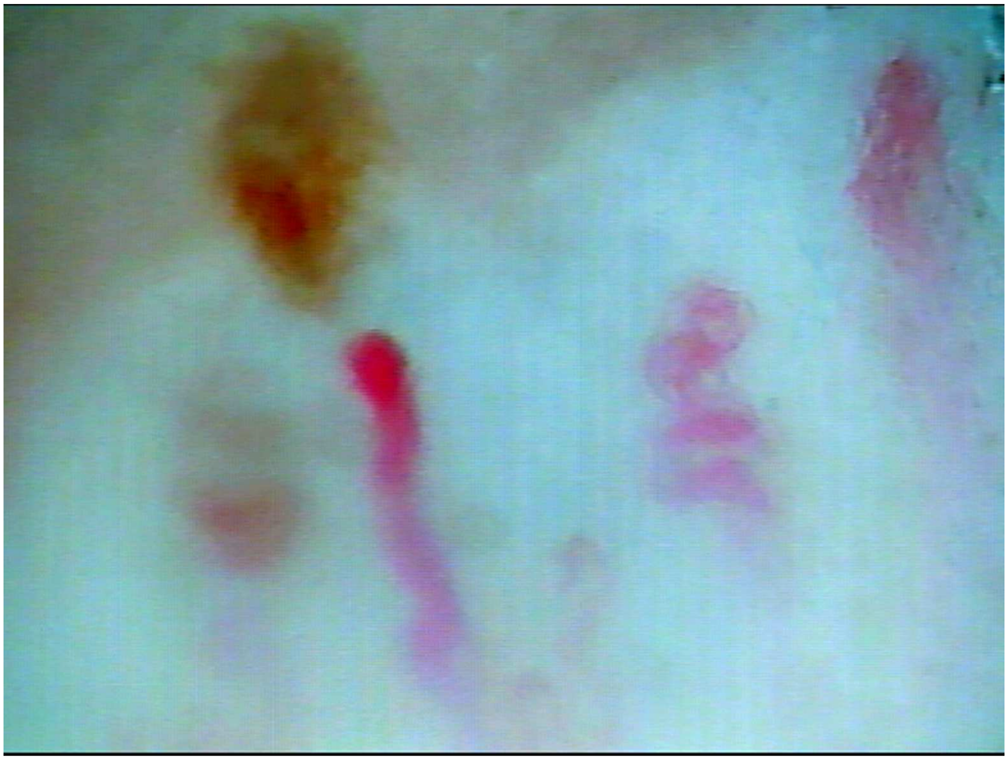


Avascular area

225x169mm (300 x 300 DPI)

Enlarged capillary

172x129mm (300 x 300 DPI)



microhemorrhage

172x130mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	Nailfold capillary morphological characteristics of hand-arm vibration syndrome: a cross-sectional study
Introduction		
Background/rationale	2	Raynaud's phenomenon has been observed in patients who suffer from enduring exposure to high-frequency hand-arm vibration. The skin, arteries, and nerves of the upper extremities can be modified by such vibration exposure. In this study, we focus on the impact of vibration exposure on the arteries. Vibration-induced white finger (VWF) is the most typical clinical manifestation of vascular injuries in hand-arm vibration syndrome (HAVS). The pathology of VWF is not well understood. However, the vascular disorders resulting from HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities. Capillaroscopy is widely accepted and utilized in the clinical diagnosis of Raynaud's syndrome. It has also been introduced for the assessment of HAVS in recent years, due to its advantages of non-invasive vivo examination, ability to follow disease progression overtime, uncomplicated and non-time-consuming performance, provision of various characteristics for analysis, and inexpensive costs.
Objectives	3	The purpose of this study was to investigate the characteristics of nailfold capillaroscopy associate to hand-arm vibration syndrome.
Methods		
Study design	4	Nailfold capillaroscopy; Hand-arm vibration syndrome; Vibration-induced white finger; vascular injuries
Setting	5	The study, including nailfold capillaroscopy image capture and cold water provocation, were carried out in the same winter in 2014 in Shandong province, China. Nailfold capillaroscopy data measurement and analysis were performed from January to May, 2015.
Participants	6	All participants were males, workers exposed to hand-arm vibration with at least 12 continuous months seniorities. Subjects with any of the following conditions were not recruited in this study: history of or current hypertension, diabetes, hepatitis, nephritis, immunological diseases and trauma (muscular, neural, and osseous); who smoked or had alcohol intake within 24 hours or with fingers affected by recent local trauma did not recruit in the experiment, neither. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covered 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited by a particular health exam. In total, 113 subjects were recruited and participated in this study: 35 patients, defined by the revealed vibration white finger symptom after the cold provocation as well as the workers themselves description of VWF history with evidence (photo or third party witnesses), were categorized as the HAVS group. In addition, 39 age-matched participants, occupied the same hand-arm vibration exposed position but without symptoms of HAVS, were categorized as the vibration-exposed controls (VEC) group. Finally, 39 age-matched participants who were employed in positions without hand-arm vibration exposure were classified as the non-vibration-exposed controls (NVEC) group.

Variables	7	For every image, the following parameters were taken into analysis: the number of capillaries/mm, avascular areas, haemorrhages, enlarged capillaries, input limb width, output limb width, apical width, capillary limbs length, capillary total width, and ratio of output limb and input limb. Only the distal row of capillaries was used for imagery and data counting. Capillary with a limb larger than 20μm was defined as "enlarged capillaries", and the lack of at least 2 successive capillaries was defined as an avascular area. Total hand-transmitted vibration exposure dose was calculated as cumulative exposure index (CEI), Napierian logarithm CEI (LN (CEI)) was used as a final index to calculate the vibration exposure dose.
Data sources/ measurement	8*	Qualitative variables (haemorrhages, enlarged capillaries, and avascular areas) were counted in number if the symptom revealed, then presented it in percentage. Quantitative variables (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are counted in capillary number or measured in Photoshop software. Input limb width was the measurement of the input limb the widest limb part linear distance, it's the same procedure for measuring output limb width, and apical width. Capillary limbs length was the measurement of the vertical linear distance between the apex of the apical capillary part and the visible point of the capillary base. Capillary total width was the measurement of the linear distance between leftmost point of one limb and rightmost point of another limb of the widest part of capillary.
Bias	9	The experiments were carried out in the same winter. All nailfold capillary characteristics were evaluated under blinded conditions.
Study size	10	It was determined by cluster sampling.
Quantitative variables	11	There are several quantitative variables in the analyses: the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width. They are expressed as mean ± standard deviation (with 95% CI). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distribution ones. Grouping these quantitative variables basically followed the suggestions of Maricq and Leroy, and Cutolo et al. articles on nailfold capillaroscopy.
Statistical methods	12	The measurement results (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are expressed as mean ± standard deviation (with 95% CI); categorical information (avascular areas, haemorrhages, and enlarged capillaries) is presented as proportion (with percentage). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distribution ones. The Chi-square test was used to analyse categorical information. The measurement results were also evaluated via ROC curves, and then the optimal specificity and sensitivity were selected as cut-off points determined by Youden index for each variable for discriminating between the HAVS group and NVEC group. Logistic regression was applied to observe the association of habits and nailfold capillary characteristics. A probability value of $P<0.05$ (two-sided) was considered statistically significant. All analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

		Logistic regression was used to analyse several nailfold capillary characteristics associated with the habits of smoking and alcohol intake in the HAVS and NVEC groups. Bivariate correlation was applied to examine several nailfold capillaroscopy characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, avascular areas, and VWF) between the HAVS and NVEC groups
		There are no missing data.
		A one gold industry consists of several Sub Companies in the whole Shandong province. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covered 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited by a particular health exam.
		Sensitivity analyses were applied to assess various nailfold capillary parameters (Number of capillaries/mm, Output limb width, Input limb width, Apical width, Capillary limbs length, Capillary total width, Ratio of output limb and input limb)
Results		
Participants	13*	There are 90 rock drillers in the 3 Sub Companies, thus there are 90 potentially eligible population; then there are 88 examined for eligibility workers, 74 confirmed eligible subjects finally. 74 subjects and no missing individual throughout in the study and completing follow-up and analysed.
		2 asked for private affairs leaves in the first stage; 14 workers were not willing to attend the study.
Descriptive data	14*	In total, 113 male goldminers were recruited in this study. As shown in Table 1, the average ages of the HAVS, VEC, and NVEC groups were 42.8, 40.72, and 41.37 years, respectively. The HAVS group had longer seniority than the VEC group; the average seniority of the HAVS and VEC groups was 127.60 and 70.77 months, respectively. The BMIs of the HAVS group (22.52), VEC group (23.92), and NVEC group (24.59) were slightly different. Approximately 80% of participants were smokers and more than 50% of participants drank alcohol. The LNCEI of the HAVS group was 10.52, which is marginally higher than that of the VEC group at 9.91.
		None.
Outcome data	15*	5 outcome events in total.
Main results	16	Alcohol intake and cigarette will affect vascular function, therefore, these two confounders were adjusted for estimates. For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (P>0.05). Some studies have revealed that there is a relation with vessel wall injuries and alcohol intake, which may contributes to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed.
Other analyses	17	Of the 74 subjects in the HAVS and NVEC groups who had a nailfold capillaroscopy, there was an even distribution (P>0.05) of participants who smoke or drank alcohol, see Table 3. In total, 80% and 87.2% of subjects in the HAVS and NVEC groups were smokers and 54.3% and 53.8% of subjects in drank alcohol. For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (P>0.05). Some studies have

revealed that there is a relation with vessel wall injuries and alcohol intake, which may contributes to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed (Table 4).

We assumed that the capillary characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, and avascular areas) might correlated with each other and with the symptoms of VWF. Therefore we explored the bivariate correlations of the variables mentioned above (Table 5).

Haemorrhages, enlarged capillaries, and avascular areas positively correlated with VWF (r=0.607, P <0.001; r=0.348, P<0.05; and r=0.486, P<0.001, respectively), while the number of capillaries/mm (r=-0.601, P<0.001) negatively correlated with VWF. Avascular areas negatively correlated with number of capillaries/mm (r=-0.568, P<0.001) and positively correlated with haemorrhages (r=0.416, P<0.001). Haemorrhages negatively correlated with number of capillaries/mm (r=-0.458, P<0.001). Avascular areas did not correlate with enlarged capillaries, and enlarged capillaries did not correlate either number of capillaries/mm or haemorrhages, neither.

The results of the sensitivity analysis are presented in Table 2. The average number of capillaries/mm in the HAVS group was 7.41±1.79 limbs, compared with 8.87±1.80 limbs in the VEC group and 9.69±1.22 limbs in the NVEC group; the cut-off value was 8.75 capillaries/mm with a sensitivity of 76.9% and a specificity of 77.1%, the area under the ROC curve was 0.846 with a 95% confidence interval ranging from 0.753 to 0.939. The average output limb width (μm) in the HAVS group was 21.09±4.44 compared with 16.06±2.05 and 15.74±2.21 in the VEC and NVEC groups, respectively. The cut-off value of this characteristic was 16.72, with 80.0% sensitivity and 74.4% specificity; the area under the ROC curve was 0.866 and the 95% confidence interval ranged from 0.781 to 0.950. Input limb width (μm) had an average of 15.77±3.26 in the HAVS group compared with 13.05±1.75 and 12.84±1.60 in the VEC and NVEC groups, respectively. The cut-off value was 13.73, with similar sensitivity (77.1%) and specificity (74.7%), and the area under the ROC curve was 0.782 with a 95% confidence interval ranging from 0.669 to 0.895. The mean values of apical width (μm) were 21.40±4.34, 16.65±3.41, and 16.92±2.53 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value of this parameter was 18.51, with a sensitivity of 80.0% and a specificity of 79.5%; the area under the ROC curve was 0.823 with a 95% confidence interval ranging from 0.714 to 0.932. The average of capillary limbs lengths (μm) was 191.77±52.88 in the HAVS group compared with 180.21±64.99 and 159.83±29.40 in the VEC and NVEC groups, respectively. The cut-off value of capillary limbs length was 165.37, while the sensitivity (65.7%) and specificity (56.4%) were relatively lower than the other characteristics. The trend in the capillary total widths (μm) was similar to that of the capillary limbs length, the averages of the HAVS, VEC, and NVEC groups were 50.71±10.92, 42.33±6.25, and 43.16±5.32, respectively; the cut-off value was 44.46 with a low sensitivity (62.9%) and specificity (53.8%). The mean values of the output and input limb ratio were 1.36±0.13, 1.25±0.08, and 1.24±0.10 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value was 1.29 with a sensitivity of 74.0% and specificity of 71.8%; the area under the ROC curve was 0.775, and the 95% confidence interval ranged from 0.669 to 0.881.

It can be seen that the characteristics of output limb width, input limb width and apical width had quite good sensitivity (80.0%, 77.1% and 80.0%), while the number of capillaries/mm and apical width had the greatest specificity. Apical width was graded the best sensitivity (80.0%) and specificity (79.5) at the same time among the 4 characteristics. Thus, number of capillaries/mm, output limb width, input limb width and apical width are considered to be the

		optimum parameters to the HAVS diagnosis. Significant differences in all nailfold capillary characteristics existed between all groups (P<0.05).
Discussion		
Key results	18	In this study, we attempted to obtain a set of reliable reference values of vascular measurements, and also to find out the relations between each vascular pattern and vibration white finger pathological change. We infer that: operating hand-held vibrating tools up to a certain degree, might leads to neurological and vascular damage of the hands, characterized by vasoconstriction caused by endothelium damage, followed by morphological alterations of the capillaries. Pathological changes of capillary alterations manifest as enlarged or tortuous capillaries occurring as blood hypoxia, which arise from vascular compensation. Moreover, as a consequence of chronical vessel wall impairment and rupture, erythrocytes are able to cross over the vessel wall then form haemorrhages, which give rise to degeneration of capillaries. Finally, capillaries begin to drop off, which cause vascular areas. We suggest that the appearance of enlarged capillaries and haemorrhages triggered vascular degeneration, forming the avascular areas. Thus, the proportions of haemorrhages and enlarged capillaries were greater than the presence of avascular areas. However, it is possible that, as many of the HAVS subjects were still undergoing the process of developing HAVS, the existence of enlarged capillaries and haemorrhages but not avascular areas could be observed. In the VEC and NVEC groups, the avascular areas were greater than or equal to the existence of haemorrhages and enlarged capillaries. The effectiveness in counting the number of capillaries per millimetre in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects. We recommend that the parameters of number of capillaries/mm, output limb width, input limb width, and apical width can be taken into consideration as HAVS examination parameters, especially the apical width.
Limitations	19	There are some limitations in this study: demographic characteristics of populations may vary from the northern to the southern China. As an example, the subjects in this study were from northern China, who adapt to the extreme cold winters while the population in the southern China, who do not adapt to cold temperature, this will lead to different vascular measurement results. Hence, the measurement results in this study probably represent for population in northern place, and further studies are required for the southern place population. Nevertheless, in future studies, we highly recommend that a weighted index can be considered as one of the assessment rules, which may enhance the nailfold capillaroscopy analysis with more accuracy, and also raise the HAVS assessment procedure efficiency.
Interpretation	20	There were three steps before we choose subjects in this study: firstly, all workers participated in the pre-examination by clinical doctors before employment that make sure all candidates are healthy and no contraindications involved (which include primary Raynaud phenomenon, scleroderma and other kinds of immunological diseases) to handle this job. Secondly, before the any experiments in this study, all workers have taken a face-to-face questionnaire which includes questions about immunological hereditary diseases that helps us to make sure no subjects get involved with diseases like systematic scleroderma or Raynaud phenomenon. And finally, we also set up control group, which subjects were also employee in the industry other than working in the gold mine tunnel.
Generalisability	21	Morphological alterations can be observed on nailfold capillaroscopy. A previous study indicated that haemorrhages are the bridge between enlarged capillaries and avascular areas [18]. Our study consists with this, where, in the HAVS group, ratio of haemorrhages was similar to that of enlarged capillaries, while avascular areas increased up to 74.3%.

The effectiveness in counting the number of capillaries per millimeter in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects [31, 32]. Ingegnoli et al. indicated that the number of capillaries per millimeter in healthy subjects ranged from 7 to 10. Our results, 7.41±1.79/mm in the HAVS group, 8.87±1.80/mm in the VEC group, and 9.69±1.22/mm in the NVEC group, are consistent with the previous data, and we also believe that counting the number of capillaries per millimeter is a proper way to judge capillary abnormalities. The results of number of capillaries per millimeter published here have reference significance for HAVS, with a cut-off value of 8.75 capillaries within 1mm, of which sensitivity and specificity are 76.9% and 77.1%, respectively.

Other information		
Funding	22	This study was founded by National Science Foundation of China (NSFC No. 81202183); and by Science and Technology Program of Guangdong, China (STPG No.2013B051000065); and by the Open Projects Program of Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment (No.2012A061400007-03). No original study was based on.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Complete List of Authors:	Chen, Qingsong; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Chen, Guiping; Guangdong Province Hospital for Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China Xiao, Bin; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Lin, Hansheng; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Qu, Hongying; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Zhang, Danying; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Shi, Maogong; Yantaishan Hospital, Yantai, Shandong264000, China Lang, Li; Guangdong Province Hospital for Occupational Disease Prevention and Treatment Yang, Bei; Yantaishan Hospital, Yantai, Shandong264000, China Yan, Maosheng; Guangdong Province Hospital for Occupational Disease Prevention and Treatment
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Nailfold capillary morphological characteristics of hand-arm vibration syndrome: a cross-sectional study

Qingsong Chen [†,*,1], Guiping Chen[†,1], Bin Xiao[1], Hansheng Lin[1], Hongying Qu[1],

Danying Zhang[1], Maogong Shi[2], Li Lang[1], Bei Yang[2], Maosheng Yan[1]

[1] *Guangdong Province Hospital for Occupational Disease Prevention and Treatment；And Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China;*

[2] *Yantaishan Hospital, Yantai, Shandong 264000, China.*

[†] *These authors contributed equally to this work.*

* *Author to whom correspondence should be addressed; E-Mail: qschen@gdoh.org;*

Add.: No.68, Xingang Xi Road, Haikang Street, Haizhu District, Guangzhou, China.

Tel.: +86-20-8926-6013 (Q.C.); Fax: +86-20-8902-1031 (Q.C.).

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[1] *Guangdong Province Hospital for Occupational Disease Prevention and Treatment; And Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangzhou, Guangdong 510300, China;*

[2] *Yantaishan Hospital, Yantai, Shandong 264000, China.*

† *These authors contributed equally to this work.*

* *Author to whom correspondence should be addressed; E-Mail: qschen@gdoh.org;*

Add.: No.68, Xingang Xi Road, Haikang Street, Haizhu District, Guangzhou, China.
Tel.: +86-20-8926-6013 (Q.C.); Fax: +86-20-8902-1031 (Q.C.).

Abstract

Objective: The purpose of this study was to investigate the characteristics of nailfold capillaroscopy associated with hand-arm vibration syndrome.

Methods: In total, 113 male goldminers were recruited: 35 workers who were chronically exposed to vibration then developed VWF were defined as the HAVS group, 39 workers who were exposed to vibration but did not have HAVS were classified as the vibration-exposed controls group (VEC), and 39 workers without vibration exposure were categorized as the non-vibration-exposed controls group (NVEC). Video capillaroscopy was used to capture images of the 2nd, 3rd, and 4th fingers of both hands. The following nailfold capillary characteristics were included: number of capillaries/mm, avascular areas, haemorrhages, and enlarged capillaries. The experiments were carried out in the same winter. All characteristics were evaluated under blinded conditions.

Results: Significant differences in all morphological characteristics existed between the groups (P<0.05). Avascular areas in the HAVS, VEC, and NVEC groups appeared in 74.3%, 43.6%, and 25.0% of subjects, respectively. A higher percentage of subjects had haemorrhages in the HAVS group (65.7%) compared with the other groups (VEC: 7.7% and NVEC: 7.5%). The number of capillaries/mm, input limb width, output limb width, apical width, and ratio of output limb and input limb all had more than 70% sensitivity or specificity of their cut-off value.

Conclusion: Nailfold capillary characteristics, especially the number of capillaries/mm, avascular areas, haemorrhages, output limb width, input limb width, and apical width alterations revealed significant associations with hand-arm vibration syndrome.

Keywords: Nailfold capillaroscopy; Hand-arm vibration syndrome; Vibration-induced white finger; vascular injuries;

Strengths and limitations of this study

1. This is the first study providing several cut-off values of nailfold capillary morphological characteristics to systematically evaluate hand distal vascular alterations resulting from hand-arm vibration exposure.
2. The measurement results in this study are probably representative only for the population in northern areas, as demographic characteristics of populations may vary according to workers' location (e.g. tropical areas).
3. Further studies targeting tropical workers or a variety of vibrating tools are required in the future.

Introduction

Hand-arm vibration syndrome (HAVS) is characterized by asymmetrical vasospasms of the digital arteries. Vibration-induced white finger (VWF), the most dominant symptom and the most typical clinical manifestation of vascular injuries of HAVS, is a secondary form of Raynaud's phenomenon (RP), resulting from enduring occupational exposure to hand-held vibrating tools (e.g. drills, buffs, and riveters). VWF was first described as an industrial disease in 1985 [1, 2]. Prior to this, HAVS had been recognized legally as and occupational disease since 1957 in China [3]. The pathogenesis of HAVS is not fully understood, but it is clear that vibration can result in the damage of vascular, neurological, and musculoskeletal systems of the upper limbs [4, 5]. Workers who suffer from HAVS may undergo neurological tingling, numbness in the fingers, sensory perception reductions, tactile discrimination and manipulative dexterity, musculoskeletal swelling, and stiffness in hands or loss of grip strength. HAVS may also result in deterioration of manipulative dexterity [6] or operation performance impairment.

In this study, we focused on the impact of vibration exposure on arteries. Raynaud's phenomenon has been observed in patients who suffer from enduring exposure to high frequency hand-arm vibration [7-9]. A positive relationship between high level hand-arm vibration exposure and the appearance of HAVS vascular symptoms has been reported [10]. Exposure to high intensity vibration may be related to vascular changes in the digits, which include vasospasms, fixed narrowing of the vessel lumen, and ischemia [11]. The pathophysiology of RP in HAVS is complicated, but it is presumed that chronic vibration exposure may trigger an exaggerated central sympathetic vasoconstrictor reflex and local changes in the digital vessels, vasoactive substances, including endothelin and immunologic factors, and alterations in blood viscosity. Furthermore, vascular disorders of HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities [2, 12].

Nailfold capillaries, which are typically characterized by their hair pin shape, are mostly parallel to the skin surface (Figure 1). Their structure can be easily seen in vivo by means of nailfold capillaroscopy. Nailfold capillary abnormalities associated with HAVS can be recognized by structural alterations, such as degeneration of capillary density, avascular areas (Figure 2), appearance of enlarged capillaries (Figure 3), local haemorrhages (Figure 4), and angiogenesis. To assess the condition of capillaries, both morphological characteristic changes and measurements are able to provide disease identification at every stage. The history of capillaroscopy dates back approximately 200 years, and was extensively used after the advent of the work of Maricq and Leroy [13]. Cutolo et al. began their studies on capillaries [14], eventually detailing a capillaroscopy procedure [15] and suggesting a set of parameters which should be taken into consideration, including presence of enlarged and giant capillaries, haemorrhages, loss of capillaries, disorganization of the vascular array, and ramified/bushy capillaries. These researchers also defined the major sclerodermic nailfold capillaroscopy patterns in the "early", "active" and "late" stages. Despite the general description and measurement of nailfold capillaries, the categorization of nailfold capillaries is also worthwhile.

Sakaguchi et al. classified workers' nailfold capillary morphology into 5 types [16]. Based on previous studies, their capillaroscopy studies involved other Raynaud diseases [14, 15, 17], such as connective autoimmune rheumatic diseases [18, 19], systemic lupus erythematosus [20], dermatomyositis [21], and Sjögren syndrome [22]. This method was later introduced in the scanning of VWF. In 2011, Mahbub and Harada reported that few capillaroscopic studies on VWF existed, and the ones that did commonly focused on capillary abnormalities on limb dropouts and/or morphology alterations [23]. Smith et al. standardized the method of interpreting individual capillaries reliably and introduced a more practical way for novices to distinguish normal capillaries and abnormal capillaries individually after a quick training course [24].

Compared with existing HAVS diagnostic methods, capillaroscopy provides a relatively objective and feasible way to assess HAVS. Vascular Doppler and duplex scanning, cold provocation tests, finger skin temperature (FST), finger systolic blood pressure (FSBP), and plethysmography are extensively applied in HAVS diagnosis. However, some of these methods have been questioned for several reasons. For example, the performance conditions of the cold provocation test are quite unpleasant, which might be unbearable for the subjects, and its test outcomes may lead to unreproducible measurement results [25]. Some studies have indicated that use of FSBP without pre- or post-cold stress digit plethysmography or cold provocation might underestimate the severity of a patient's condition. Even though a demonstrable decrease in FSBP occurs upon cooling, it is not specific to HAVS, which means other diseases or factors may contribute to the FSBP drop [10]. The diagnostic ability of FSBP in the assessment of HAVS remains a controversial topic [10, 23, 26, 27]. Plethysmography requires expensive equipment and it is complicated to perform [23]. The effectiveness of FST on HAVS diagnosis has been doubted for its accuracy of readings, which heavily depend on the thermistor probe position. The specificity and sensitivity of the results were found not to be consistent and reproducible between studies [25, 26]. VWF is the "combination of sympathetic hyperactivity and lesion to structures and functions in the walls of blood vessels in the skin" [5]. The capillary alterations reveal the vascular injuries of HAVS. Thus, the morphology of injured vessels can be visually observed through capillaroscopy, which cannot be achieved by using other methods. Capillaroscopy provides an accurate way to measure and analysis data objectively. Also, capillaroscopy has advantages of low cost, non-invasiveness, and simple manipulation [28, 29].

Thus, this study aimed to investigate the characteristics of nailfold capillaroscopy associated with HAVS.

Methods

Subjects

All participants were male workers exposed to hand-arm vibration for at least 12 continuous months. Subjects with any of the following conditions were not recruited in this study: history of or current hypertension, diabetes, hepatitis, nephritis, immunological diseases, or trauma (muscular, neural, and osseous). Those who smoked or had alcohol intake within 24 hours or with fingers affected by recent local trauma were not recruited in the experiment. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covering 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited to a participate in a health exam. A cold provocation test was performed on all subjects after nailfold capillaroscopy examination.

In total, 113 subjects were recruited and participated in this study: 35 patients, defined by the revealed VWF symptoms after the cold provocation test, as well as the workers' personal description of VWF history with evidence (photo or third party witnesses), were categorized as the HAVS group. In addition, 39 age-matched participants, occupying the same hand-arm vibration exposed position but without symptoms of HAVS, were categorized as the vibration-exposed controls (VEC) group. Finally, 39 age-matched participants who were employed in positions without hand-arm vibration exposure were classified as the non-vibration-exposed controls (NVEC) group.

Goldminer information was obtained by face to face interview. The questionnaire, based on the questionnaire by SWS, included 4 parts: basic personal information, employment status, habits, and medical history and clinical characteristics. Basic personal information included name, nationality, age, height, weight, degree of education, and dominant hand. Employment status covered detailed history of past and current work ability, job titles, types of applied vibrating tool exposure, and duration of vibration exposure. Habits consisted of smoking, alcohol intake, and daily transportation. Medical histories and clinical characteristics were documented in detail, and included hypertension, diabetes, immunological diseases, hepatitis, nephritis, primary Raynaud's diseases, medicine intake, and digit alteration (appearance of VWF or cyanosis, tingling, numbness, etc.).

Informed consent was obtained from each respondent, and ethics approval was received from the Ethical Committee of Guangdong Province Hospital for Occupational Disease Prevention and Treatment.

Vibration measurement

Vibration produced by rock drill operation was measured with a VI-400 Pro 3-axial human vibration monitor (QUEST Technologies, Oconomowoc, Wisconsin, USA) by a qualified technician. Daily 8 hours' time-weighted average vibration exposure level (A (8)) was calculated according to ISO 5439-2:2001. Total hand-transmitted vibration exposure dose was calculated as cumulative exposure index (*CEI*) under the following formula:

$$CEI = \sum A(8)^2{}_i t_{di} t_{yi}$$

Where t_{di} *is the working days per year of each tool,* t_{yi} *is the years of using vibrating machine.* Napierian logarithm *CEI* (LN (*CEI*)) was used as a final index to calculate the vibration exposure dose.

Nailfold capillaroscopy

Vibration exposure should have occurred at least 24 hours prior to the nailfold capillaroscopy examination. At the beginning of the examination procedure, all subjects adapted to room temperature (20-22℃) with their hands placed on the table at heart level for at least 10 minutes. Meanwhile, finger skin temperature measurements of the 2nd knuckle of were also taken. The experiments were all carried out in the same winter.

Nailfold capillaroscopy was performed by capillaroscopy (CSW-XW2000 video-capillaroscopy, Shenzhen, Guangdong Province, China), which was equipped with a ×400 optical probe, on the 2nd, 3rd, and 4th fingers of both hands which are prone to developing representative morphological patterns, including enlarged capillaries, capillary density, angiogenesis, local haemorrhages, and avascular areas. A drop of immersion oil was applied to the nailfold bed in order to increase the translucency of the keratin layer. Images of capillaries in the middle areas of the nailfold within 2 mm were captured for a continuous 4 pictures per digit, 24 pictures per subject. Capillaroscopy was performed by a trained technician.

For every image, the following parameters were taken into analysis: the number of capillaries/mm, avascular areas, haemorrhages, enlarged capillaries, input limb width, output limb width, apical width, capillary limbs length, capillary total width, and ratio of output limb and input limb. Only the distal row of capillaries was used for imagery and data counting. Capillaries with a limb larger than 20μm were defined as "enlarged capillaries", and the lack of at least 2 successive capillaries was defined as an avascular area [30]. All characteristics were evaluated under blinded conditions.

The measurements of input limb width, output limb width, apical width, capillary limbs length, and capillary total width were calculated by the Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA). Before calculation the images were modified: the edges were sharpened, and brightness and contrast were adjusted properly.

Statistical analysis

The measurement results (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are expressed as mean ± standard deviation (with 95% CI); categorical information (avascular areas, haemorrhages, and enlarged capillaries) is presented as proportion (with percentage). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distributed ones. The Chi-square test was used to analyse categorical information. The measurement results were also evaluated via sensitivity analysis, and then the optimal specificity and sensitivity were selected as cut-off points determined by Youden index for each variable for discriminating between the HAVS group and NVEC group. Logistic regression was applied to observe the association of habits and nailfold capillary characteristics. A probability value of $P<0.05$ (two-sided) was considered statistically significant. All analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics of participants

In total, 113 male goldminers were recruited in this study. As shown in Table 1, the average ages of the HAVS, VEC, and NVEC groups were 42.8, 40.72, and 41.37 years, respectively. The HAVS group had longer seniority than the VEC group; the average seniority of the HAVS and VEC groups was 127.60 and 70.77 months, respectively. The BMIs of the HAVS group (22.52), VEC group (23.92), and NVEC group (24.59) were slightly different. Approximately 80% of participants were smokers and more than 50% of participants drank alcohol. The LN*CEI* of the HAVS group was 10.52, which is marginally higher than that of the VEC group at 9.91.

Table 1: Demographic characteristics and nailfold capillary characteristics of participants

	HAVS (n=35)	VEC (n=39)	NVEC (n=39)	*P*
Demographic characteristics				
Age (years), mean ± SD	42.80±5.96	40.72±7.35	41.37±8.74	>0.05
Seniority (months), mean ± SD	127.60±63.68[a]	70.77±50.27	—	<0.001†
BMI, mean ± SD	22.52±2.59[a, c]	23.92±2.75	24.59±3.18	0.008
Smoking, n (%)	28 (80)	34 (87.2)	33 (82.5)	>0.05
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	29 (72.5)	>0.05
LN*CEI*, mean ± SD	10.52±1.07	9.91±1.12	—	<0.05†
Nailfold capillary characteristics				
Haemorrhages, n (%)	23 (65.7)[a,c]	3 (7.7)	3 (7.5)	<0.001
Enlarged capillaries, n (%)	21 (60.0)[c]	9.98 (25.6)[b]	9.75 (25.0)	0.04
Avascular areas, n (%)	26 (74.3)[c]	17 (43.6)[b]	10 (25.0)	<0.001
Number of capillaries/mm, mean±SD	7.41±1.79[a,c]	8.87±1.80[b]	9.69±1.22	<0.001
Output limb width (μm), mean±SD	21.09±4.44[a,c]	16.06±2.05	15.74±2.21	<0.001
Input limb width (μm), mean±SD	15.77±3.26[a,c]	13.05±1.75	12.84±1.60	<0.001
Apical width (μm), mean±SD	21.40±4.34[a,c]	16.65±3.41	16.92±2.53	<0.001
Capillary limbs length (μm), mean±SD	191.77±52.88[a,c]	180.21±64.99	159.83±29.40	<0.05
Capillary total width (μm),	50.71±10.92[c]	42.33±6.25	43.16±5.32	0.001

mean±SD				
Ratio of output limb and input limb (μm), mean±SD	1.36±0.13[a,c]	1.25±0.08	1.24±0.10	<0.001

[a]HAVS vs. VEC: $P<0.05$;[b]VEC vs. NVEC:$P<0.05$;[c]HAVS vs. NVEC:$P<0.05$.

†Comparison between HAVS vs. VEC.

Note: Napierian logarithm CEI (LN [CEI]) was used as a final index to calculate the vibration exposure dose.

Results of Nailfold capillary characteristics

Nailfold capillary characteristics in this study are shown in Table 1. Haemorrhages existed to a greater extent in the HAVS group (65.7%) compared with the VEC and NVEC groups (7.7% and 7.5%, respectively). Enlarged capillaries were also observed more frequently in the HAVS group (60.0%) compared with the VEC (25.6%) and NVEC (25.0%) groups. Avascular areas were evident in 74.3% of HAVS participants, but these areas were only apparent in 43.6% and 25.0% of participants in the VEC and NVEC groups, respectively.

The results of the sensitivity analysis are presented in Table 2. The average number of capillaries/mm in the HAVS group was 7.41±1.79 limbs, compared with 8.87±1.80 limbs in the VEC group and 9.69±1.22 limbs in the NVEC group; the cut-off value was 8.75 capillaries/mm with a sensitivity of 76.9% and a specificity of 77.1%, the area under the ROC curve was 0.846 with a 95% confidence interval ranging from 0.753 to 0.939. The average output limb width (μm) in the HAVS group was 21.09±4.44 compared with 16.06±2.05 and 15.74±2.21 in the VEC and NVEC groups, respectively. The cut-off value of this characteristic was 16.72, with 80.0% sensitivity and 74.4% specificity; the area under the ROC curve was 0.866 and the 95% confidence interval ranged from 0.781 to 0.950. Input limb width (μm) had an average of 15.77±3.26 in the HAVS group compared with 13.05±1.75 and 12.84±1.60 in the VEC and NVEC groups, respectively. The cut-off value was 13.73, with similar sensitivity (77.1%) and specificity (74.7%), and the area under the ROC curve was 0.782 with a 95% confidence interval ranging from 0.669 to 0.895. The mean values of apical width (μm) were 21.40±4.34, 16.65±3.41, and 16.92±2.53 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value of this parameter was 18.51, with a sensitivity of 80.0% and a specificity of 79.5%; the area under the ROC curve was 0.823 with a 95% confidence interval ranging from 0.714 to 0.932. The average of capillary limb lengths (μm) was 191.77±52.88 in the HAVS group compared with 180.21±64.99 and 159.83±29.40 in the VEC and NVEC groups, respectively. The cut-off value of capillary limb length was 165.37, while the sensitivity (65.7%) and specificity (56.4%) were relatively lower than the other characteristics. The trend in the capillary total widths (μm) was similar to that of the capillary limb length, the averages of the HAVS, VEC, and NVEC groups were 50.71±10.92, 42.33±6.25, and 43.16±5.32, respectively; the cut-off value was 44.46 with a low sensitivity (62.9%) and specificity (53.8%). The mean values of the output and input limb ratio were 1.36±0.13, 1.25±0.08, and 1.24±0.10 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value was 1.29 with a sensitivity of 74.0% and specificity of 71.8%; the area under the ROC curve was 0.775, and the 95% confidence interval ranged from 0.669 to 0.881.

It can be seen that the characteristics of output limb width, input limb width, and apical width had quite good sensitivity (80.0%, 77.1% and 80.0%), while the number of capillaries/mm and apical width had the greatest specificity. Apical width was graded the best sensitivity (80.0%) and specificity (79.5) at the same time among the 4 characteristics. Thus, number of capillaries/mm, output limb width, input limb width and apical width are considered to be the optimum parameters to the HAVS diagnosis. Significant differences in all nailfold capillary characteristics existed between all groups ($P<0.05$).

Table 2: Sensitivity analysis of various nailfold capillary parameters

	Cut-Off Value	Sensitivity (%)	Specificity (%)	Area Under the ROC Curve	95%CI
Number of capillaries/mm	8.75	76.9	77.1	0.846	0.753-0.939
Output limb width, μm	16.72	80.0	74.4	0.866	0.781-0.950
Input limb width, μm	13.73	77.1	74.7	0.782	0.669-0.895
Apical width, μm	18.51	80.0	79.5	0.823	0.714-0.932
Capillary limb length, μm	165.37	65.7	56.4	0.686	0.561-0.812
Capillary total width, μm	44.46	62.9	53.8	0.704	0.580-0.829
Ratio of output limb and input limb	1.29	74.0	71.8	0.775	0.669-0.881

Results of association of nailfold capillaroscopy with habits

Of the 74 subjects in the HAVS and NVEC groups who had a nailfold capillaroscopy, there was an even distribution (*P*>0.05) of participants who smoke or drank alcohol (Table 3). In total, 80% and 87.2% of subjects in the HAVS and NVEC groups were smokers and 54.3% and 53.8% of subjects drank alcohol.

Table 3: Distribution of smoking and alcohol intake in the HAVS and NVEC groups

	HAVS (n=35)	NVEC (n=39)	*P*
Smoking, n (%)	28 (80.0)	34 (87.2)	0.403
Alcohol drinking, n (%)	19 (54.3)	21 (53.8)	0.970

Results of logistic regression results

For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (*P*>0.05). Some studies have revealed that there is a relationship between vessel wall injuries and alcohol intake, which may contribute to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed (Table 4).

Table 4: Logistic regression results of several nailfold capillary characteristics associated with the habits of smoking and alcohol intake in the HAVS and NVEC groups

	Control factor(s)	N (n = 74)	Wald	*P*	OR (95% CI)
Haemorrhages, n (%)	Smoking	62 (83.8)	0.286	>0.05	1.412 (0.398,5.007)
	Alcohol drinking	40 (54.1)	0.232	>0.05	0.789 (0.301,2.071)
Enlarged capillaries, n (%)	Smoking	62 (83.8)	1.485	>0.05	2.192 (0.621,7.740)
	Alcohol drinking	40 (54.1)	0.629	>0.05	1.462 (0.572,3.739)
Avascular areas, n (%)	Smoking	62 (83.8)	0.017	>0.05	1.085 (0.313,3.755)
	Alcohol drinking	40 (54.1)	0.522	>0.05	0.713 (0.284,1.787)
VWF, n (%)	Smoking	62 (83.8)	0.693	>0.05	1.703 (0.486,5.960)

Alcohol drinking	40 (54.1)	0.005	>0.05	0.968 (0.386,2.432)

Results of bivariate correlations

We assumed that the capillary characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, and avascular areas) might be correlated with each other and with the symptoms of VWF. Therefore, we explored the bivariate correlations of the variables mentioned above (Table 5).

Haemorrhages, enlarged capillaries, and avascular areas positively correlated with VWF (r=0.607, *P* <0.001; r=0.348, *P*<0.05; and r=0.486, *P*<0.001, respectively), while the number of capillaries/mm (r=-0.601, *P*<0.001) negatively correlated with VWF. Avascular areas negatively correlated with number of capillaries/mm (r=-0.568, *P*<0.001) and positively correlated with haemorrhages (r=0.416, *P*<0.001). Haemorrhages negatively correlated with number of capillaries/mm (r=-0.458, *P*<0.001). Avascular areas did not correlate with enlarged capillaries, and enlarged capillaries did not correlate either number of capillaries/mm or haemorrhages.

Table 5: Bivariate correlation of several nailfold capillaroscopy characteristics between the HAVS and NVEC groups

	Number of Capillaries/mm	Haemorrhages	Enlarged Capillaries	Avascular Areas	VWF
Number of capillaries/mm	1	r=-0.458**	r=-0.157	r=-0.568**	r=-0.601**
Haemorrhages	—	1	r=0.178	r=0.416**	r=0.607**
Enlarged capillaries	—	—	1	r=-0.004	r=0.348*
Avascular areas	—	—	—	1	r=0.486**
VWF	—	—	—	—	1

N=74, * *P*< 0.05, ** *P*< 0.001.

Discussion

Nailfold capillaroscopy examination has been applied for RP for years. The observed abnormal specific vascular patterns are distinctive from one pathological stage to another. Therefore, this method should include a number of parameters, and the proper value range of each parameter needs to be set up in order to provide a more complete assessment.

Hand-arm vibration syndrome is a secondary form of RP. Although some studies have explored nailfold capillaroscopy in persons with HAVS, its parameters were either not comprehensive or were mainly focused on the classification of variables for levels of disease categorization. Few studies suggested parameters for clinical cut-off values, and most of the nailfold capillaroscopy studies focused on the semi-quantitative scale methods of nailfold capillary patterns, rather than giving exact values of nailfold capillary measurements. The capillary alterations in immunological diseases with respect to RP are quite apparent, and they contrast in every pathological stage. However, the situation is different in people with HAVS, and the prevention of HAVS development needs reference values as thresholds for judging vibration exposure to workers. This will ultimately help to decide if a worker is still suitable for his/her position.

In this study, we attempted to obtain a set of reliable reference values of vascular measurements, and also to find out the relationship between each vascular pattern and VWF pathological changes.

As the bivariate correlation analysis results showed, the appearance of haemorrhages negatively correlated with the number of capillaries/mm, which meant that the more haemorrhages that were observed, the less capillaries were present within 1mm. The number of capillaries/mm (r=-0.568, P<0.001) and haemorrhages (r=0.416, P<0.001), correspondingly, negatively and positively correlated with avascular areas, which indicated that the avascular areas arose with the appearance of haemorrhages and the degeneration of capillary number. The manifestation of VWF positively correlated with haemorrhages (r=0.607, P<0.001), enlarged capillaries (0.348, P<0.05), and avascular areas (r=0.486, P<0.001), but negatively correlated with number of capillaries/mm (r=-0.601, P<0.001). This revealed that more haemorrhages, enlarged capillaries, and avascular areas associated with more severe VWF (and vice versa). In addition, fewer capillaries appearing within 1 mm associated with more serious VWF.

Thus, we infer that: operating hand-held vibrating tools, to a certain degree, might lead to neurological and vascular damage of the hands, characterized by vasoconstriction caused by endothelium damage, followed by morphological alterations of the capillaries. Pathological changes of capillary alterations manifest as enlarged or tortuous capillaries occurring as blood hypoxia, which arise from vascular compensation. Moreover, as a consequence of chronical vessel wall impairment and rupture, erythrocytes are able to cross over the vessel wall then form haemorrhages, which give rise to degeneration of capillaries. Finally, capillaries begin to drop off, which cause avascular areas.

Shandong province, located in the northeast of China, has extreme cold weather in the winter. Goldminers who are exposed to hand-arm vibration under such weather and working conditions may develop capillary impairment, characterized as VWF, which is seen as vasoconstriction.

Morphological alterations can be observed on nailfold capillaroscopy. A previous study indicated that haemorrhages are the bridge between enlarged capillaries and avascular areas [18]. Our study agrees with this, as, in the HAVS group, the ratio of haemorrhages was similar to that of enlarged capillaries, while avascular areas increased up to 74.3%. We suggest that the appearance of enlarged capillaries and haemorrhages triggered vascular degeneration, forming the avascular areas. Thus, the proportions of haemorrhages and enlarged capillaries were greater than the presence of avascular areas. However, it is possible that, as many of the HAVS subjects were still undergoing the process of developing HAVS, the existence of enlarged capillaries and haemorrhages but not avascular areas could be observed. In the VEC and NVEC groups, the avascular areas were greater than or equal to the existence of haemorrhages and enlarged capillaries, which were in accordance with our hypothesis.

Hand-arm vibration syndrome subjects had more haemorrhages and enlarged capillaries than those in the VEC and NVEC groups. Generally, enlarged capillaries are much more likely to be observed than haemorrhages in subjects who are chronically exposed to hand arm vibration, and those subjects tend to develop HAVS. Once participants are exposed to hand-arm vibration for a certain duration, for example those in the HAVS group, haemorrhages are more likely to be observed. We propose that haemorrhages form after enlarged capillaries appear, as previous studies have mentioned. Thus, it is feasible that haemorrhages and enlarged capillaries should be included as reference characteristics.

Avascular areas likely related to capillary density (r=-0.568, *P*<0.001). The inhomogeneous distribution of capillaries results in drop off, which decreases the amount of capillaries, creating avascular areas.

The effectiveness in counting the number of capillaries per millimeter in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects [31, 32]. Ingegnoli et al. indicated that the number of capillaries per millimeter in healthy subjects ranged from 7 to 10. Our results, 7.41±1.79/mm in the HAVS group, 8.87±1.80/mm in the VEC group, and 9.69±1.22/mm in the NVEC group, are consistent with the previous data, and we also believe that counting the number of capillaries per millimeter is a proper way to judge capillary abnormalities. The results of number of capillaries per millimeter published here have reference significance for HAVS, with a cut-off value of 8.75 capillaries within 1mm, of which sensitivity and specificity are 76.9% and 77.1%, respectively.

In the process of setting reliable parameters of HAVS reference values, the sensitivity analysis in our study showed several parameters with considerable sensitivity or specificity. Parameters with considerable sensitivity (more than 77%) were output limb width, input limb width, and apical width, while the parameters with considerable specificity were number of capillaries/mm and apical width. It is worth mentioning that apical width shares a considerable sensitivity and a considerable specificity. Here, we recommend that the parameters of number of capillaries/mm, output limb width, input limb width, and apical width can be taken into consideration as HAVS examination parameters, especially the apical width. What makes the sensitivity and specificity of these parameters so different is the limb shape itself. Generally, the output or input limb is uneven, which means that the measurement point varies, thus resulting in diverse measurements. However, the capillary apex is fixed in a narrow range, thus the measurement of apical width would be relatively more objective than measurement of the limbs.

The results of this study are generally in accordance with previous RP studies. We believe it is because HAVS is a secondary form of RP that shares a familiar pathology with other Raynaud diseases [33, 34]. Therefore, we suppose our results are reliable in the assessment of HAVS.

There are some limitations in this study. Demographic characteristics of populations may vary according to workers' location in China. As an example, the subjects in this study were from northern China, who adapted to the extreme cold winters, while the population in the tropical China, do not adapt to cold temperature; this will lead to different vascular measurement results. Hence, the measurement results in this study are probably representative only for population in northern areas, and further studies are required for the tropical populations. Nevertheless, in future studies, we highly recommend that a weighted index be considered as one of the assessment rules, which may enhance the nailfold capillaroscopy analysis with more accuracy, and also raise the HAVS assessment procedure efficiency.

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Data sharing*: Extra data are available by emailing qschen@gdoh.org*

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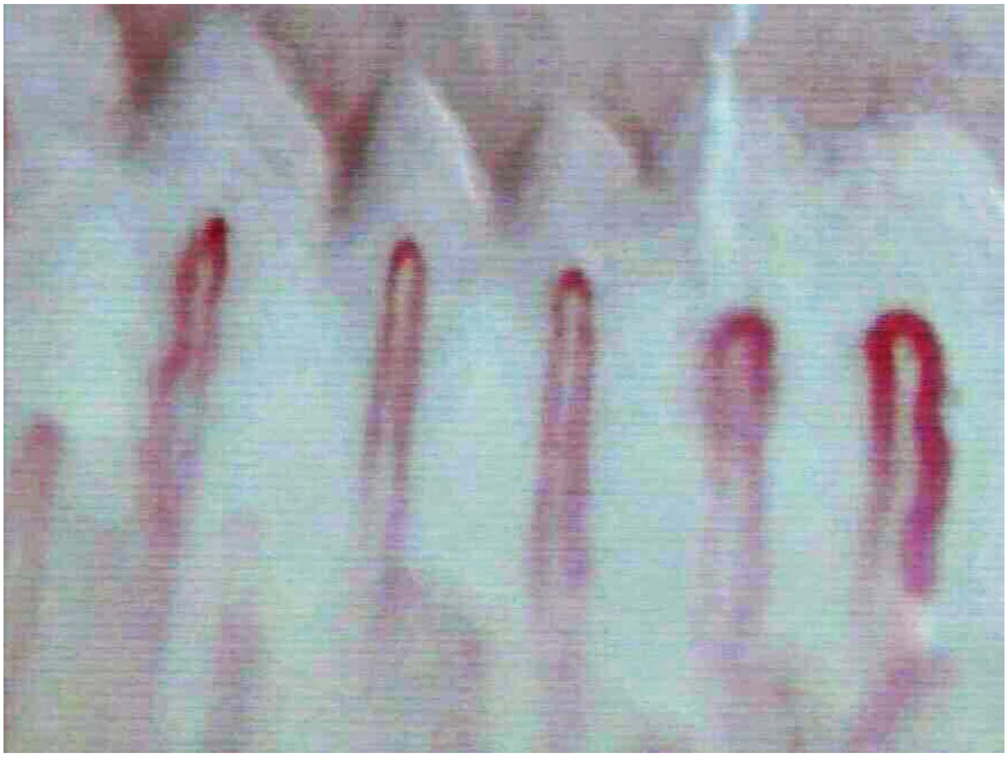
Contributors*: Qingsong Chen and Guiping Chen conceived of the study and were responsible for the design and search strategy. Guiping Chen, Danying Zhang, and Bei Yang were responsible for conducting the search. Maosheng Yan and Guiping Chen conducted the data analysis and interpretation. The initial draft of the manuscript was prepared by Qingsong Chen and Guiping Chen. All authors provided input to the analysis plans, interpretation of data, and critical revision of the successive drafts of the manuscript.*

References

1. Cutolo, M., et al., *Capillaroscopy.* Best Pract Res Clin Rheumatol, 2008. **22**(6): p. 1093-108.do
2. Noel, B., *Pathophysiology and classification of the vibration white finger.* Journal of the Peripheral Nervous System, 2000. **5**(4): p. 242-242.
3. Lin, W., et al., *The study on hand-arm vibration syndrome in China.* Industrial health, 2005. **43**(3): p. 480-483.
4. Griffin, M.J., *Handbook of human vibration*. 2012: Academic press.
5. Gemne, G., *Diagnostics of hand-arm system disorders in workers who use vibrating tools.* Occupational and Environmental Medicine, 1997. **54**(2): p. 90-95.
6. Sauni, R., et al., *Work disability after diagnosis of hand-arm vibration syndrome.* Int Arch Occup Environ Health, 2015. **88**(8): p. 1061-8.
7. Nyantumbu, B., et al., *Hand-arm vibration syndrome in South African gold miners.* Occup Med (Lond), 2007. **57**(1): p. 25-9.
8. Harada, N. and M.H. Mahbub, *Diagnosis of vascular injuries caused by hand-transmitted vibration.* Int Arch Occup Environ Health, 2008. **81**(5): p. 507-18.
9. Hatron, P., P. Frimat, and E. Hachulla, *[Raynaud's phenomena of occupational origin].* La Revue du praticien, 1998. **48**(15): p. 1653-1658.
10. Heaver, C., et al., *Hand–arm vibration syndrome: a common occupational hazard in industrialized countries.* Journal of Hand Surgery (European Volume), 2011. **36**(5): p. 354-363.
11. Maundrell, A. and S.M. Proudman, *Epidemiology of Raynaud's Phenomenon*, in *Raynaud's Phenomenon*. 2015, Springer. p. 21-35.
12. Olsen, N., *Centrally and locally mediated vasomotor activities in Raynaud's phenomenon.* Scandinavian journal of work, environment & health, 1987: p. 309-312.
13. Maricq, H.R. and E. Carwile LeRoy, *Patterns of finger capillary abnormalities in connective tissue disease by "wide - field" microscopy.* Arthritis & Rheumatism, 1973. **16**(5): p. 619-628.
14. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis & rheumatism, 2003. **48**(11): p. 3023-3030.
15. Cortes, S. and M. Cutolo, *Capillarosecopic patterns in rheumatic diseases.* Acta reumatológica portuguesa, 2006. **32**(1): p. 29-36.
16. Sakaguchi, S., et al., *Morphologic classification of nailfold capillary microscopy in workers exposed to hand-arm vibration.* Industrial health, 2011. **49**(5): p. 614-618.
17. Smith, V., et al., *Nailfold capillaroscopy for day-to-day clinical use: construction of a simple scoring modality as a clinical prognostic index for digital trophic lesions.* Annals of the rheumatic diseases, 2010: p. annrheumdis132431.
18. Cutolo, M., et al., *The contribution of capillaroscopy to the differential diagnosis of connective autoimmune diseases.* Best Practice & Research Clinical Rheumatology, 2007. **21**(6): p. 1093-1108.
19. Cutolo, M., et al., *Nailfold capillaroscopy is useful for the diagnosis and follow-up of autoimmune rheumatic diseases. A future tool for the analysis of microvascular heart involvement?* Rheumatology, 2006. **45**(suppl 4): p. iv43-iv46.
20. Bongard, O., et al., *Association of anticardiolipin antibodies and abnormal nailfold capillaroscopy in patients with systemic lupus erythematosus.* Lupus, 1995. **4**(2): p. 142-144.
21. Marie, I., et al., *Polymyositis and dermatomyositis: short term and longterm outcome, and predictive factors of prognosis.* The Journal of rheumatology, 2001. **28**(10): p. 2230-2237.
22. Tektonidou, M., et al., *Microvascular abnormalities in Sjögren's syndrome: nailfold capillaroscopy.*

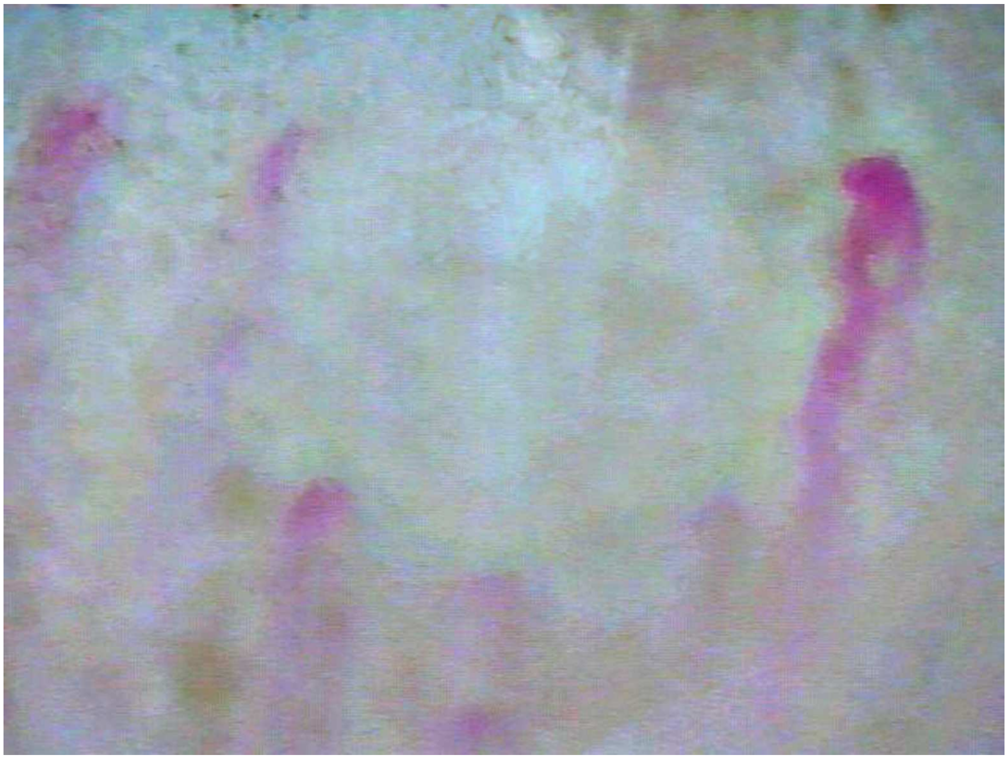
Rheumatology, 1999. **38**(9): p. 826-830.

23. Mahbub, M. and N. Harada, *Review of different quantification methods for the diagnosis of digital vascular abnormalities in hand-arm vibration syndrome.* Journal of occupational health, 2011. **53**(4): p. 241-249.
24. Smith, V., et al., *An EULAR study group pilot study on reliability of simple capillaroscopic definitions to describe capillary morphology in rheumatic diseases.* Rheumatology, 2016. **55**(5): p. 883-890.
25. Harada, N., *Cold-stress tests involving finger skin temperature measurement for evaluation of vascular disorders in hand-arm vibration syndrome: review of the literature.* International archives of occupational and environmental health, 2002. **75**(1-2): p. 14-19.
26. Poole, K., J. Elms, and H. Mason, *The diagnostic value of finger systolic blood pressure and cold-provocation testing for the vascular component of hand–arm vibration syndrome in health surveillance.* Occupational Medicine, 2004. **54**(8): p. 520-527.
27. Thompson, A., R. House, and M. Manno, *The sensitivity and specificity of thermometry and plethysmography in the assessment of hand–arm vibration syndrome.* Occupational medicine, 2008. **58**(3): p. 181-186.
28. Etehad Tavakol, M., et al., *Nailfold Capillaroscopy in Rheumatic Diseases: Which Parameters Should Be Evaluated?* Biomed Res Int, 2015. **2015**: p. 974530.
29. Filosa, G. and R. De Angelis, *Capillaroscopy.* 2015: p. 1107-1114.
30. Ingegnoli, F., et al. *Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations*. in *Seminars in arthritis and rheumatism*. 2009. Elsevier.
31. Ingegnoli, F., et al., *Nailfold capillary patterns in healthy subjects: a real issue in capillaroscopy.* Microvascular research, 2013. **90**: p. 90-95.
32. Hoerth, C., et al., *Qualitative and quantitative assessment of nailfold capillaries by capillaroscopy in healthy volunteers.* Vasa, 2012. **41**(1): p. 19-26.
33. Herrick, A.L., *Pathogenesis of Raynaud's phenomenon.* Rheumatology (Oxford), 2005. **44**(5): p. 587-96.
34. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis Rheum, 2003. **48**(11): p. 3023-30.



Normal capillaries

232x174mm (300 x 300 DPI)

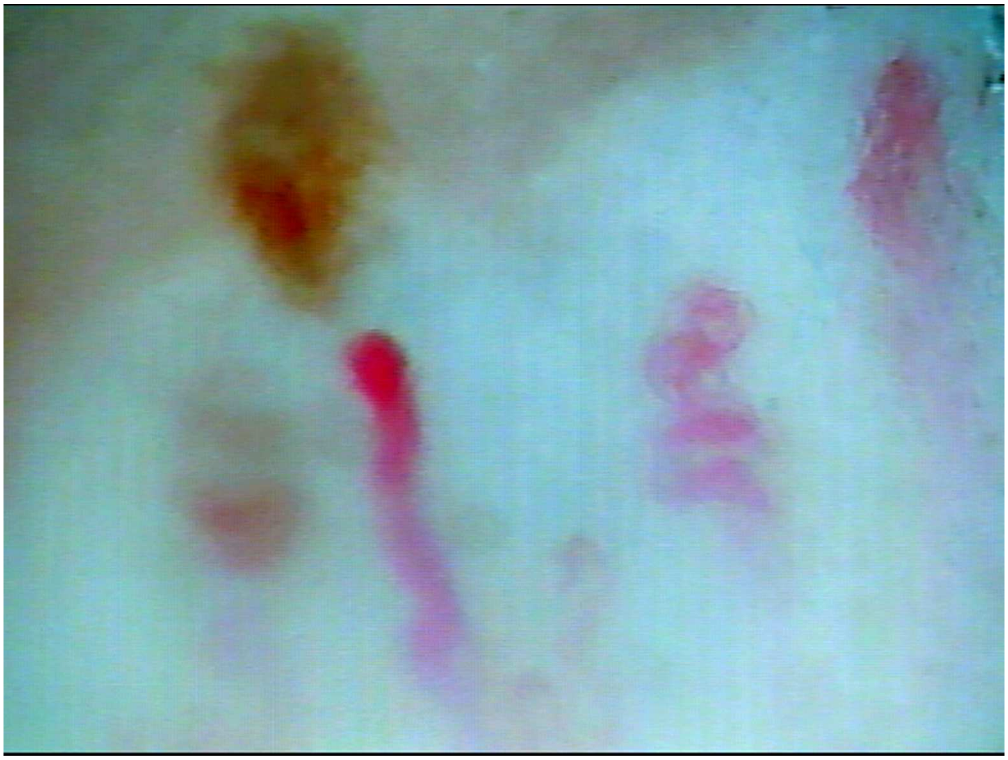


Avascular area

225x169mm (300 x 300 DPI)

Enlarged capillary

172x129mm (300 x 300 DPI)



microhemorrhage

172x130mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	Nailfold capillary morphological characteristics of hand-arm vibration syndrome: a cross-sectional study
Introduction		
Background/rationale	2	Raynaud's phenomenon has been observed in patients who suffer from enduring exposure to high-frequency hand-arm vibration. The skin, arteries, and nerves of the upper extremities can be modified by such vibration exposure. In this study, we focus on the impact of vibration exposure on the arteries. Vibration-induced white finger (VWF) is the most typical clinical manifestation of vascular injuries in hand-arm vibration syndrome (HAVS). The pathology of VWF is not well understood. However, the vascular disorders resulting from HAVS can be observed in at least three ways: digital organic micro-angiopathy, digital vasospastic phenomenon, and arterial thrombosis in the upper extremities. Capillaroscopy is widely accepted and utilized in the clinical diagnosis of Raynaud's syndrome. It has also been introduced for the assessment of HAVS in recent years, due to its advantages of non-invasive vivo examination, ability to follow disease progression overtime, uncomplicated and non-time-consuming performance, provision of various characteristics for analysis, and inexpensive costs.
Objectives	3	The purpose of this study was to investigate the characteristics of nailfold capillaroscopy associate to hand-arm vibration syndrome.
Methods		
Study design	4	Nailfold capillaroscopy; Hand-arm vibration syndrome; Vibration-induced white finger; vascular injuries
Setting	5	The study, including nailfold capillaroscopy image capture and cold water provocation, were carried out in the same winter in 2014 in Shandong province, China. Nailfold capillaroscopy data measurement and analysis were performed from January to May, 2015.
Participants	6	All participants were males, workers exposed to hand-arm vibration with at least 12 continuous months seniorities. Subjects with any of the following conditions were not recruited in this study: history of or current hypertension, diabetes, hepatitis, nephritis, immunological diseases and trauma (muscular, neural, and osseous); who smoked or had alcohol intake within 24 hours or with fingers affected by recent local trauma did not recruit in the experiment, neither. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covered 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited by a particular health exam. In total, 113 subjects were recruited and participated in this study: 35 patients, defined by the revealed vibration white finger symptom after the cold provocation as well as the workers themselves description of VWF history with evidence (photo or third party witnesses), were categorized as the HAVS group. In addition, 39 age-matched participants, occupied the same hand-arm vibration exposed position but without symptoms of HAVS, were categorized as the vibration-exposed controls (VEC) group. Finally, 39 age-matched participants who were employed in positions without hand-arm vibration exposure were classified as the non-vibration-exposed controls (NVEC) group.

Variables	7	For every image, the following parameters were taken into analysis: the number of capillaries/mm, avascular areas, haemorrhages, enlarged capillaries, input limb width, output limb width, apical width, capillary limbs length, capillary total width, and ratio of output limb and input limb. Only the distal row of capillaries was used for imagery and data counting. Capillary with a limb larger than 20μm was defined as "enlarged capillaries", and the lack of at least 2 successive capillaries was defined as an avascular area. Total hand-transmitted vibration exposure dose was calculated as cumulative exposure index (CEI), Napierian logarithm CEI (LN (CEI)) was used as a final index to calculate the vibration exposure dose.
Data sources/ measurement	8*	Qualitative variables (haemorrhages, enlarged capillaries, and avascular areas) were counted in number if the symptom revealed, then presented it in percentage. Quantitative variables (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are counted in capillary number or measured in Photoshop software. Input limb width was the measurement of the input limb the widest limb part linear distance, it's the same procedure for measuring output limb width, and apical width. Capillary limbs length was the measurement of the vertical linear distance between the apex of the apical capillary part and the visible point of the capillary base. Capillary total width was the measurement of the linear distance between leftmost point of one limb and rightmost point of another limb of the widest part of capillary.
Bias	9	The experiments were carried out in the same winter. All nailfold capillary characteristics were evaluated under blinded conditions.
Study size	10	It was determined by cluster sampling.
Quantitative variables	11	There are several quantitative variables in the analyses: the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width. They are expressed as mean ± standard deviation (with 95% CI). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distribution ones. Grouping these quantitative variables basically followed the suggestions of Maricq and Leroy, and Cutolo et al. articles on nailfold capillaroscopy.
Statistical methods	12	The measurement results (the number of capillaries/mm, input limb width, output limb width, apical width, capillary limbs length, and capillary total width) are expressed as mean ± standard deviation (with 95% CI); categorical information (avascular areas, haemorrhages, and enlarged capillaries) is presented as proportion (with percentage). The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the measurement results, a t-test or one-way ANOVA was used to analyse the normally distributed quantitative values, and the Mann-Whitney U test was used to analyse the non-normally distribution ones. The Chi-square test was used to analyse categorical information. The measurement results were also evaluated via ROC curves, and then the optimal specificity and sensitivity were selected as cut-off points determined by Youden index for each variable for discriminating between the HAVS group and NVEC group. Logistic regression was applied to observe the association of habits and nailfold capillary characteristics. A probability value of $P<0.05$ (two-sided) was considered statistically significant. All analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).

		Logistic regression was used to analyse several nailfold capillary characteristics associated with the habits of smoking and alcohol intake in the HAVS and NVEC groups. Bivariate correlation was applied to examine several nailfold capillaroscopy characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, avascular areas, and VWF) between the HAVS and NVEC groups
		There are no missing data.
		A one gold industry consists of several Sub Companies in the whole Shandong province. Subjects were recruited by cluster sampling from 3 Sub gold Companies within one gold industry covered 90 rock drillers in Zhaoyuan district of Shandong province, China. The workers who participated in this study were invited by a particular health exam.
		Sensitivity analyses were applied to assess various nailfold capillary parameters (Number of capillaries/mm, Output limb width, Input limb width, Apical width, Capillary limbs length, Capillary total width, Ratio of output limb and input limb)
Results		
Participants	13*	There are 90 rock drillers in the 3 Sub Companies, thus there are 90 potentially eligible population; then there are 88 examined for eligibility workers, 74 confirmed eligible subjects finally. 74 subjects and no missing individual throughout in the study and completing follow-up and analysed.
		2 asked for private affairs leaves in the first stage; 14 workers were not willing to attend the study.
Descriptive data	14*	In total, 113 male goldminers were recruited in this study. As shown in Table 1, the average ages of the HAVS, VEC, and NVEC groups were 42.8, 40.72, and 41.37 years, respectively. The HAVS group had longer seniority than the VEC group; the average seniority of the HAVS and VEC groups was 127.60 and 70.77 months, respectively. The BMIs of the HAVS group (22.52), VEC group (23.92), and NVEC group (24.59) were slightly different. Approximately 80% of participants were smokers and more than 50% of participants drank alcohol. The LNCEI of the HAVS group was 10.52, which is marginally higher than that of the VEC group at 9.91.
		None.
Outcome data	15*	5 outcome events in total.
Main results	16	Alcohol intake and cigarette will affect vascular function, therefore, these two confounders were adjusted for estimates. For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (P>0.05). Some studies have revealed that there is a relation with vessel wall injuries and alcohol intake, which may contributes to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed.
Other analyses	17	Of the 74 subjects in the HAVS and NVEC groups who had a nailfold capillaroscopy, there was an even distribution (P>0.05) of participants who smoke or drank alcohol, see Table 3. In total, 80% and 87.2% of subjects in the HAVS and NVEC groups were smokers and 54.3% and 53.8% of subjects in drank alcohol. For both habits (smoking and alcohol drinking), univariate analysis of individual habits did not yield any association with nailfold capillary characteristics (P>0.05). Some studies have

revealed that there is a relation with vessel wall injuries and alcohol intake, which may contributes to the development of enlarged capillaries; however, no significant differences between these 2 factors were observed in our study, thus no further multivariable analysis was performed (Table 4).

We assumed that the capillary characteristics (number of capillaries/mm, haemorrhages, enlarged capillary, and avascular areas) might correlated with each other and with the symptoms of VWF. Therefore we explored the bivariate correlations of the variables mentioned above (Table 5).

Haemorrhages, enlarged capillaries, and avascular areas positively correlated with VWF (r=0.607, P <0.001; r=0.348, P<0.05; and r=0.486, P<0.001, respectively), while the number of capillaries/mm (r=-0.601, P<0.001) negatively correlated with VWF. Avascular areas negatively correlated with number of capillaries/mm (r=-0.568, P<0.001) and positively correlated with haemorrhages (r=0.416, P<0.001). Haemorrhages negatively correlated with number of capillaries/mm (r=-0.458, P<0.001). Avascular areas did not correlate with enlarged capillaries, and enlarged capillaries did not correlate either number of capillaries/mm or haemorrhages, neither.

The results of the sensitivity analysis are presented in Table 2. The average number of capillaries/mm in the HAVS group was 7.41±1.79 limbs, compared with 8.87±1.80 limbs in the VEC group and 9.69±1.22 limbs in the NVEC group; the cut-off value was 8.75 capillaries/mm with a sensitivity of 76.9% and a specificity of 77.1%, the area under the ROC curve was 0.846 with a 95% confidence interval ranging from 0.753 to 0.939. The average output limb width (μm) in the HAVS group was 21.09±4.44 compared with 16.06±2.05 and 15.74±2.21 in the VEC and NVEC groups, respectively. The cut-off value of this characteristic was 16.72, with 80.0% sensitivity and 74.4% specificity; the area under the ROC curve was 0.866 and the 95% confidence interval ranged from 0.781 to 0.950. Input limb width (μm) had an average of 15.77±3.26 in the HAVS group compared with 13.05±1.75 and 12.84±1.60 in the VEC and NVEC groups, respectively. The cut-off value was 13.73, with similar sensitivity (77.1%) and specificity (74.7%), and the area under the ROC curve was 0.782 with a 95% confidence interval ranging from 0.669 to 0.895. The mean values of apical width (μm) were 21.40±4.34, 16.65±3.41, and 16.92±2.53 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value of this parameter was 18.51, with a sensitivity of 80.0% and a specificity of 79.5%; the area under the ROC curve was 0.823 with a 95% confidence interval ranging from 0.714 to 0.932. The average of capillary limbs lengths (μm) was 191.77±52.88 in the HAVS group compared with 180.21±64.99 and 159.83±29.40 in the VEC and NVEC groups, respectively. The cut-off value of capillary limbs length was 165.37, while the sensitivity (65.7%) and specificity (56.4%) were relatively lower than the other characteristics. The trend in the capillary total widths (μm) was similar to that of the capillary limbs length, the averages of the HAVS, VEC, and NVEC groups were 50.71±10.92, 42.33±6.25, and 43.16±5.32, respectively; the cut-off value was 44.46 with a low sensitivity (62.9%) and specificity (53.8%). The mean values of the output and input limb ratio were 1.36±0.13, 1.25±0.08, and 1.24±0.10 in the HAVS, VEC, and NVEC groups, respectively. The cut-off value was 1.29 with a sensitivity of 74.0% and specificity of 71.8%; the area under the ROC curve was 0.775, and the 95% confidence interval ranged from 0.669 to 0.881.

It can be seen that the characteristics of output limb width, input limb width and apical width had quite good sensitivity (80.0%, 77.1% and 80.0%), while the number of capillaries/mm and apical width had the greatest specificity. Apical width was graded the best sensitivity (80.0%) and specificity (79.5) at the same time among the 4 characteristics. Thus, number of capillaries/mm, output limb width, input limb width and apical width are considered to be the

		optimum parameters to the HAVS diagnosis. Significant differences in all nailfold capillary characteristics existed between all groups (P<0.05).
Discussion		
Key results	18	In this study, we attempted to obtain a set of reliable reference values of vascular measurements, and also to find out the relations between each vascular pattern and vibration white finger pathological change. We infer that: operating hand-held vibrating tools up to a certain degree, might leads to neurological and vascular damage of the hands, characterized by vasoconstriction caused by endothelium damage, followed by morphological alterations of the capillaries. Pathological changes of capillary alterations manifest as enlarged or tortuous capillaries occurring as blood hypoxia, which arise from vascular compensation. Moreover, as a consequence of chronical vessel wall impairment and rupture, erythrocytes are able to cross over the vessel wall then form haemorrhages, which give rise to degeneration of capillaries. Finally, capillaries begin to drop off, which cause vascular areas. We suggest that the appearance of enlarged capillaries and haemorrhages triggered vascular degeneration, forming the avascular areas. Thus, the proportions of haemorrhages and enlarged capillaries were greater than the presence of avascular areas. However, it is possible that, as many of the HAVS subjects were still undergoing the process of developing HAVS, the existence of enlarged capillaries and haemorrhages but not avascular areas could be observed. In the VEC and NVEC groups, the avascular areas were greater than or equal to the existence of haemorrhages and enlarged capillaries. The effectiveness in counting the number of capillaries per millimetre in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects. We recommend that the parameters of number of capillaries/mm, output limb width, input limb width, and apical width can be taken into consideration as HAVS examination parameters, especially the apical width.
Limitations	19	There are some limitations in this study: demographic characteristics of populations may vary from the northern to the southern China. As an example, the subjects in this study were from northern China, who adapt to the extreme cold winters while the population in the southern China, who do not adapt to cold temperature, this will lead to different vascular measurement results. Hence, the measurement results in this study probably represent for population in northern place, and further studies are required for the southern place population. Nevertheless, in future studies, we highly recommend that a weighted index can be considered as one of the assessment rules, which may enhance the nailfold capillaroscopy analysis with more accuracy, and also raise the HAVS assessment procedure efficiency.
Interpretation	20	There were three steps before we choose subjects in this study: firstly, all workers participated in the pre-examination by clinical doctors before employment that make sure all candidates are healthy and no contraindications involved (which include primary Raynaud phenomenon, scleroderma and other kinds of immunological diseases) to handle this job. Secondly, before the any experiments in this study, all workers have taken a face-to-face questionnaire which includes questions about immunological hereditary diseases that helps us to make sure no subjects get involved with diseases like systematic scleroderma or Raynaud phenomenon. And finally, we also set up control group, which subjects were also employee in the industry other than working in the gold mine tunnel.
Generalisability	21	Morphological alterations can be observed on nailfold capillaroscopy. A previous study indicated that haemorrhages are the bridge between enlarged capillaries and avascular areas [18]. Our study consists with this, where, in the HAVS group, ratio of haemorrhages was similar to that of enlarged capillaries, while avascular areas increased up to 74.3%.

The effectiveness in counting the number of capillaries per millimeter in this study is consistent with previous studies of patients with systemic scleroderma and healthy subjects [31, 32]. Ingegnoli et al. indicated that the number of capillaries per millimeter in healthy subjects ranged from 7 to 10. Our results, 7.41±1.79/mm in the HAVS group, 8.87±1.80/mm in the VEC group, and 9.69±1.22/mm in the NVEC group, are consistent with the previous data, and we also believe that counting the number of capillaries per millimeter is a proper way to judge capillary abnormalities. The results of number of capillaries per millimeter published here have reference significance for HAVS, with a cut-off value of 8.75 capillaries within 1mm, of which sensitivity and specificity are 76.9% and 77.1%, respectively.

Other information		
Funding	22	This study was founded by National Science Foundation of China (NSFC No. 81202183); and by Science and Technology Program of Guangdong, China (STPG No.2013B051000065); and by the Open Projects Program of Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment (No.2012A061400007-03). No original study was based on.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.