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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002785
Article Type:	Research
Date Submitted by the Author:	27-Feb-2013
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Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Public health
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, CHEMICAL PATHOLOGY, PUBLIC HEALTH

The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

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Running Title: Capillaroscopy post-exposure to VCM

Words count for introduction methods results discussion conclusion: 2298

Number of tables: 1

Number of figures: 2

Words count for abstract: 258

ABSTRACT

Objectives: To assess residual long-term microcirculation abnormalities by capillaroscopy, 15 years after retiring from occupational exposure to vinyl chloride monomer (VCM).

Design: Cross-sectional study.

Setting: Allier, one of the major area of PVC production in France.

Participants: We enrolled 761 male retired workers exposed to chemical toxics. Exposure to chemical other than VCM excluded potential participants.

Primary and secondary outcomes measures: These participants underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities, as well as a survey to determine exposure time, direct or indirect contact, type of occupation, smoking status and time after exposure. A double blind analysis of capillaroscopic images was done. A control group was matched in age, sex, type of occupation.

Results: 179/761 retired workers were only exposed to VCM at their work, with 21 meeting the inclusion criteria and included. Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Retired workers previously exposed to VCM had significantly higher capillaroscopic modifications than 35 controls: enlarged capillaries (19% vs. 0%, p<.001), dystrophy (28.6% vs. 0%, p=.0012), and augmented length (33% vs. 0%, p<.001). Time exposure was linked with enlarged capillaries (R^2 adjusted = 53%, p<.0001). They also had higher symptoms of Raynaud (19% vs. 0%, p=.007) without correlation with capillaroscopic modifications.

Conclusion: Although VCM exposure was already known to affect microcirculation, our study demonstrates residual long term abnormalities following an average of 15 years retirement, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure was not related to capillaroscopic modifications; its origin remains to be determined.

Keywords Exposition, Vinyl chloride monomer, Capillaroscopy, Raynaud

ARTICLE SUMMARY

Article focus

- Vinyl chloride monomer exposure induces microcirculation abnormalities, which can be diagnosed by capillaroscopy.
- Residual long-term abnormalities following retirement required investigation.

Key messages

- Our results demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time-related exposure response.
- Symptoms of Raynaud, although statistically associated with exposure, was not related to capillaroscopic modifications; its origin remains to be determined.

Strengths and limitations of this study

- The strong points is that this study had a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factor and the focus on retired workers at least 15 years after the end of occupational VCM exposure.
- The main limitation is that pathophysiology of Raynaud after VCM exposure remains unclear.

INTRODUCTION

Vinyl chloride monomer (VCM) is primarily used in the manufacture of plastics and also serves as a raw material in organic synthesis. VCM is an aliphatic hydrocarbon also known as chloroethene. Its polymerization lead to a synthetic resin called polyvinyl chloride, commonly abbreviated PVC. PVC, is the third-most widely produced plastic, after polyethylene and polypropylene.[1] PVC can be made softer and more flexible by the addition of phthalates, and may also replace rubber. Thus, PVC is widely used including: pipes and water distribution, a substitute for painted wood (e.g. window frames, sills, flooring), electrical cable insulation, inflatable products, waterproof clothing (e.g. coats, skiing equipment, shoes), healthcare products (e.g. containers, tubing, catheters), food packaging, dental appliances and vinyl records.[1]

Harmless in its polymeric form, workers handling the finished PVC product are perfectly safe. In contrast, the at-risk phase lies in the manual descaling of autoclaves used for the polymerization where workers can contact it during its monomer state.[2] The chronic intoxication by gaseous monomer VCM is linked to several symptoms such as:[3] asthenia and dizziness,[3] Raynaud's syndrome,[4 5] digestive ulcera with nausea and anorexia,[3] systemic symptoms of arthralgia and myalgia[3], trophic cutaneous symptoms and sclerosis. It has also been suspected in the onset of acroosteolysis[4 6 7] and hepatocellular carcinoma.[8 9] More generally, VCM exposure involves chromosomal aberrations and increased carcinogenic risk.[10] Even if VCM related diseases may have a genetic bases, they are also linked to prolonged occupational VCM exposure.[5 11]

Scleroderma-like microvascular abnormalities have been also described on exposed workers.[12-14] The most common and non-invasive means of investigating these abnormalities is capillaroscopy. Widespread identification of individuals most at risk could enable early detection and treatment. The residual effects of VCM on microcirculation have been shown

only once on 15 workers who had ceased their VCM exposure six months prior to testing.[13] However, residual long-term abnormalities following retirement are unknown. Our hypothesis was higher capillaroscopic abnormalities in the VCM exposed group than in the control group.

Therefore, the aim of our cross- sectional study was to investigate residual long-term capillaroscopic abnormalities following retirement, after 15 years without VCM exposure.

METHODS

Participants

We enrolled male retired workers exposed to VCM in PVC production. They provided written informed consent. The study was approved by the human ethics committees from Clermont-Ferrand university hospital, France. To be eligible, participants had to be: male, retired, aged over 60 years, with at least a 5-year occupational exposure to VCM, a time after VCM exposure of at least 5 years, and no exposure to chemicals other than VCM. Moreover, participants with diabetes mellitus were also excluded as it may interfere with microcirculation,[15-20] as well as individuals declaring the use or previous use of treatments which may alter microcirculation.

Participants responded to a survey to determine exposure time, direct or indirect contact, type of occupation, time after exposure and smoking history. They underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities such as pathologies that may interfere with microcirculation (arterial hypertension, dyslipidemia) or pathologies potentially linked with VCM exposure (cardiovascular or respiratory diseases).[21]

A control group was matched in age, sex, type of occupation. They were recruited via advertisements. Selection criteria for this group also included no occupational or leisure chemical exposure, and no diabetes mellitus.[15-20]

Capillaroscopy

A nailfold capillaroscopy was performed on all fingers of each patient, excluding thumbs.[22] The nailfold capillaroscopies of the fingers were captured in images and electronically stored. The same investigator conducted all the capillaroscopies. A double blind analysis of capillaroscopic images was completed on deidentified data.

The outcomes were the five following classical criteria used in capillaroscopy:[23] density, length, diameter, dystrophy and hemorrhage. Criteria for abnormalities were defined as: decreased capillary density <10/mm (avascular zone <7/mm), augmented capillary length >300µm, increased capillary diameter >30µm (megacapillary >50µm), and dystrophy was associated with capillary branching >15%.

Statistics

Data are presented as mean percentage change and standard deviation (SD).

The main judgment criterion for abnormal microcirculation was the presence of at least one abnormality in capillaroscopy. The most common abnormality is capillary dystrophy.[23] Previous data[2 23] and personal observation on residual long-term abnormalities following VCM exposure showed that a percentage of dystrophy of approximately 25% was required to differentiate between the exposed group and the controls. Using this value as the main outcome, we calculated that a sample of 10 participants per group allows a statistical power greater than 80% with an alpha level less than 5%.

Statistical analyses were performed with SPSS software, v19. The Gaussian distribution for each parameter was assessed by a Shapiro-Wilk test. Comparisons between groups (exposed vs. control) were made through the usual tests: Chi2 test for categorical variables (or Fisher's exact test where appropriate) and Student's t test for quantitative variables (or Kruskal-Wallis if assumptions of normal distribution were violated). Significance was accepted for a p-value < 5%. A matrix of the correlations between the outcomes and exposure was determined using a non parametric Spearman test. Multivariate models were used to predict the relationship between capillary parameters and other parameters such as exposure time and time after exposure.

RESULTS

Participants

We enrolled 761male retired workers exposed to chemical toxics from two leading enterprises involved in PVC production (n=435 and n=91), as well as participants from many subcontracting companies (n=235), also known for VCM exposure. The strict selection criteria of exposure only to VCM reduced the sample size to 21 (figure 1).

Thirty five age-matched controls were also recruited without occupational or leisure time exposure to chemical toxics.

Main capillaroscopic outcomes

There was no missing data. Double blinded analysis showed no statistical difference between the two investigators.

Compared with controls, retired workers previously exposed to VCM had higher capillaroscopic abnormalities: enlarged capillaries (0% vs. 19%, p<.001), dystrophy (0% vs. 28.6%, p=.0012) (figure 2), and augmented length (0% vs. 33%, p<.001). The mean length was higher in the exposed group than in controls (291±14µ vs. 254±9µ, p=.020), as well as the mean diameter of capillaries (28.9±0.9µ vs. 25.7±0.6µ, p=.006) (table 1).

Exposure

Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Time of exposure to VCM was strongly linked with enlarged capillaries (R^2 adjusted = 53%, p<.0001) and modestly linked with capillary length (R^2 adjusted = 8%; p=.031) (table 1). Age was not associated with capillaroscopic abnormalities.

Symptoms of Raynaud

VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).

Comorbidities and smoking

Neither respiratory nor cardiovascular diseases were associated with VCM exposure. However, we combined both groups to explore potential associations between capillaroscopic parameters and high blood pressure, dyslipidemia, and smoking. Capillary length in participants medicated for arterial hypertension (n=20/61) did not differ from participants without hypertension. Participants treated with lipid lowering drugs with dyslipidemia (n=10/61) also showed a trend for a higher capillary length than participants without dyslipidemia (p=.079). Finally, there were no capillaroscopic difference between smokers and

non smokers. However, smokers who had been exposed to VCM tended to have a higher capillary length than non smokers (p=.073).

DISCUSSION

Principal findings of the study

Changes in microcirculation persist for at least 15 years following occupational VCM exposure, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure, was not related to pathological capillaroscopic changes.

what the study adds

The microcirculation changes following VCM exposure has been previously shown on 15 workers who ceased their occupational exposure six months prior to testing.[13] Our study supports these results over a longer period following VCM exposure – 15 years.

The dose responsiveness of VCM exposure and compromised capillarisation is well accepted.[13] Although daily VCM doses may have been more informative, the current study was restricted to years of exposure. Thus, we are limited to describing associations with an exposure time – response rather than a dose-response.

The absence of changes in microcirculation on less exposed workers[24] resulted in a suggestion that a threshold of exposure exists. This finding is supported by previous studies showing that long-term exposure (>8 years) induced greater chromosomal aberrations.[10 25] Further, not all workers exposed to VCM develop microvascular abnormalities, suggestive of an underlying genetic susceptibility (polymorphism of glutathione S-transferase).[5 11] A finding that female

VCM-exposed workers were more susceptible than males to the risk of increased chromosome damage also reinforced genetic susceptibility theory.[25]

Comparison with other studies

After 50 years, minor dystrophies could alter readability and interpretation of capillaroscopic analyses.[26] Nevertheless, we controlled this parameter by matching the exposed group and the controls on age and we conducted a double-blind analysis by two experienced readers. Moreover, capillaroscopic abnormalities among workers exposed to VCM in the present study were not influenced by age.[13]

VCM has been suspected of causing respiratory and circulatory diseases.[27] However, similar frequencies of these diseases in both groups do not support this hypothesis.

Diabetes mellitus,[15-20] high blood pressure,[17 28-30] dyslipidemia,[29] or some comorbidities/medications[21] could interfere with microcirculation. In line with previous studies, we showed a diminished capillary length in participants treated for arterial hypertension,[17 28-30] and a trend for participants treated with lipid lowering drugs.[29] Diabetes mellitus was an exclusion criterion and thus, could not interfere with our results. Perhaps due to low numbers of participants, our results failed to support previous findings of compromised microcirculation in people with high blood pressure. The trend for increased capillary length observed in our participants with dyslipidemia could be a response to increased peripheral vascular resistance, in order to maintain their function of metabolic exchange.[30] Similarly, smoking could induce a decrease in tissue perfusion,[31] and dystrophia.[32] We did not observe differences between smokers and non-smokers, with the exception of a trend for abnormal microcirculation among smokers exposed to VCM. The potential of a synergistic effect of tobacco and VCM-exposure warrants further investigations. It should be noted that VCM

exposure in the current study was more strongly associated with compromised microcirculation than high blood pressure, dyslipidemia and smoking.

A higher prevalence of symptoms of Raynaud has been established in workers with VCM exposure,[27] up to one third of the exposed workers.[13] Our results support these data, and extend knowledge by demonstrating the prevalence of symptoms of Raynaud remained higher at least 15 years following VCM exposure. Furthermore, in the current study, all the participants who suffered from symptoms of Raynaud had never taken medications or suffered from other diseases conducive to Raynaud.

Unanswered questions

Although symptoms of Raynaud are statistically associated with VCM exposure, we could not report a link with capillaroscopic modifications. The pathophysiology of Raynaud's phenomenon remains unknown. There seems to be primary or secondary vascular failure influenced by a hereditary factor.[33] Decreased perfusion pressure could be secondary to systemic hypotension or be caused by proximal arterial occlusion, influenced by many factors; both vascular and intra vascular, neural, environmental or hereditary.[34] Angiography of the hands of patients exposed to VCM showed occlusions, stenosis and narrowing of distal arteries with the development of collateral circulation.[35] Lack of statistical power in our study could contribute to the lack of relationship between capillaroscopic changes and symptoms of Raynaud.

Strengths and limitations of study

This study presents some major strengths: a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factors, well-matched controls, a double blind analyses, sufficient number of participants to detect the capillaroscopic differences between

groups, the focus on retired workers at least 15 years after the end of occupational VCM exposure. The attendance rate of 30% (53 of 179 individuals exclusively exposed to VCM at work agreed to participate in our study) seems very high compared with other studies[36-40] taking into account their age (75 years), distance from the location of the medical examination (averaging approximately 80 km), and that no financial compensation was offered.

There are limitations to this study: a cross-sectional design however, proof of concept was important and achieved (with a possibility of longitudinal follow-up); differences in some but not all capillary outcomes may be explained by a lack of power; the results are insufficient to propose guidelines for all workers exposed to VCM; more accurate quantifiable measures of VCM exposure are not available, however for the purpose of this study we used industry established lists of exposure legally required in France; we combined our knoledgeonly use a binary pathophysiology of Raynaud after VCM exposure remains unclear, most of the retired workers exposed to VCM were from the same enterprise; and potentially more at-risk manufacturing processes remained undetected.

CONCLUSION

Although VCM exposure was already known to affect microcirculation, our study demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time exposure – response. Symptoms of Raynaud, although statistically associated with exposure were not associated with capillaroscopic modifications; its origin remains to be determined. Future research could focus on other chemical products which have a similar structure than VCM and more extensive research on type of occupations at-risk of VCM exposure.

Role of the Funding Source

The Occupational Medicine department of CHU G. Montpied, Clermont-Ferrand, France funded this study. No other funding source had a role in the design, conduct, or reporting of the study.

Contributors

VL has participated as a MD student and principal investigator. FD and AC obtained research funding and generated the intellectual development of the study. FD knew potential exposed workers to VCM. VL, FD, AC and SH contributed to the conception of the protocol. VL, FD and SH made data analysis. VL, FD, MT contributed to manuscript drafting. VL recruited all participants and performed all capillaroscopies. VL and MT completed the double blind analyses of capillaroscopy. FD, AC, VL, GN and MT revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Our thanks to Geraldine Naughton for help with manuscript English proof reading. We also want to acknowledge the “Association of the sick from chemicals” (Association des Malades de la Chimie), 15 Av Albert Poncet 03410 Domerat, France, which helped us recruit the retired workers from VCM exposure.

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Table 1 Characteristics of participants, exposure to VCM and capillaroscopic outcomes.

	Retired from exposure to VCM (n=21)	**Controls (n=40)**	**p-value**
Age – years	74.4±2.9	76.3±3.2	NS
type of **occupation**: **blue collar** workers – "manual"	100%	100%	NS
Exposure to VCM:			
direct contact with VCM	100%	0	**<.001**
exposure time – years	29.8±1.9	0	**<.001**
time after exposure – years	15.9±2.4	-	-
Main capillaroscopic outcomes:			
1. **density**			
mean density – mm	8.6±0.4	8.8±0.3	NS
decreased capillary density <10/mm – n (%)	9 (43)	16 (46)	NS
avascular zone <7/mm – n(%)	0	0	NS
2. **length**			
mean length – mm	291±14µ	254±9µ	**.020**
augmented capillary length >300µm – n(%)	7 (33)	0	**<.001**
3. **diameter**			
mean diameter of capillaries – mm	28.9±0.9µ	25.7±0.6µ	**.006**
enlarged capillaries >30µm – n(%)	4 (19)	0	**<.001**
megacapillary >50µm – n(%)	0	0	NS
4. **dystrophy**			
capillary branching >15% – n(%)	6 (29)	0	**<.001**
5. **hemorrhage** – n(%)	0	0	NS
Symptoms of Raynaud:			
n(%) with Raynaud	4 (19)	0	**.007**
Participants with medications which could induce Raynaud – n(%)	2 (9)	4 (11)	NS
Other causes of Raynaud	0	0	NS
Comorbidities			
Respiratory diseases	2 (9)	6 (17)	NS
Cardiovascular diseases (except high blood pressure)	4 (19)	5 (14)	NS
Myocardial infraction	3 (14)	3(9)	NS
Routine medications – n(%) of patients treated for:			
Blood pressure	7 (33)	13 (37)	NS
Lipid lowering	4 (19)	6 (17)	NS
Smoking – n(%)	9 (43)	18 (51)	NS

Figure 1 Participant flow chart.

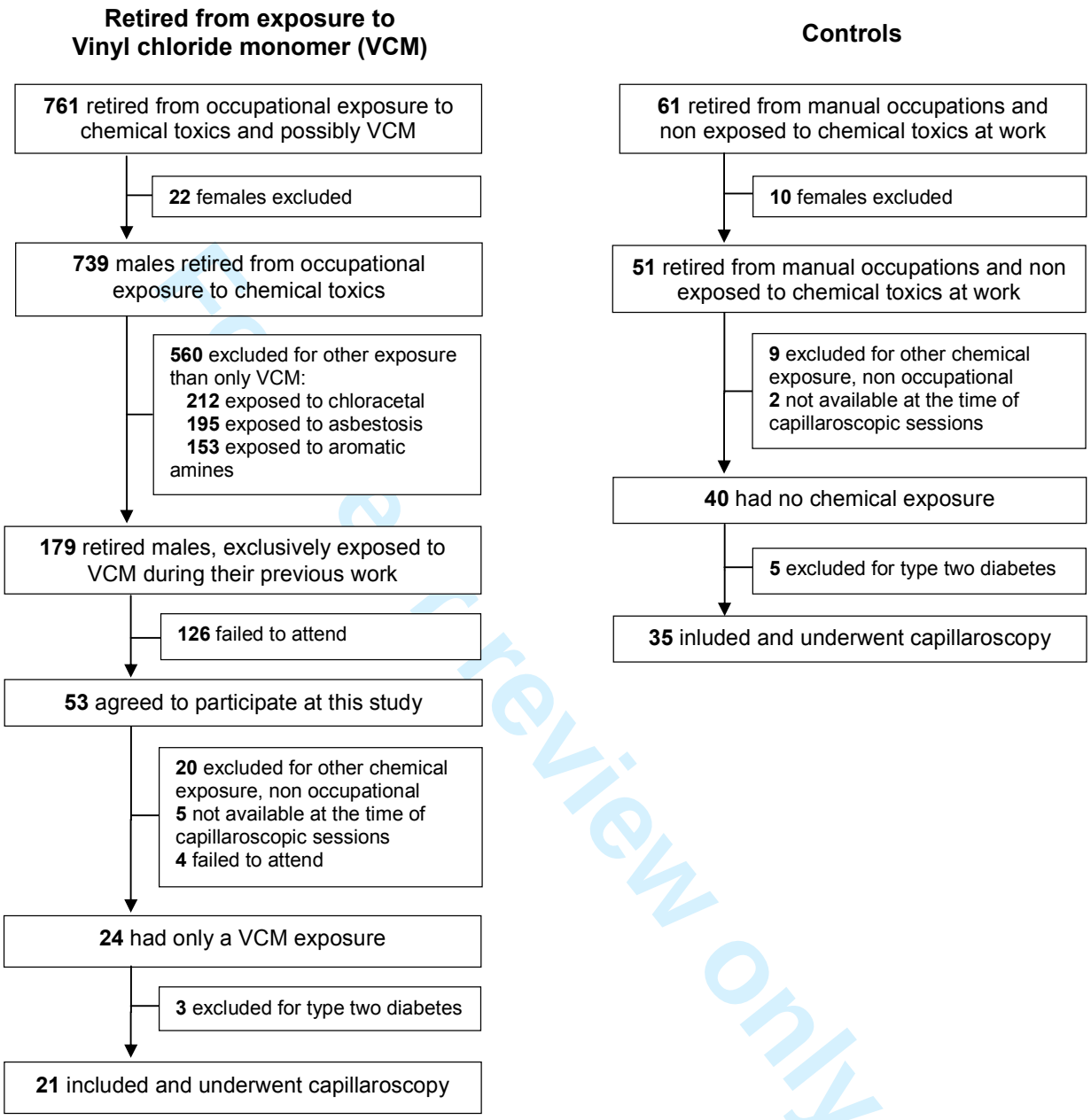
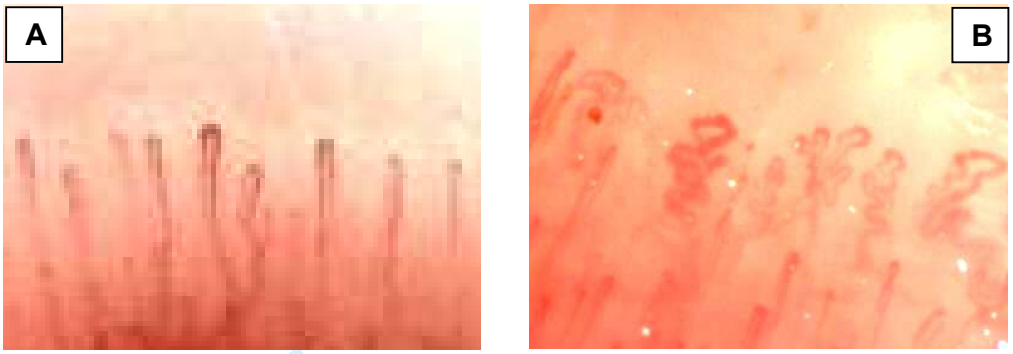


Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	19 (figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	8-9
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8-9
		(*e*) Describe any sensitivity analyses	8-9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	19 (figure 1)
		(b) Give reasons for non-participation at each stage	19 (figure 1)
		(c) Consider use of a flow diagram	19 (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7;9; 19 (figure 1)
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-10; 19 (figure 1)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10; 19 (figure 1)
		(*b*) Report category boundaries when continuous variables were categorized	9-10; 19 (figure 1)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*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.

The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002785.R1
Article Type:	Research
Date Submitted by the Author:	04-Apr-2013
Complete List of Authors:	Lopez, Vincent; CHU G. Montpied, Chamoux, Alain; CHU G. Montpied, Tempier, Marion; CHU G. Montpied, Thiel, Helene; CHU G. Montpied, Ughetto, Sylvie; CHU G. Montpied, Trousselard, Marion; IRBA, Naughton, Geraldine; Australian Catholic University, School of Exercise Science Dutheil, Frederic; CHU G. Montpied,
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Public health
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, CHEMICAL PATHOLOGY, PUBLIC HEALTH

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	19 (figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	8-9
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The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

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Running Title: Capillaroscopy post-exposure to VCM

Words count for introduction methods results discussion conclusion: 2298

Number of tables: 1

Number of figures: 2

Words count for abstract: 258

ABSTRACT

Objectives: To assess residual long-term microcirculation abnormalities by capillaroscopy, 15 years after retiring from occupational exposure to vinyl chloride monomer (VCM).

Design: Cross-sectional study.

Setting: Allier, one of the major area of PVC production in France.

Participants: We screened 761 (97% males) retired workers exposed to chemical toxics. Exposure to chemical other than VCM excluded potential participants.

Primary and secondary outcomes measures: These participants underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities, as well as a survey to determine exposure time, direct or indirect contact, type of occupation, smoking status and time after exposure. A double blind analysis of capillaroscopic images was done. A control group was matched in age, sex, type of occupation.

Results: 179/761 retired workers were only exposed to VCM at their work, with 21 meeting the inclusion criteria and included. Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Retired workers previously exposed to VCM had significantly higher capillaroscopic modifications than 35 controls: enlarged capillaries (19% vs. 0%, p<.001), dystrophy (28.6% vs. 0%, p=.0012), and augmented length (33% vs. 0%, p<.001). Time exposure was linked with enlarged capillaries (R^2 adjusted = 63%, p<.0001). They also had higher symptoms of Raynaud (19% vs. 0%, p=.007) without correlation with capillaroscopic modifications.

Conclusion: Although VCM exposure was already known to affect microcirculation, our study demonstrates residual long term abnormalities following an average of 15 years retirement, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure was not related to capillaroscopic modifications; its origin remains to be determined.

Keywords Exposition, Vinyl chloride monomer, Capillaroscopy, Raynaud

ARTICLE SUMMARY

Article focus

- Vinyl chloride monomer exposure induces microcirculation abnormalities, which can be diagnosed by capillaroscopy.
- Residual long-term abnormalities following retirement required investigation.

Key messages

- Our results demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time-related exposure response.
- Symptoms of Raynaud, although statistically associated with exposure, was not related to capillaroscopic modifications; its origin remains to be determined.

Strengths and limitations of this study

- The strong points is that this study had a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factor and the focus on retired workers at least 15 years after the end of occupational VCM exposure.
- The main limitation is that pathophysiology of Raynaud after VCM exposure remains unclear.

INTRODUCTION

Vinyl chloride monomer (VCM) is primarily used in the manufacture of plastics and also serves as a raw material in organic synthesis. VCM is an aliphatic hydrocarbon also known as chloroethene. Its polymerization lead to a synthetic resin called polyvinyl chloride, commonly abbreviated PVC. PVC, is the third-most widely produced plastic, after polyethylene and polypropylene.[1] PVC can be made softer and more flexible by the addition of phthalates, and may also replace rubber. Thus, PVC is widely used including: pipes and water distribution, a substitute for painted wood (e.g. window frames, sills, flooring), electrical cable insulation, inflatable products, waterproof clothing (e.g. coats, skiing equipment, shoes), healthcare products (e.g. containers, tubing, catheters), food packaging, dental appliances and vinyl records.[1]

Harmless in its polymeric form, workers handling the finished PVC product are perfectly safe. In contrast, the at-risk phase lies in the manual descaling of autoclaves used for the polymerization where workers can contact it during its monomer state.[2] The chronic intoxication by gaseous monomer VCM is linked to several symptoms such as:[3] asthenia and dizziness,[3] Raynaud's syndrome,[4 5] digestive ulcera with nausea and anorexia,[3] systemic symptoms of arthralgia and myalgia[3], trophic cutaneous symptoms and sclerosis. It has also been suspected in the onset of acroosteolysis[4 6 7] and hepatocellular carcinoma.[8 9] More generally, VCM exposure involves chromosomal aberrations and increased carcinogenic risk.[10] Even if VCM related diseases may have a genetic bases, they are also linked to prolonged occupational VCM exposure.[5 11]

Scleroderma-like microvascular abnormalities have been also described on exposed workers.[12-14] The most common and non-invasive means of investigating these abnormalities is capillaroscopy. Widespread identification of individuals most at risk could enable early detection and management strategy.[15] The residual effects of VCM on microcirculation have

been shown only once on 15 workers who had ceased their VCM exposure six months prior to testing.[13] However, residual long-term abnormalities following retirement are unknown. Our hypothesis was higher capillaroscopic abnormalities in the VCM exposed group than in the control group.

Therefore, the aim of our cross- sectional study was to investigate residual long-term capillaroscopic abnormalities following retirement, after 15 years without VCM exposure.

METHODS

Participants

We enrolled male retired workers exposed to VCM in PVC production. They provided written informed consent. The study was approved by the human ethics committees from Clermont-Ferrand university hospital, France. To be eligible, participants had to be: male (due to male dominance in this workforce), retired, aged over 60 years, with at least a 5-year occupational exposure to VCM, a time after VCM exposure of at least 5 years, and no exposure to chemicals other than VCM. Moreover, participants with diabetes mellitus were also excluded as it may interfere with microcirculation,[16-21] as well as individuals declaring the use or previous use of treatments which may alter microcirculation.

Participants responded to a survey to determine exposure time, direct or indirect contact, type of occupation, time after exposure and smoking history. They underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities such as pathologies that may interfere with microcirculation (arterial hypertension, dyslipidemia) or pathologies potentially linked with VCM exposure (cardiovascular or respiratory diseases).[22]

A control group was matched in age, sex, type of occupation. They were recruited via advertisements. Selection criteria for this group also included no occupational or leisure chemical exposure, and no diabetes mellitus.[16-21]

Capillaroscopy

A nailfold capillaroscopy was performed on all fingers of each patient, excluding thumbs.[23] The nailfold capillaroscopies of the fingers were captured in images and electronically stored. The same investigator conducted all the capillaroscopies. A double blind analysis of capillaroscopic images was completed on deidentified data.

The outcomes were the five following classical criteria used in capillaroscopy: density, length, diameter, dystrophy and hemorrhage. Criteria for abnormalities were defined as: decreased capillary density <10/mm (avascular zone <7/mm),[24] augmented capillary length >300µm,[24 25] increased capillary diameter >25µm[25] (megacapillary >50µm)[26 27], and dystrophy was associated with capillary branching >15%.[28] Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall.[27]

Statistics

Data are presented as mean percentage change and standard deviation (SD).

The main judgment criterion for abnormal microcirculation was the presence of at least one abnormality in capillaroscopy. The most common abnormality is capillary dystrophy.[25] Previous data[2 25] and personal observation on residual long-term abnormalities following VCM exposure showed that a percentage of dystrophy of approximately 25% was required to differentiate between the exposed group and the controls. Using this value as the main outcome, we calculated that a sample of 10 participants per group allows a statistical power greater than 80% with an alpha level less than 5%.

Statistical analyses were performed with SPSS software, v19. Correlations were used for inter-observer reliability. The Gaussian distribution for each parameter was assessed by a Shapiro-Wilk test. Comparisons between groups (exposed vs. control) were made through the usual tests: Chi2 test for categorical variables (or Fisher's exact test where appropriate) and Student's t test for quantitative variables (or Kruskal-Wallis if assumptions of normal distribution were violated). Significance was accepted for a p-value < 5%. The links between continuous variables were analyzed using linear regression. The links between binary and continuous variables were analyzed with logistic regression. Multivariate models were used to predict the relationship between capillary parameters and other parameters such as exposure time and time after exposure.

RESULTS

Participants

We screened 761 (97% males) retired workers exposed to chemical toxics from two leading enterprises involved in PVC production (n=435 and n=91), as well as participants from many subcontracting companies (n=235), also known for VCM exposure. The strict selection criteria of exposure only to VCM reduced the sample size to 21 (figure 1).

Thirty five age-matched controls were also recruited without occupational or leisure time exposure to chemical toxics.

Main capillaroscopic outcomes

There was no missing data. Inter-rater reliability was confirmed with correlations exceeding 0.70 for each parameter. The mean of the values of the 2 investigators is presented in table 1.

Concerning the qualitative data, when a disagreement occurred, the two observers analyzed again together and requested the opinion of a third expert. The disagreement occurred only for 2 decreased capillary density <10/mm, and 1 capillary branching >15%.

Compared with controls, retired workers previously exposed to VCM had higher capillaroscopic abnormalities: enlarged capillaries (0% vs. 19%, p<.001), dystrophy (0% vs. 28.6%, p=.0012) (figure 2), and augmented length (0% vs. 33%, p<.001). The mean length was 15% higher in the exposed group than in controls (p=.020), as well as a 12% greater diameter of capillaries (p=.006) (table 1).

Exposure

Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Time of exposure to VCM was strongly linked with enlarged capillaries (R^2 adjusted = 63%, p<.0001) and modestly linked with capillary length (R^2 = 8%; p=.031) (table 1). Age was not associated with capillaroscopic abnormalities. No multivariate models improved results from simple regressions.

Symptoms of Raynaud

VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).

Comorbidities and smoking

Neither respiratory nor cardiovascular diseases were associated with VCM exposure. However, we combined both groups to explore potential associations between capillaroscopic parameters and high blood pressure, dyslipidemia, and smoking. Capillary length in participants medicated for arterial hypertension (n=20/61) did not differ from participants

without hypertension. Participants treated with lipid lowering drugs with dyslipidemia (n=10/61) also showed a trend for a higher capillary length than participants without dyslipidemia (p=.079). Finally, there were no capillaroscopic difference between smokers and non smokers. However, smokers who had been exposed to VCM tended to have a higher capillary length than non smokers (p=.073).

DISCUSSION

Principal findings of the study

Changes in microcirculation persist for at least 15 years following occupational VCM exposure, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure, was not related to pathological capillaroscopic changes.

What the study adds

The microcirculation changes following VCM exposure has been previously shown on 15 workers who ceased their occupational exposure six months prior to testing.[13] Our study supports these results over a longer period following VCM exposure – 15 years.

The dose responsiveness of VCM exposure and compromised capillarisation is generally,[13] but not always,[12] reported. Although daily VCM doses may have been more informative, the current study was restricted to years of exposure. Thus, we are limited to describing associations with an exposure time – response rather than a dose-response. Years of exposure is an easy question for physicians to ask of workers during risk assessment protocols.

The absence of changes in microcirculation on less exposed workers[29] resulted in a suggestion that a threshold of exposure exists. This finding is supported by previous studies showing that

long-term exposure (>8 years) induced greater chromosomal aberrations.[10 30] Further, not all workers exposed to VCM develop microvascular abnormalities, suggestive of an underlying genetic susceptibility (polymorphism of glutathione S-transferase).[5 11] A finding that female VCM-exposed workers were more susceptible than males to the risk of increased chromosome damage also reinforced genetic susceptibility theory.[30]

Comparison with other studies

After 50 years of age, minor dystrophies could alter readability and interpretation of capillaroscopic analyses.[31] Nevertheless, we controlled this parameter by matching the exposed group and the controls on age and we conducted a double-blind analysis by two experienced readers. Moreover, capillaroscopic abnormalities among workers exposed to VCM in the present study were not influenced by age.[13]

VCM has been suspected of causing respiratory and circulatory diseases.[32] However, similar frequencies of these diseases in both groups do not support this hypothesis.

Diabetes mellitus,[16-21] high blood pressure,[18 33-35] dyslipidemia,[34] or some comorbidities/medications[22] could interfere with microcirculation. In line with previous studies, we showed a diminished capillary length in participants treated for arterial hypertension,[18 33-35] and a trend for participants treated with lipid lowering drugs.[34] Diabetes mellitus was an exclusion criterion and thus, could not interfere with our results. Perhaps due to low numbers of participants, our results failed to support previous findings of compromised microcirculation in people with high blood pressure. The trend for increased capillary length observed in our participants with dyslipidemia could be a response to increased peripheral vascular resistance, in order to maintain their function of metabolic exchange.[35] Similarly, smoking could induce a decrease in tissue perfusion,[36] and dystrophia.[37] We did not observe differences between smokers and non-smokers, with the exception of a trend for abnormal

microcirculation among smokers exposed to VCM. The potential of a synergistic effect of tobacco and VCM-exposure warrants further investigations. It should be noted that VCM exposure in the current study was more strongly associated with compromised microcirculation than high blood pressure, dyslipidemia and smoking.

Previous research into the links between systemic sclerosis and VCM exposure is limited by single case design[38] and somewhat dated analyses of a population exposed to solvents.[39] The broader use of term such as solvents is less specific than the VCM exposure carefully isolated for investigation in the present study.

A higher prevalence of symptoms of Raynaud has been established in workers with VCM exposure,[32] up to one third of the exposed workers.[13] The comparison between studies with different selection criteria and different sample sizes is difficult. There is also the possibility of selection bias in non randomized recruitment. Within these limitations, our results support previous data, and extend knowledge by demonstrating the prevalence of symptoms of Raynaud remained higher at least 15 years following VCM exposure. Furthermore, in the current study, all the participants who suffered from symptoms of Raynaud had never taken medications or suffered from other diseases conducive to Raynaud.

Unanswered questions

Although symptoms of Raynaud are statistically associated with VCM exposure, we could not report a link with capillaroscopic modifications. The pathophysiology of Raynaud's phenomenon remains unknown. There seems to be primary or secondary vascular failure influenced by a hereditary factor.[40] Decreased perfusion pressure could be secondary to systemic hypotension or be caused by proximal arterial occlusion, influenced by many factors; both vascular and intra vascular, neural, environmental or hereditary.[41] Angiography of the hands of patients exposed to VCM showed occlusions, stenosis and narrowing of distal

arteries with the development of collateral circulation.[42] Lack of statistical power in our study could contribute to the lack of relationship between capillaroscopic changes and symptoms of Raynaud.

Strengths and limitations of study

This study presents some major strengths: a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factors, well-matched controls, a double blind analyses, sufficient number of participants to detect the capillaroscopic differences between groups, the focus on retired workers at least 15 years after the end of occupational VCM exposure. The attendance rate of 30% (53 of 179 individuals exclusively exposed to VCM at work agreed to participate in our study) seems very high compared with other studies[43-47] taking into account their age (75 years), distance from the location of the medical examination (averaging approximately 80 km), and that no financial compensation was offered.

There are limitations to this study: a cross-sectional design however, proof of concept was important and achieved (with a possibility of longitudinal follow-up); differences in some but not all capillary outcomes may be explained by a lack of power; the results are insufficient to propose guidelines for all workers exposed to VCM; more accurate quantifiable measures of VCM exposure are not available, however for the purpose of this study we used industry established lists of exposure legally required in France; we combined our knoledgeonly use a binary pathophysiology of Raynaud after VCM exposure remains unclear, most of the retired workers exposed to VCM were from the same enterprise; and potentially more at-risk manufacturing processes remained undetected.

CONCLUSION

Although VCM exposure was already known to affect microcirculation, our study demonstrated the potential for residual long-term abnormalities following an average of 15 years retirement, with a time exposure – response. Symptoms of Raynaud, although statistically associated with exposure were not associated with capillaroscopic modifications; its origin remains to be determined. Future research could focus on other chemical products which have a similar structure than VCM and more extensive research on type of occupations at-risk of VCM exposure.

Role of the Funding Source

The Occupational Medicine department of CHU G. Montpied, Clermont-Ferrand, France funded this study. No other funding source had a role in the design, conduct, or reporting of the study.

Contributors

VL has participated as a MD student and principal investigator. FD and AC obtained research funding and generated the intellectual development of the study. FD knew potential exposed workers to VCM. VL, FD, AC and SH contributed to the conception of the protocol. VL, FD and SH made data analysis. VL, FD, MT contributed to manuscript drafting. VL recruited all participants and performed all capillaroscopies. VL and MT completed the double blind analyses of capillaroscopy. FD, AC, VL, GN and MT revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Our thanks to Geraldine Naughton for help with manuscript English proof reading. We also want to acknowledge the "Association of the sick from chemicals" (Association des Malades de la Chimie), 15 Av Albert Poncet 03410 Domerat, France, which helped us recruit the retired workers from VCM exposure.

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Table 1 Characteristics of participants, exposure to VCM and capillaroscopic outcomes.

	Retired from exposure to VCM (n=21)	Controls (n=40)	p-value
Age – years	74.4±2.9	76.3±3.2	NS
type of **occupation**: **blue collar** workers – "manual"	100%	100%	NS
Exposure to VCM:			
direct contact with VCM	100%	0	**<.001**
exposure time – years	29.8±1.9	0	**<.001**
time after exposure – years	15.9±2.4	-	-
Main capillaroscopic outcomes:			
1. **density**			
mean density – mm	8.6±0.4	8.8±0.3	NS
decreased capillary density <10/mm – n (%)	9 (43)	16 (46)	NS
avascular zone <7/mm – n(%)	0	0	NS
2. **length**			
mean length – µm	291±14	254±9	**.020**
augmented capillary length >300µm – n(%)	7 (33)	0	**<.001**
3. **diameter**			
mean diameter of capillaries – µm	28.9±0.9	25.7±0.6	**.006**
enlarged capillaries >25µm – n(%)	4 (19)	0	**<.001**
megacapillary >50µm – n(%)	0	0	NS
4. **dystrophy**			
capillary branching >15% – n(%)	6 (29)	0	**<.001**
5. **hemorrhage** – n(%)	0	0	NS
Symptoms of Raynaud:			
n(%) with Raynaud	4 (19)	0	**.007**
Participants with medications which could induce Raynaud – n(%)	2 (9)	4 (11)	NS
Other causes of Raynaud	0	0	NS
Comorbidities			
Respiratory diseases	2 (9)	6 (17)	NS
Cardiovascular diseases (except high blood pressure)	4 (19)	5 (14)	NS
Myocardial infraction	3 (14)	3(9)	NS
Routine medications – n(%) of patients treated for:			
Blood pressure	7 (33)	13 (37)	NS
Lipid lowering	4 (19)	6 (17)	NS
Smoking – n(%)	9 (43)	18 (51)	NS

Figure 1 Participant flow chart.

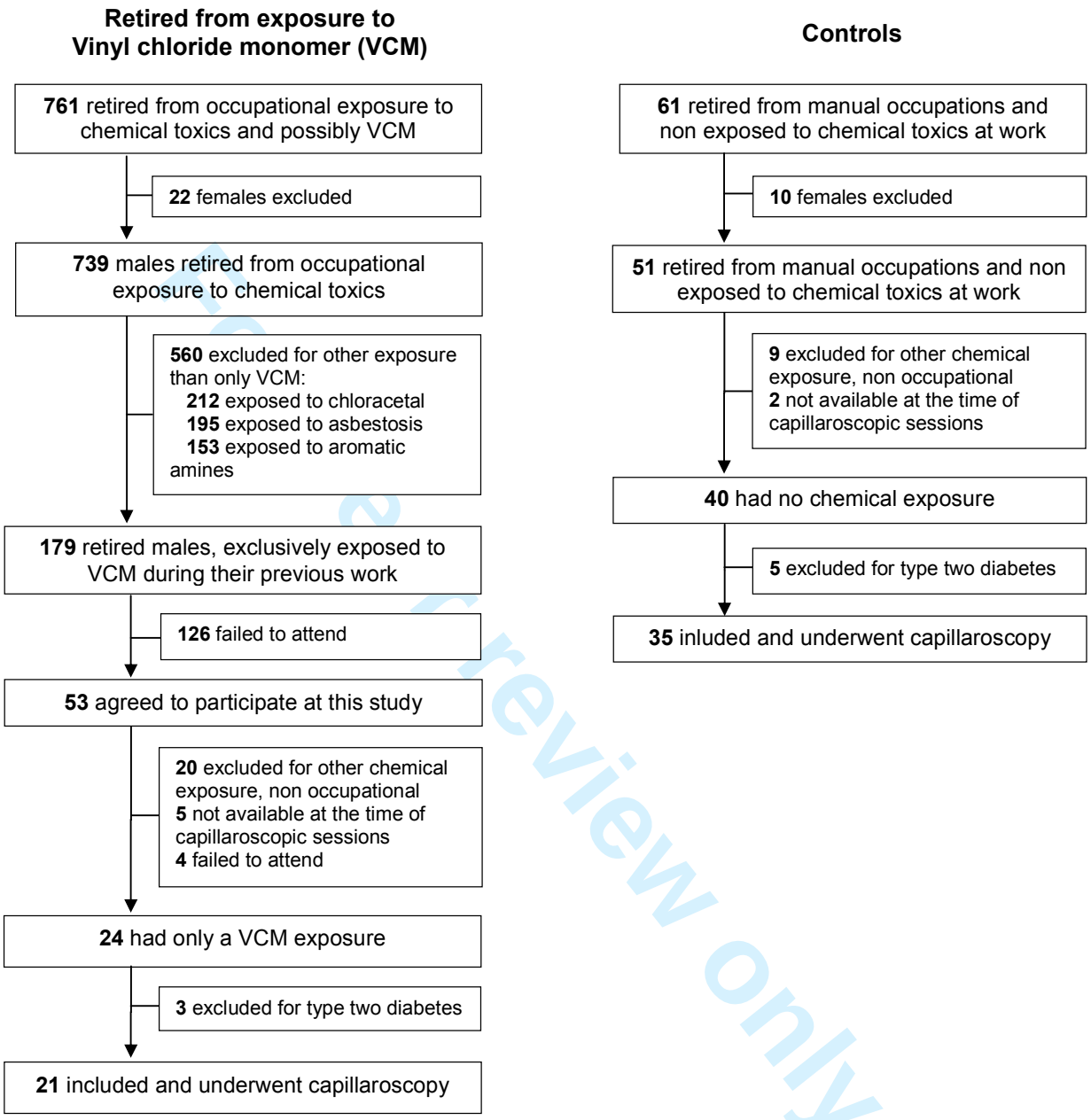
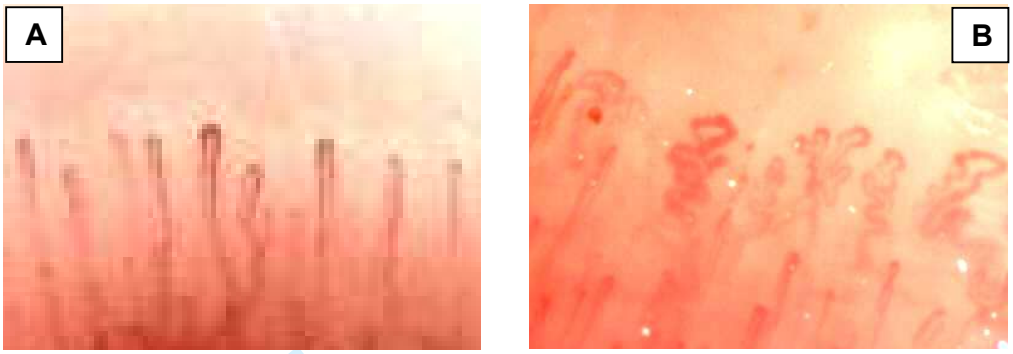


Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).



The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

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Running Title: Capillaroscopy post-exposure to VCM

Words count for introduction methods results discussion conclusion: 2298

Number of tables: 1

Number of figures: 2

Words count for abstract: 258

ABSTRACT

Objectives: To assess residual long-term microcirculation abnormalities by capillaroscopy, 15 years after retiring from occupational exposure to vinyl chloride monomer (VCM).

Design: Cross-sectional study.

Setting: Allier, one of the major area of PVC production in France.

Participants: We screened ~~enrolled~~ 761 (97% males) ~~male~~ retired workers exposed to chemical toxics. Exposure to chemical other than VCM excluded potential participants.

Primary and secondary outcomes measures: These participants underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities, as well as a survey to determine exposure time, direct or indirect contact, type of occupation, smoking status and time after exposure. A double blind analysis of capillaroscopic images was done. A control group was matched in age, sex, type of occupation.

Results: 179/761 retired workers were only exposed to VCM at their work, with 21 meeting the inclusion criteria and included. Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Retired workers previously exposed to VCM had significantly higher capillaroscopic modifications than 35 controls: enlarged capillaries (19% vs. 0%, p<.001), dystrophy (28.6% vs. 0%, p=.0012), and augmented length (33% vs. 0%, p<.001). Time exposure was linked with enlarged capillaries (R^2 adjusted = 6353%, p<.0001). They also had higher symptoms of Raynaud (19% vs. 0%, p=.007) without correlation with capillaroscopic modifications.

Conclusion: Although VCM exposure was already known to affect microcirculation, our study demonstrates residual long term abnormalities following an average of 15 years retirement, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure was not related to capillaroscopic modifications; its origin remains to be determined.

Keywords Exposition, Vinyl chloride monomer, Capillaroscopy, Raynaud

ARTICLE SUMMARY

Article focus

- Vinyl chloride monomer exposure induces microcirculation abnormalities, which can be diagnosed by capillaroscopy.
- Residual long-term abnormalities following retirement required investigation.

Key messages

- Our results demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time-related exposure response.
- Symptoms of Raynaud, although statistically associated with exposure, was not related to capillaroscopic modifications; its origin remains to be determined.

Strengths and limitations of this study

- The strong points is that this study had a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factor and the focus on retired workers at least 15 years after the end of occupational VCM exposure.
- The main limitation is that pathophysiology of Raynaud after VCM exposure remains unclear.

INTRODUCTION

Vinyl chloride monomer (VCM) is primarily used in the manufacture of plastics and also serves as a raw material in organic synthesis. VCM is an aliphatic hydrocarbon also known as chloroethene. Its polymerization lead to a synthetic resin called polyvinyl chloride, commonly abbreviated PVC. PVC, is the third-most widely produced plastic, after polyethylene and polypropylene.[1] PVC can be made softer and more flexible by the addition of phthalates, and may also replace rubber. Thus, PVC is widely used including: pipes and water distribution, a substitute for painted wood (e.g. window frames, sills, flooring), electrical cable insulation, inflatable products, waterproof clothing (e.g. coats, skiing equipment, shoes), healthcare products (e.g. containers, tubing, catheters), food packaging, dental appliances and vinyl records.[1]

Harmless in its polymeric form, workers handling the finished PVC product are perfectly safe. In contrast, the at-risk phase lies in the manual descaling of autoclaves used for the polymerization where workers can contact it during its monomer state.[2] The chronic intoxication by gaseous monomer VCM is linked to several symptoms such as:[3] asthenia and dizziness,[3] Raynaud's syndrome,[4 5] digestive ulcera with nausea and anorexia,[3] systemic symptoms of arthralgia and myalgia[3], trophic cutaneous symptoms and sclerosis. It has also been suspected in the onset of acroosteolysis[4 6 7] and hepatocellular carcinoma.[8 9] More generally, VCM exposure involves chromosomal aberrations and increased carcinogenic risk.[10] Even if VCM related diseases may have a genetic bases, they are also linked to prolonged occupational VCM exposure.[5 11]

Scleroderma-like microvascular abnormalities have been also described on exposed workers.[12-14] The most common and non-invasive means of investigating these abnormalities is capillaroscopy. Widespread identification of individuals most at risk could enable early detection and management strategy~~treatment~~.[15] The residual effects of VCM on

microcirculation have been shown only once on 15 workers who had ceased their VCM exposure six months prior to testing.[13] However, residual long-term abnormalities following retirement are unknown. Our hypothesis was higher capillaroscopic abnormalities in the VCM exposed group than in the control group.

Therefore, the aim of our cross- sectional study was to investigate residual long-term capillaroscopic abnormalities following retirement, after 15 years without VCM exposure.

METHODS

Participants

We enrolled male retired workers exposed to VCM in PVC production. They provided written informed consent. The study was approved by the human ethics committees from Clermont-Ferrand university hospital, France. To be eligible, participants had to be: male (due to male dominance in this workforce), retired, aged over 60 years, with at least a 5-year occupational exposure to VCM, a time after VCM exposure of at least 5 years, and no exposure to chemicals other than VCM. Moreover, participants with diabetes mellitus were also excluded as it may interfere with microcirculation,[16-21] as well as individuals declaring the use or previous use of treatments which may alter microcirculation.

Participants responded to a survey to determine exposure time, direct or indirect contact, type of occupation, time after exposure and smoking history. They underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities such as pathologies that may interfere with microcirculation (arterial hypertension, dyslipidemia) or pathologies potentially linked with VCM exposure (cardiovascular or respiratory diseases).[22]

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A control group was matched in age, sex, type of occupation. They were recruited via advertisements. Selection criteria for this group also included no occupational or leisure chemical exposure, and no diabetes mellitus.[16-21]

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Capillaroscopy

A nailfold capillaroscopy was performed on all fingers of each patient, excluding thumbs.[23]

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The nailfold capillaroscopies of the fingers were captured in images and electronically stored. The same investigator conducted all the capillaroscopies. A double blind analysis of capillaroscopic images was completed on deidentified data.

The outcomes were the five following classical criteria used in capillaroscopy:[23] density, length, diameter, dystrophy and hemorrhage. Criteria for abnormalities were defined as: decreased capillary density <10/mm (avascular zone <7/mm),[24] augmented capillary length >300µm,[24 25] –increased capillary diameter >2530µm[25] (megacapillary >50µm)[26 27], and dystrophy was associated with capillary branching >15%.[28] Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall.[27]

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Statistics

Data are presented as mean percentage change and standard deviation (SD).

The main judgment criterion for abnormal microcirculation was the presence of at least one abnormality in capillaroscopy. The most common abnormality is capillary dystrophy.[25] Previous data[2 25] and personal observation on residual long-term abnormalities following VCM exposure showed that a percentage of dystrophy of approximately 25% was required to differentiate between the exposed group and the controls. Using this value as the main outcome, we calculated that a sample of 10 participants per group allows a statistical power greater than 80% with an alpha level less than 5%.

Statistical analyses were performed with SPSS software, v19. Correlations were used for inter-observer reliability. The Gaussian distribution for each parameter was assessed by a Shapiro-Wilk test. Comparisons between groups (exposed vs. control) were made through the usual tests: Chi2 test for categorical variables (or Fisher's exact test where appropriate) and Student's t test for quantitative variables (or Kruskal-Wallis if assumptions of normal distribution were violated). Significance was accepted for a p-value < 5%. ~~A matrix of the correlations between the outcomes and exposure was determined using a non parametric Spearman test.~~ The links between continuous variables were analyzed using linear regression. The links between b~~-~~inary and continuous variables were analyzed with logistic regression. Multivariate models were used to predict the relationship between capillary parameters and other parameters such as exposure time and time after exposure.

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RESULTS

Participants

We screened ~~enrolled~~ 761 (97% males) retired workers exposed to chemical toxics from two leading enterprises involved in PVC production (n=435 and n=91), as well as participants from many subcontracting companies (n=235), also known for VCM exposure. The strict selection criteria of exposure only to VCM reduced the sample size to 21 (figure 1).

Thirty five age-matched controls were also recruited without occupational or leisure time exposure to chemical toxics.

Main capillaroscopic outcomes

There was no missing data. ~~Double blinded analysis showed no statistical difference between the two investigators.~~ Inter-rater reliability was confirmed with correlations exceeding 0.70 for each parameter. The mean of the values of the 2 investigators is presented in table 1. Concerning the qualitative data, when a disagreement occurred, the two observers analyzed again together and requested the opinion of a third expert. The disagreement occurred only for 2 decreased capillary density <10/mm, and 1 capillary branching >15%.

Compared with controls, retired workers previously exposed to VCM had higher capillaroscopic abnormalities: enlarged capillaries (0% vs. 19%, p<.001), dystrophy (0% vs. 28.6%, p=.0012) (figure 2), and augmented length (0% vs. 33%, p<.001). The mean length was 15% higher in the exposed group than in controls (~~291±14µ vs. 254±9µ,~~ p=.020), as well as a 12% greater ~~the mean~~ diameter of capillaries (~~28.9±0.9µ vs. 25.7±0.6µ,~~ p=.006) (table 1).

Exposure

Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Time of exposure to VCM was strongly linked with enlarged capillaries (R^2 adjusted = 63~~53~~%, p<.0001) and modestly linked with capillary length (R^2 ~~adjusted~~ = 8%; p=.031) (table 1). Age was not associated with capillaroscopic abnormalities. No multivariate models improved results from simple regressions.

Symptoms of Raynaud

VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).

Comorbidities and smoking

Neither respiratory nor cardiovascular diseases were associated with VCM exposure. However, we combined both groups to explore potential associations between capillaroscopic parameters and high blood pressure, dyslipidemia, and smoking. Capillary length in participants medicated for arterial hypertension (n=20/61) did not differ from participants without hypertension. Participants treated with lipid lowering drugs with dyslipidemia (n=10/61) also showed a trend for a higher capillary length than participants without dyslipidemia (p=.079). Finally, there were no capillaroscopic difference between smokers and non smokers. However, smokers who had been exposed to VCM tended to have a higher capillary length than non smokers (p=.073).

DISCUSSION

Principal findings of the study

Changes in microcirculation persist for at least 15 years following occupational VCM exposure, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure, was not related to pathological capillaroscopic changes.

Ww~~w~~hat the study adds

The microcirculation changes following VCM exposure has been previously shown on 15 workers who ceased their occupational exposure six months prior to testing.[13] Our study supports these results over a longer period following VCM exposure – 15 years.

The dose responsiveness of VCM exposure and compromised capillarisation is generally,[13] but not always,[12] ~~well~~ reported~~accepted~~.~~[13]~~ Although daily VCM doses may have been more

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informative, the current study was restricted to years of exposure. Thus, we are limited to describing associations with an exposure time – response rather than a dose-response. Years of exposure is an easy question for physicians to ask of workers during risk assessment protocols.

The absence of changes in microcirculation on less exposed workers[29] resulted in a suggestion that a threshold of exposure exists. This finding is supported by previous studies showing that long-term exposure (>8 years) induced greater chromosomal aberrations.[10 30] Further, not all workers exposed to VCM develop microvascular abnormalities, suggestive of an underlying genetic susceptibility (polymorphism of glutathione S-transferase).[5 11] A finding that female VCM-exposed workers were more susceptible than males to the risk of increased chromosome damage also reinforced genetic susceptibility theory.[30]

Comparison with other studies

After 50 years of age, minor dystrophies could alter readability and interpretation of capillaroscopic analyses.[31] Nevertheless, we controlled this parameter by matching the exposed group and the controls on age and we conducted a double-blind analysis by two experienced readers. Moreover, capillaroscopic abnormalities among workers exposed to VCM in the present study were not influenced by age.[13]

VCM has been suspected of causing respiratory and circulatory diseases.[32] However, similar frequencies of these diseases in both groups do not support this hypothesis.

Diabetes mellitus,[16-21] high blood pressure,[18 33-35] dyslipidemia,[34] or some comorbidities/medications[22] could interfere with microcirculation. In line with previous studies, we showed a diminished capillary length in participants treated for arterial hypertension,[18 33-35] and a trend for participants treated with lipid lowering drugs.[34] Diabetes mellitus was an exclusion criterion and thus, could not interfere with our results. Perhaps due

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to low numbers of participants, our results failed to support previous findings of compromised microcirculation in people with high blood pressure. The trend for increased capillary length observed in our participants with dyslipidemia could be a response to increased peripheral vascular resistance, in order to maintain their function of metabolic exchange.[35] Similarly, smoking could induce a decrease in tissue perfusion,[36] and dystrophia.[37] We did not observe differences between smokers and non-smokers, with the exception of a trend for abnormal microcirculation among smokers exposed to VCM. The potential of a synergistic effect of tobacco and VCM-exposure warrants further investigations. It should be noted that VCM exposure in the current study was more strongly associated with compromised microcirculation than high blood pressure, dyslipidemia and smoking.

Previous research into the links between systemic sclerosis and VCM exposure is limited by single case design[38] and somewhat dated analyses of a population exposed to solvents.[39] The broader use of term such as solvents is less specific than the VCM exposure carefully isolated for investigation in the present study.

A~~A~~ higher prevalence of symptoms of Raynaud has been established in workers with VCM exposure,[32] up to one third of the exposed workers.[13] The comparison between studies with different selection criteria and different sample sizes is difficult. There is also the possibility of selection bias in non randomized recruitment. Within these limitations, o~~O~~ur results support previous ~~these~~ data, and extend knowledge by demonstrating the prevalence of symptoms of Raynaud remained higher at least 15 years following VCM exposure. Furthermore, in the current study, all the participants who suffered from symptoms of Raynaud had never taken medications or suffered from other diseases conducive to Raynaud.

Unanswered questions

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Although symptoms of Raynaud are statistically associated with VCM exposure, we could not report a link with capillaroscopic modifications. The pathophysiology of Raynaud's phenomenon remains unknown. There seems to be primary or secondary vascular failure influenced by a hereditary factor.[40] Decreased perfusion pressure could be secondary to systemic hypotension or be caused by proximal arterial occlusion, influenced by many factors; both vascular and intra vascular, neural, environmental or hereditary.[41] Angiography of the hands of patients exposed to VCM showed occlusions, stenosis and narrowing of distal arteries with the development of collateral circulation.[42] Lack of statistical power in our study could contribute to the lack of relationship between capillaroscopic changes and symptoms of Raynaud.

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Strengths and limitations of study

This study presents some major strengths: a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factors, well-matched controls, a double blind analyses, sufficient number of participants to detect the capillaroscopic differences between groups, the focus on retired workers at least 15 years after the end of occupational VCM exposure. The attendance rate of 30% (53 of 179 individuals exclusively exposed to VCM at work agreed to participate in our study) seems very high compared with other studies[43-47] taking into account their age (75 years), distance from the location of the medical examination (averaging approximately 80 km), and that no financial compensation was offered.

There are limitations to this study: a cross-sectional design however, proof of concept was important and achieved (with a possibility of longitudinal follow-up); differences in some but not all capillary outcomes may be explained by a lack of power; the results are insufficient to propose guidelines for all workers exposed to VCM; more accurate quantifiable measures of VCM exposure are not available, however for the purpose of this study we used industry

established lists of exposure legally required in France; we combined our knoledgeonly use a binary pathophysiology of Raynaud after VCM exposure remains unclear, most of the retired workers exposed to VCM were from the same enterprise; and potentially more at-risk manufacturing processes remained undetected.

CONCLUSION

Although VCM exposure was already known to affect microcirculation, our study demonstrated the potential for residual long-term abnormalities following an average of 15 years retirement, with a time exposure – response. Symptoms of Raynaud, although statistically associated with exposure were not associated with capillaroscopic modifications; its origin remains to be determined. Future research could focus on other chemical products which have a similar structure than VCM and more extensive research on type of occupations at-risk of VCM exposure.

Role of the Funding Source

The Occupational Medicine department of CHU G. Montpied, Clermont-Ferrand, France funded this study. No other funding source had a role in the design, conduct, or reporting of the study.

Contributors

VL has participated as a MD student and principal investigator. FD and AC obtained research funding and generated the intellectual development of the study. FD knew potential exposed workers to VCM. VL, FD, AC and SH contributed to the conception of the protocol. VL, FD

and SH made data analysis. VL, FD, MT contributed to manuscript drafting. VL recruited all participants and performed all capillaroscopies. VL and MT completed the double blind analyses of capillaroscopy. FD, AC, VL, GN and MT revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Our thanks to Geraldine Naughton for help with manuscript English proof reading. We also want to acknowledge the "Association of the sick from chemicals" (Association des Malades de la Chimie), 15 Av Albert Poncet 03410 Domerat, France, which helped us recruit the retired workers from VCM exposure.

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Table 1 Characteristics of participants, exposure to VCM and capillaroscopic outcomes.

	Retired from exposure to VCM (n=21)	Controls (n=40)	p-value
Age – years	74.4±2.9	76.3±3.2	NS
type of **occupation**: **blue collar** workers – "manual"	100%	100%	NS
Exposure to VCM:			
direct contact with VCM	100%	0	**<.001**
exposure time – years	29.8±1.9	0	**<.001**
time after exposure – years	15.9±2.4	-	-
Main capillaroscopic outcomes:			
1. **density**			
mean density – mm	8.6±0.4	8.8±0.3	NS
decreased capillary density <10/mm – n (%)	9 (43)	16 (46)	NS
avascular zone <7/mm – n(%)	0	0	NS
2. **length**			
mean length – µmm	291±14µ	254±9µ	**.020**
augmented capillary length >300µm – n(%)	7 (33)	0	**<.001**
3. **diameter**			
mean diameter of capillaries – µmm	28.9±0.9µ	25.7±0.6µ	**.006**
enlarged capillaries >2530µm – n(%)	4 (19)	0	**<.001**
megacapillary >50µm – n(%)	0	0	NS
4. **dystrophy**			
capillary branching >15% – n(%)	6 (29)	0	**<.001**
5. **hemorrhage** – n(%)	0	0	NS
Symptoms of Raynaud:			
n(%) with Raynaud	4 (19)	0	**.007**
Participants with medications which could induce Raynaud – n(%)	2 (9)	4 (11)	NS
Other causes of Raynaud	0	0	NS
Comorbidities			
Respiratory diseases	2 (9)	6 (17)	NS
Cardiovascular diseases (except high blood pressure)	4 (19)	5 (14)	NS
Myocardial infraction	3 (14)	3(9)	NS
Routine medications – n(%) of patients treated for:			
Blood pressure	7 (33)	13 (37)	NS
Lipid lowering	4 (19)	6 (17)	NS
Smoking – n(%)	9 (43)	18 (51)	NS

Figure 1 Participant flow chart.

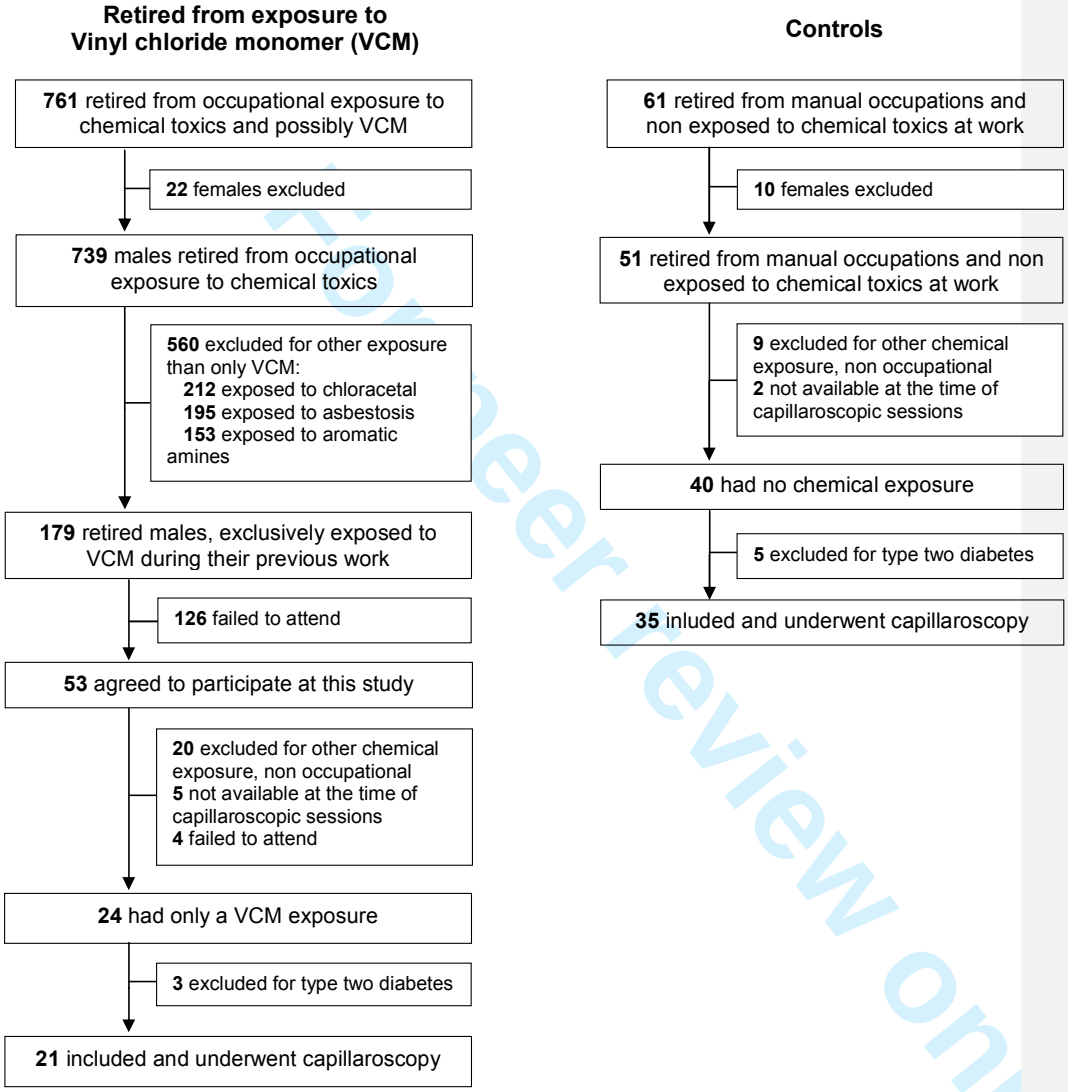
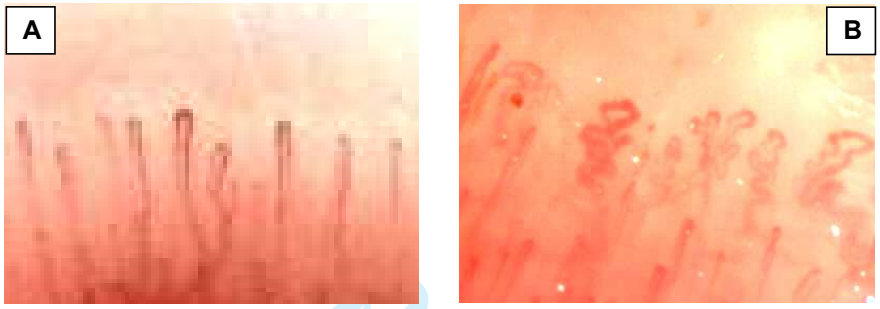


Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).



BMJ open

The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-002785.R2
Article Type:	Research
Date Submitted by the Author:	23-Apr-2013
Complete List of Authors:	Lopez, Vincent; CHU G. Montpied, Chamoux, Alain; CHU G. Montpied, Tempier, Marion; CHU G. Montpied, Thiel, Helene; CHU G. Montpied, Ughetto, Sylvie; CHU G. Montpied, Trousselard, Marion; IRBA, Naughton, Geraldine; Australian Catholic University, School of Exercise Science Dutheil, Frederic; CHU G. Montpied,
Primary Subject Heading:	Occupational and environmental medicine
Secondary Subject Heading:	Public health
Keywords:	OCCUPATIONAL & INDUSTRIAL MEDICINE, CHEMICAL PATHOLOGY, PUBLIC HEALTH

The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

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Running Title: Capillaroscopy post-exposure to VCM

Words count for introduction methods results discussion conclusion: 2402

Number of tables: 1

Number of figures: 2

Words count for abstract: 262

ABSTRACT

Objectives: To assess residual long-term microcirculation abnormalities by capillaroscopy, 15 years after retiring from occupational exposure to vinyl chloride monomer (VCM).

Design: Cross-sectional study.

Setting: Allier, one of the major area of PVC production in France.

Participants: We screened 761 (97% males) retired workers exposed to chemical toxics. Exposure to chemical other than VCM excluded potential participants.

Primary and secondary outcomes measures: These participants underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities, as well as a survey to determine exposure time, direct or indirect contact, type of occupation, smoking status and time after exposure. A double blind analysis of capillaroscopic images was done. A control group was matched in age, sex, type of occupation.

Results: 179/761 retired workers were only exposed to VCM at their work, with 21 meeting the inclusion criteria and included. Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Retired workers previously exposed to VCM had significantly higher capillaroscopic modifications than 35 controls: enlarged capillaries (19% vs. 0%, p<.001), dystrophy (28.6% vs. 0%, p=.0012), and augmented length (33% vs. 0%, p<.001). Time exposure was linked (p<.0001) with enlarged capillaries (R^2=.63), dystrophy (R^2=.51), and capillary length (R^2=.36). They also had higher symptoms of Raynaud (19% vs. 0%, p=.007) without correlation with capillaroscopic modifications.

Conclusion: Although VCM exposure was already known to affect microcirculation, our study demonstrates residual long term abnormalities following an average of 15 years retirement, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure was not related to capillaroscopic modifications; its origin remains to be determined.

Keywords Exposition, Vinyl chloride monomer, Capillaroscopy, Raynaud

ARTICLE SUMMARY

Article focus

- Vinyl chloride monomer exposure induces microcirculation abnormalities, which can be diagnosed by capillaroscopy.
- Residual long-term abnormalities following retirement required investigation.

Key messages

- Our results demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time-related exposure response.
- Symptoms of Raynaud, although statistically associated with exposure, was not related to capillaroscopic modifications; its origin remains to be determined.

Strengths and limitations of this study

- The strong points is that this study had a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factor and the focus on retired workers at least 15 years after the end of occupational VCM exposure.
- The main limitation is that pathophysiology of Raynaud after VCM exposure remains unclear.

INTRODUCTION

Vinyl chloride monomer (VCM) is primarily used in the manufacture of plastics and also serves as a raw material in organic synthesis. VCM is an aliphatic hydrocarbon also known as chloroethene. Its polymerization lead to a synthetic resin called polyvinyl chloride, commonly abbreviated PVC. PVC, is the third-most widely produced plastic, after polyethylene and polypropylene.[1] PVC can be made softer and more flexible by the addition of phthalates, and may also replace rubber. Thus, PVC is widely used including: pipes and water distribution, a substitute for painted wood (e.g. window frames, sills, flooring), electrical cable insulation, inflatable products, waterproof clothing (e.g. coats, skiing equipment, shoes), healthcare products (e.g. containers, tubing, catheters), food packaging, dental appliances and vinyl records.[1]

Harmless in its polymeric form, workers handling the finished PVC product are perfectly safe. In contrast, the at-risk phase lies in the manual descaling of autoclaves used for the polymerization where workers can contact it during its monomer state.[2] The chronic intoxication by gaseous monomer VCM is linked to several symptoms such as:[3] asthenia and dizziness,[3] Raynaud's syndrome,[4 5] digestive ulcera with nausea and anorexia,[3] systemic symptoms of arthralgia and myalgia[3], trophic cutaneous symptoms and sclerosis. It has also been suspected in the onset of acroosteolysis[4 6 7] and hepatocellular carcinoma.[8 9] More generally, VCM exposure involves chromosomal aberrations and increased carcinogenic risk.[10] Even if VCM related diseases may have a genetic bases, they are also linked to prolonged occupational VCM exposure.[5 11]

Scleroderma-like microvascular abnormalities have been also described on exposed workers.[12-14] The most common and non-invasive means of investigating these abnormalities is capillaroscopy. Widespread identification of individuals most at risk could enable early detection and management strategy.[15] The residual effects of VCM on microcirculation have

been shown only once on 15 workers who had ceased their VCM exposure six months prior to testing.[13] However, residual long-term abnormalities following retirement are unknown. Our hypothesis was higher capillaroscopic abnormalities in the VCM exposed group than in the control group.

Therefore, the aim of our cross- sectional study was to investigate residual long-term capillaroscopic abnormalities following retirement, after 15 years without VCM exposure.

METHODS

Participants

We enrolled male retired workers exposed to VCM in PVC production. They provided written informed consent. The study was approved by the human ethics committees from Clermont-Ferrand university hospital, France. To be eligible, participants had to be: male (due to male dominance in this workforce), retired, aged over 60 years, with at least a 5-year occupational exposure to VCM, a time after VCM exposure of at least 5 years, and no exposure to chemicals other than VCM. Moreover, participants with diabetes mellitus were also excluded as it may interfere with microcirculation,[16-21] as well as individuals declaring the use or previous use of treatments which may alter microcirculation.

Participants responded to a survey to determine exposure time, direct or indirect contact, type of occupation, time after exposure and smoking history. They underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities such as pathologies that may interfere with microcirculation (arterial hypertension, dyslipidemia) or pathologies potentially linked with VCM exposure (cardiovascular or respiratory diseases).[22]

A control group was matched in age, sex, type of occupation. They were recruited via advertisements. Selection criteria for this group also included no occupational or leisure chemical exposure, and no diabetes mellitus.[16-21]

Capillaroscopy

A nailfold capillaroscopy was performed on all fingers of each patient, excluding thumbs.[23] The nailfold capillaroscopies of the fingers were captured in images and electronically stored. The same investigator conducted all the capillaroscopies. A double blind analysis of capillaroscopic images was completed on deidentified data.

The outcomes were the five following classical criteria used in capillaroscopy: density, length, diameter, dystrophy and hemorrhage. Criteria for abnormalities were defined as: decreased capillary density <10/mm (avascular zone <7/mm),[24] augmented capillary length >300µm,[24 25] increased capillary diameter >25µm[25] (megacapillary >50µm)[26 27], and dystrophy was associated with capillary branching >15%.[28] Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall.[27]

Statistics

Data are presented as mean percentage change and standard deviation (SD).

The main judgment criterion for abnormal microcirculation was the presence of at least one abnormality in capillaroscopy. Under the assumption of similar proportions of abnormalities as that reported during a VCM exposure,[12-14] our sample calculation indicated that we would need 27 participants in each of the exposed and non-exposed groups to find a change in probability of 35% (i.e., 40% in exposed versus 5% in non-exposed) for a power of 80% and a two-sided alpha of 5%. When we considered an exposed to non-exposed sample ratio of 1:2, 19 and 38 participants, respectively were needed in the exposed and non-exposed groups.

Under the assumption of 0% prevalence in the non-exposed group, sample sizes of 13 exposed and 26 non-exposed were required.

Statistical analyses were performed with SPSS software, v19. Correlations were used for inter-observer reliability. The Gaussian distribution for each parameter was assessed by a Shapiro-Wilk test. Comparisons between groups (exposed vs. control) were made through the usual tests: Chi2 test for categorical variables (or Fisher's exact test where appropriate) and Student's t test for quantitative variables (or Kruskal-Wallis if assumptions of normal distribution were violated). Significance was accepted for a p-value < 5%. The links between continuous variables were analyzed using linear regression. The links between binary and continuous variables were analyzed with logistic regression (Nagelkerke R Square). Multivariate models were used to predict the relationship between capillary parameters and other parameters such as exposure time and time after exposure.

RESULTS

Participants

We screened 761 (97% males) retired workers exposed to chemical toxics from two leading enterprises involved in PVC production (n=435 and n=91), as well as participants from many subcontracting companies (n=235), also known for VCM exposure. The strict selection criteria of exposure only to VCM reduced the sample size to 21 (figure 1).

Thirty five age-matched controls were also recruited without occupational or leisure time exposure to chemical toxics.

Main capillaroscopic outcomes

There was no missing data. Inter-rater reliability was confirmed with correlations exceeding 0.70 for each parameter. The mean of the values of the 2 investigators is presented in table 1. Concerning the qualitative data, when a disagreement occurred, the two observers analyzed again together and requested the opinion of a third expert. The disagreement occurred only for 2 decreased capillary density <10/mm, and 1 capillary branching >15%.

Compared with controls, retired workers previously exposed to VCM had higher capillaroscopic abnormalities: enlarged capillaries (0% vs. 19%, p<.001), dystrophy (0% vs. 28.6%, p=.0012) (figure 2), and augmented length (0% vs. 33%, p<.001). The mean length was 15% higher in the exposed group than in controls (p=.020), as well as a 12% greater diameter of capillaries (p=.006) (table 1).

Exposure

Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Time of exposure to VCM was strongly linked with enlarged capillaries (Nagelkerke R Square approximation of 63%, p<.0001), with dystrophy (Nagelkerke R Square approximation of 51%, p<.0001), and modestly linked with capillary length expressed as binary data (Nagelkerke R Square approximation of 36%, p<.0001) or as quantitative data (R^2 = 8%; p=.031) (table 1). Age was not associated with capillaroscopic abnormalities. No multivariate models improved results from simple regressions.

Symptoms of Raynaud

VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).

Comorbidities and smoking

Neither respiratory nor cardiovascular diseases were associated with VCM exposure. However, we combined both groups to explore potential associations between capillaroscopic parameters and high blood pressure, dyslipidemia, and smoking. Capillary length in participants medicated for arterial hypertension (n=20/61) did not differ from participants without hypertension. Participants treated with lipid lowering drugs with dyslipidemia (n=10/61) also showed a trend for a higher capillary length than participants without dyslipidemia (p=.079). Finally, there were no capillaroscopic difference between smokers and non smokers. However, smokers who had been exposed to VCM tended to have a higher capillary length than non smokers (p=.073).

DISCUSSION

Principal findings of the study

Changes in microcirculation persist for at least 15 years following occupational VCM exposure, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure, was not related to pathological capillaroscopic changes.

What the study adds

The microcirculation changes following VCM exposure has been previously shown on 15 workers who ceased their occupational exposure six months prior to testing.[13] Our study supports these results over a longer period following VCM exposure – 15 years.

The dose responsiveness of VCM exposure and compromised capillarisation is generally,[13] but not always,[12] reported. Although daily VCM doses may have been more informative, the

current study was restricted to years of exposure. Thus, we are limited to describing associations with an exposure time – response rather than a dose-response. Years of exposure is an easy question for physicians to ask of workers during risk assessment protocols.

The absence of changes in microcirculation on less exposed workers[29] resulted in a suggestion that a threshold of exposure exists. This finding is supported by previous studies showing that long-term exposure (>8 years) induced greater chromosomal aberrations.[10 30] Further, not all workers exposed to VCM develop microvascular abnormalities, suggestive of an underlying genetic susceptibility (polymorphism of glutathione S-transferase).[5 11] A finding that female VCM-exposed workers were more susceptible than males to the risk of increased chromosome damage also reinforced genetic susceptibility theory.[30]

Comparison with other studies

After 50 years of age, minor dystrophies could alter readability and interpretation of capillaroscopic analyses.[31] Nevertheless, we controlled this parameter by matching the exposed group and the controls on age and we conducted a double-blind analysis by two experienced readers. Moreover, capillaroscopic abnormalities among workers exposed to VCM in the present study were not influenced by age.[13]

VCM has been suspected of causing respiratory and circulatory diseases.[32] However, similar frequencies of these diseases in both groups do not support this hypothesis.

Diabetes mellitus,[16-21] high blood pressure,[18 33-35] dyslipidemia,[34] or some comorbidities/medications[22] could interfere with microcirculation. In line with previous studies, we showed a diminished capillary length in participants treated for arterial hypertension,[18 33-35] and a trend for participants treated with lipid lowering drugs.[34] Diabetes mellitus was an exclusion criterion and thus, could not interfere with our results. Perhaps due to low numbers of participants, our results failed to support previous findings of compromised

microcirculation in people with high blood pressure. The trend for increased capillary length observed in our participants with dyslipidemia could be a response to increased peripheral vascular resistance, in order to maintain their function of metabolic exchange.[35] Similarly, smoking could induce a decrease in tissue perfusion,[36] and dystrophia.[37] We did not observe differences between smokers and non-smokers, with the exception of a trend for abnormal microcirculation among smokers exposed to VCM. The potential of a synergistic effect of tobacco and VCM-exposure warrants further investigations. It should be noted that VCM exposure in the current study was more strongly associated with compromised microcirculation than high blood pressure, dyslipidemia and smoking.

Previous research into the links between systemic sclerosis and VCM exposure is limited by single case design[38] and somewhat dated analyses of a population exposed to solvents.[39] The broader use of term such as solvents is less specific than the VCM exposure carefully isolated for investigation in the present study.

A higher prevalence of symptoms of Raynaud has been established in workers with VCM exposure,[32] up to one third of the exposed workers.[13] The comparison between studies with different selection criteria and different sample sizes is difficult. There is also the possibility of selection bias in non randomized recruitment. Within these limitations, our results support previous data, and extend knowledge by demonstrating the prevalence of symptoms of Raynaud remained higher at least 15 years following VCM exposure. Furthermore, in the current study, all the participants who suffered from symptoms of Raynaud had never taken medications or suffered from other diseases conducive to Raynaud.

Unanswered questions

Although symptoms of Raynaud are statistically associated with VCM exposure, we could not report a link with capillaroscopic modifications. The pathophysiology of Raynaud's

phenomenon remains unknown. There seems to be primary or secondary vascular failure influenced by a hereditary factor.[40] Decreased perfusion pressure could be secondary to systemic hypotension or be caused by proximal arterial occlusion, influenced by many factors; both vascular and intra vascular, neural, environmental or hereditary.[41] Angiography of the hands of patients exposed to VCM showed occlusions, stenosis and narrowing of distal arteries with the development of collateral circulation.[42] Lack of statistical power in our study could contribute to the lack of relationship between capillaroscopic changes and symptoms of Raynaud.

Strengths and limitations of study

This study presents some major strengths: a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factors, well-matched controls, a double blind analyses, sufficient number of participants to detect the capillaroscopic differences between groups, the focus on retired workers at least 15 years after the end of occupational VCM exposure. The attendance rate of 30% (53 of 179 individuals exclusively exposed to VCM at work agreed to participate in our study) seems very high compared with other studies[43-47] taking into account their age (75 years), distance from the location of the medical examination (averaging approximately 80 km), and that no financial compensation was offered.

There are limitations to this study. The cross-sectional design has limitations; however, proof of concept was important and achieved (with a possibility of longitudinal follow-up). Differences in some but not all capillary outcomes may be explained by a lack of statistical power. The results are insufficient to propose guidelines for all workers exposed to VCM. More accurate quantifiable measures of VCM exposure are not available; however, for the purpose of this study we used industry established lists of exposure legally required in France. The pathophysiology of Raynaud after VCM exposure remains unclear. A further limitation

may lie in the fact that most of the retired workers exposed to VCM were from the same enterprise; thus, potentially more at-risk manufacturing processes remained undetected. Gender specificity may warrant future studies.

CONCLUSION

Although VCM exposure was already known to affect microcirculation, our study demonstrated the potential for residual long-term abnormalities following an average of 15 years retirement, with a time exposure – response. Symptoms of Raynaud, although statistically associated with exposure were not associated with capillaroscopic modifications; its origin remains to be determined. Future research could focus on other chemical products which have a similar structure than VCM and more extensive research on type of occupations at-risk of VCM exposure.

Role of the Funding Source

The Occupational Medicine department of CHU G. Montpied, Clermont-Ferrand, France funded this study. No other funding source had a role in the design, conduct, or reporting of the study.

Contributors

VL has participated as a MD student and principal investigator. FD and AC obtained research funding and generated the intellectual development of the study. FD knew potential exposed workers to VCM. VL, FD, AC and SH contributed to the conception of the protocol. VL, FD and SH made data analysis. VL, FD, MT contributed to manuscript drafting. VL recruited all

participants and performed all capillaroscopies. VL and MT completed the double blind analyses of capillaroscopy. FD, AC, VL, GN and MT revised the manuscript. All authors read and approved the final manuscript.

Competing Interests

None

Data sharing

No additional data available.

Acknowledgments

Our thanks to Dr George Mnatzaganian for help with statistics (Faculty of Health Sciences, Australian Catholic University, Fitzroy, Victoria, Australia, email: George.Mnatzaganian@acu.edu.au). We also want to acknowledge the "Association of the sick from chemicals" (Association des Malades de la Chimie), 15 Av Albert Poncet 03410 Domerat, France, which helped us recruit the retired workers from VCM exposure.

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Table 1 Characteristics of participants, exposure to VCM and capillaroscopic outcomes.

	Retired from exposure to VCM (n=21)	Controls (n=40)	p-value
Age – years	74.4±2.9	76.3±3.2	NS
type of **occupation**: **blue collar** workers – "manual"	100%	100%	NS
Exposure to VCM:			
direct contact with VCM	100%	0	**<.001**
exposure time – years	29.8±1.9	0	**<.001**
time after exposure – years	15.9±2.4	-	-
Main capillaroscopic outcomes:			
1. **density**			
mean density – mm	8.6±0.4	8.8±0.3	NS
decreased capillary density <10/mm – n (%)	9 (43)	16 (46)	NS
avascular zone <7/mm – n(%)	0	0	NS
2. **length**			
mean length – µm	291±14	254±9	**.020**
augmented capillary length >300µm – n(%)	7 (33)	0	**<.001**
3. **diameter**			
mean diameter of capillaries – µm	28.9±0.9	25.7±0.6	**.006**
enlarged capillaries >25µm – n(%)	4 (19)	0	**.007**
megacapillary >50µm – n(%)	0	0	NS
4. **dystrophy**			
capillary branching >15% – n(%)	6 (29)	0	**<.001**
5. **hemorrhage** – n(%)	0	0	NS
Symptoms of Raynaud:			
n(%) with Raynaud	4 (19)	0	**.007**
Participants with medications which could induce Raynaud – n(%)	2 (9)	4 (11)	NS
Other causes of Raynaud	0	0	NS
Comorbidities			
Respiratory diseases	2 (9)	6 (17)	NS
Cardiovascular diseases (except high blood pressure)	4 (19)	5 (14)	NS
Myocardial infraction	3 (14)	3(9)	NS
Routine medications – n(%) of patients treated for:			
Blood pressure	7 (33)	13 (37)	NS
Lipid lowering	4 (19)	6 (17)	NS
Smoking – n(%)	9 (43)	18 (51)	NS

Figure legends

Figure 1 Participant flow chart.

Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).

The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement

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Running Title: Capillaroscopy post-exposure to VCM

Words count for introduction methods results discussion conclusion: 2402~~298~~

Number of tables: 1

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ABSTRACT

Objectives: To assess residual long-term microcirculation abnormalities by capillaroscopy, 15 years after retiring from occupational exposure to vinyl chloride monomer (VCM).

Design: Cross-sectional study.

Setting: Allier, one of the major area of PVC production in France.

Participants: We screened 761 (97% males) retired workers exposed to chemical toxics. Exposure to chemical other than VCM excluded potential participants.

Primary and secondary outcomes measures: These participants underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities, as well as a survey to determine exposure time, direct or indirect contact, type of occupation, smoking status and time after exposure. A double blind analysis of capillaroscopic images was done. A control group was matched in age, sex, type of occupation.

Results: 179/761 retired workers were only exposed to VCM at their work, with 21 meeting the inclusion criteria and included. Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Retired workers previously exposed to VCM had significantly higher capillaroscopic modifications than 35 controls: enlarged capillaries (19% vs. 0%, p<.001), dystrophy (28.6% vs. 0%, p=.0012), and augmented length (33% vs. 0%, p<.001). Time exposure was linked (p<.0001) with enlarged capillaries (R^2=.~~R^2 adjusted =~~ 63~~%~~), dystrophy (R^2=.51), and capillary length (R^2=.36). ~~, p<.0001).~~ They also had higher symptoms of Raynaud (19% vs. 0%, p=.007) without correlation with capillaroscopic modifications.

Conclusion: Although VCM exposure was already known to affect microcirculation, our study demonstrates residual long term abnormalities following an average of 15 years retirement, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure was not related to capillaroscopic modifications; its origin remains to be determined.

Keywords Exposition, Vinyl chloride monomer, Capillaroscopy, Raynaud

ARTICLE SUMMARY

Article focus

- Vinyl chloride monomer exposure induces microcirculation abnormalities, which can be diagnosed by capillaroscopy.
- Residual long-term abnormalities following retirement required investigation.

Key messages

- Our results demonstrated residual long-term abnormalities following an average of 15 years retirement, with a time-related exposure response.
- Symptoms of Raynaud, although statistically associated with exposure, was not related to capillaroscopic modifications; its origin remains to be determined.

Strengths and limitations of this study

- The strong points is that this study had a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factor and the focus on retired workers at least 15 years after the end of occupational VCM exposure.
- The main limitation is that pathophysiology of Raynaud after VCM exposure remains unclear.

INTRODUCTION

Vinyl chloride monomer (VCM) is primarily used in the manufacture of plastics and also serves as a raw material in organic synthesis. VCM is an aliphatic hydrocarbon also known as chloroethene. Its polymerization lead to a synthetic resin called polyvinyl chloride, commonly abbreviated PVC. PVC, is the third-most widely produced plastic, after polyethylene and polypropylene.[1] PVC can be made softer and more flexible by the addition of phthalates, and may also replace rubber. Thus, PVC is widely used including: pipes and water distribution, a substitute for painted wood (e.g. window frames, sills, flooring), electrical cable insulation, inflatable products, waterproof clothing (e.g. coats, skiing equipment, shoes), healthcare products (e.g. containers, tubing, catheters), food packaging, dental appliances and vinyl records.[1]

Harmless in its polymeric form, workers handling the finished PVC product are perfectly safe. In contrast, the at-risk phase lies in the manual descaling of autoclaves used for the polymerization where workers can contact it during its monomer state.[2] The chronic intoxication by gaseous monomer VCM is linked to several symptoms such as:[3] asthenia and dizziness,[3] Raynaud's syndrome,[4 5] digestive ulcera with nausea and anorexia,[3] systemic symptoms of arthralgia and myalgia[3], trophic cutaneous symptoms and sclerosis. It has also been suspected in the onset of acroosteolysis[4 6 7] and hepatocellular carcinoma.[8 9] More generally, VCM exposure involves chromosomal aberrations and increased carcinogenic risk.[10] Even if VCM related diseases may have a genetic bases, they are also linked to prolonged occupational VCM exposure.[5 11]

Scleroderma-like microvascular abnormalities have been also described on exposed workers.[12-14] The most common and non-invasive means of investigating these abnormalities is capillaroscopy. Widespread identification of individuals most at risk could enable early detection and management strategy.[15] The residual effects of VCM on microcirculation have

been shown only once on 15 workers who had ceased their VCM exposure six months prior to testing.[13] However, residual long-term abnormalities following retirement are unknown. Our hypothesis was higher capillaroscopic abnormalities in the VCM exposed group than in the control group.

Therefore, the aim of our cross- sectional study was to investigate residual long-term capillaroscopic abnormalities following retirement, after 15 years without VCM exposure.

METHODS

Participants

We enrolled male retired workers exposed to VCM in PVC production. They provided written informed consent. The study was approved by the human ethics committees from Clermont-Ferrand university hospital, France. To be eligible, participants had to be: male (due to male dominance in this workforce), retired, aged over 60 years, with at least a 5-year occupational exposure to VCM, a time after VCM exposure of at least 5 years, and no exposure to chemicals other than VCM. Moreover, participants with diabetes mellitus were also excluded as it may interfere with microcirculation,[16-21] as well as individuals declaring the use or previous use of treatments which may alter microcirculation.

Participants responded to a survey to determine exposure time, direct or indirect contact, type of occupation, time after exposure and smoking history. They underwent a medical examination including a capillaroscopy, symptoms of Raynaud, and comorbidities such as pathologies that may interfere with microcirculation (arterial hypertension, dyslipidemia) or pathologies potentially linked with VCM exposure (cardiovascular or respiratory diseases).[22]

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A control group was matched in age, sex, type of occupation. They were recruited via advertisements. Selection criteria for this group also included no occupational or leisure chemical exposure, and no diabetes mellitus.[16-21]

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Capillaroscopy

A nailfold capillaroscopy was performed on all fingers of each patient, excluding thumbs.[23] The nailfold capillaroscopies of the fingers were captured in images and electronically stored. The same investigator conducted all the capillaroscopies. A double blind analysis of capillaroscopic images was completed on deidentified data.

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The outcomes were the five following classical criteria used in capillaroscopy: density, length, diameter, dystrophy and hemorrhage. Criteria for abnormalities were defined as: decreased capillary density <10/mm (avascular zone <7/mm),[24] augmented capillary length >300µm,[24 25] increased capillary diameter >25µm[25] (megacapillary >50µm)[26 27], and dystrophy was associated with capillary branching >15%.[28] Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall.[27]

Statistics

Data are presented as mean percentage change and standard deviation (SD).

The main judgment criterion for abnormal microcirculation was the presence of at least one abnormality in capillaroscopy. ~~The most common abnormality is capillary dystrophy.[25 12-14]~~ Under the assumption of similar proportions of abnormalities as that reported during a VCM exposure,[12-14] our sample calculation indicated that we would need 27 participants in each of the exposed and non-exposed groups to find a change in probability of 35% (i.e., 40% in exposed versus 5% in non-exposed) for a power of 80% and a two-sided alpha of 5%. When we considered an exposed to non-exposed sample ratio of 1:2, 19 and 38 participants,

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respectively were needed in the exposed and non-exposed groups. Under the assumption of 0% prevalence in the non-exposed group, sample sizes of 13 exposed and 26 non-exposed were required.

~~Previous data[2–25] and personal observation on residual long term abnormalities following VCM exposure showed that a percentage of dystrophy of approximately 25% was required to differentiate between the exposed group and the controls. Using this value as the main outcome, we calculated that a sample of 10 participants per group allows a statistical power greater than 80% with an alpha level less than 5%.~~

Statistical analyses were performed with SPSS software, v19. Correlations were used for inter-observer reliability. The Gaussian distribution for each parameter was assessed by a Shapiro-Wilk test. Comparisons between groups (exposed vs. control) were made through the usual tests: Chi2 test for categorical variables (or Fisher's exact test where appropriate) and Student's t test for quantitative variables (or Kruskal-Wallis if assumptions of normal distribution were violated). Significance was accepted for a p-value < 5%. The links between continuous variables were analyzed using linear regression. The links between binary and continuous variables were analyzed with logistic regression (Nagelkerke R Square). Multivariate models were used to predict the relationship between capillary parameters and other parameters such as exposure time and time after exposure.

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RESULTS

Participants

We screened 761 (97% males) retired workers exposed to chemical toxics from two leading enterprises involved in PVC production (n=435 and n=91), as well as participants from many

subcontracting companies (n=235), also known for VCM exposure. The strict selection criteria of exposure only to VCM reduced the sample size to 21 (figure 1).

Thirty five age-matched controls were also recruited without occupational or leisure time exposure to chemical toxics.

Main capillaroscopic outcomes

There was no missing data. Inter-rater reliability was confirmed with correlations exceeding 0.70 for each parameter. The mean of the values of the 2 investigators is presented in table 1. Concerning the qualitative data, when a disagreement occurred, the two observers analyzed again together and requested the opinion of a third expert. The disagreement occurred only for 2 decreased capillary density <10/mm, and 1 capillary branching >15%.

Compared with controls, retired workers previously exposed to VCM had higher capillaroscopic abnormalities: enlarged capillaries (0% vs. 19%, p<.001), dystrophy (0% vs. 28.6%, p=.0012) (figure 2), and augmented length (0% vs. 33%, p<.001). The mean length was 15% higher in the exposed group than in controls (p=.020), as well as a 12% greater diameter of capillaries (p=.006) (table 1).

Exposure

Exposure time was 29.8±1.9 years and time after exposure was 15.9±2.4 years. Time of exposure to VCM was strongly linked with enlarged capillaries (Nagelkerke R Square approximation of ~~R^2 adjusted =~~ 63%, p<.0001), ~~and~~ with dystrophy (Nagelkerke R Square approximation of 51%, p<.0001), and modestly linked with capillary length expressed as binary ~~(R^2 = 8%; p=.031)~~ data (Nagelkerke R Square approximation of 36%, p<.0001) or as quantitative data (R^2 = 8%; p=.031) (table 1). Age was not associated with capillaroscopic abnormalities. No multivariate models improved results from simple regressions.

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Symptoms of Raynaud

VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).

Comorbidities and smoking

Neither respiratory nor cardiovascular diseases were associated with VCM exposure. However, we combined both groups to explore potential associations between capillaroscopic parameters and high blood pressure, dyslipidemia, and smoking. Capillary length in participants medicated for arterial hypertension (n=20/61) did not differ from participants without hypertension. Participants treated with lipid lowering drugs with dyslipidemia (n=10/61) also showed a trend for a higher capillary length than participants without dyslipidemia (p=.079). Finally, there were no capillaroscopic difference between smokers and non smokers. However, smokers who had been exposed to VCM tended to have a higher capillary length than non smokers (p=.073).

DISCUSSION

Principal findings of the study

Changes in microcirculation persist for at least 15 years following occupational VCM exposure, with a time-related exposure response. Symptoms of Raynaud, although statistically associated with exposure, was not related to pathological capillaroscopic changes.

What the study adds

The microcirculation changes following VCM exposure has been previously shown on 15 workers who ceased their occupational exposure six months prior to testing.[13] Our study supports these results over a longer period following VCM exposure – 15 years.

The dose responsiveness of VCM exposure and compromised capillarisation is generally,[13] but not always,[12] reported. Although daily VCM doses may have been more informative, the current study was restricted to years of exposure. Thus, we are limited to describing associations with an exposure time – response rather than a dose-response. Years of exposure is an easy question for physicians to ask of workers during risk assessment protocols.

The absence of changes in microcirculation on less exposed workers[29] resulted in a suggestion that a threshold of exposure exists. This finding is supported by previous studies showing that long-term exposure (>8 years) induced greater chromosomal aberrations.[10 30] Further, not all workers exposed to VCM develop microvascular abnormalities, suggestive of an underlying genetic susceptibility (polymorphism of glutathione S-transferase).[5 11] A finding that female VCM-exposed workers were more susceptible than males to the risk of increased chromosome damage also reinforced genetic susceptibility theory.[30]

Comparison with other studies

After 50 years of age, minor dystrophies could alter readability and interpretation of capillaroscopic analyses.[31] Nevertheless, we controlled this parameter by matching the exposed group and the controls on age and we conducted a double-blind analysis by two experienced readers. Moreover, capillaroscopic abnormalities among workers exposed to VCM in the present study were not influenced by age.[13]

VCM has been suspected of causing respiratory and circulatory diseases.[32] However, similar frequencies of these diseases in both groups do not support this hypothesis.

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Diabetes mellitus,[16-21] high blood pressure,[18 33-35] dyslipidemia,[34] or some comorbidities/medications[22] could interfere with microcirculation. In line with previous studies, we showed a diminished capillary length in participants treated for arterial hypertension,[18 33-35] and a trend for participants treated with lipid lowering drugs.[34] Diabetes mellitus was an exclusion criterion and thus, could not interfere with our results. Perhaps due to low numbers of participants, our results failed to support previous findings of compromised microcirculation in people with high blood pressure. The trend for increased capillary length observed in our participants with dyslipidemia could be a response to increased peripheral vascular resistance, in order to maintain their function of metabolic exchange.[35] Similarly, smoking could induce a decrease in tissue perfusion,[36] and dystrophia.[37] We did not observe differences between smokers and non-smokers, with the exception of a trend for abnormal microcirculation among smokers exposed to VCM. The potential of a synergistic effect of tobacco and VCM-exposure warrants further investigations. It should be noted that VCM exposure in the current study was more strongly associated with compromised microcirculation than high blood pressure, dyslipidemia and smoking.

Previous research into the links between systemic sclerosis and VCM exposure is limited by single case design[38] and somewhat dated analyses of a population exposed to solvents.[39] The broader use of term such as solvents is less specific than the VCM exposure carefully isolated for investigation in the present study.

A higher prevalence of symptoms of Raynaud has been established in workers with VCM exposure,[32] up to one third of the exposed workers.[13] The comparison between studies with different selection criteria and different sample sizes is difficult. There is also the possibility of selection bias in non randomized recruitment. Within these limitations, our results support previous data, and extend knowledge by demonstrating the prevalence of symptoms of Raynaud remained higher at least 15 years following VCM exposure. Furthermore, in the

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current study, all the participants who suffered from symptoms of Raynaud had never taken medications or suffered from other diseases conducive to Raynaud.

Unanswered questions

Although symptoms of Raynaud are statistically associated with VCM exposure, we could not report a link with capillaroscopic modifications. The pathophysiology of Raynaud's phenomenon remains unknown. There seems to be primary or secondary vascular failure influenced by a hereditary factor.[40] Decreased perfusion pressure could be secondary to systemic hypotension or be caused by proximal arterial occlusion, influenced by many factors; both vascular and intra vascular, neural, environmental or hereditary.[41] Angiography of the hands of patients exposed to VCM showed occlusions, stenosis and narrowing of distal arteries with the development of collateral circulation.[42] Lack of statistical power in our study could contribute to the lack of relationship between capillaroscopic changes and symptoms of Raynaud.

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Strengths and limitations of study

This study presents some major strengths: a rigorous selection criteria of exclusively VCM exposed participants avoided confounding factors, well-matched controls, a double blind analyses, sufficient number of participants to detect the capillaroscopic differences between groups, the focus on retired workers at least 15 years after the end of occupational VCM exposure. The attendance rate of 30% (53 of 179 individuals exclusively exposed to VCM at work agreed to participate in our study) seems very high compared with other studies[43-47] taking into account their age (75 years), distance from the location of the medical examination (averaging approximately 80 km), and that no financial compensation was offered.

There are limitations to this study. The ~~; a~~ cross-sectional design has limitations; h~~h~~owever, proof of concept was important and achieved (with a possibility of longitudinal follow-up). D~~;~~ ~~d~~ifferences in some but not all capillary outcomes may be explained by a lack of statistical power. T~~; t~~he results are insufficient to propose guidelines for all workers exposed to VCM. M~~; m~~ore accurate quantifiable measures of VCM exposure are not available;~~;~~ however, for the purpose of this study we used industry established lists of exposure legally required in France. The ~~; we combined our knoledgeonly use a binary~~ pathophysiology of Raynaud after VCM exposure remains unclear. A further limitation may lie in the fact that m~~; m~~ost of the retired workers exposed to VCM were from the same enterprise; thus, ~~and~~ potentially more at-risk manufacturing processes remained undetected. Gender specificity may warrant future studies.

CONCLUSION

Although VCM exposure was already known to affect microcirculation, our study demonstrated the potential for residual long-term abnormalities following an average of 15 years retirement, with a time exposure – response. Symptoms of Raynaud, although statistically associated with exposure were not associated with capillaroscopic modifications; its origin remains to be determined. Future research could focus on other chemical products which have a similar structure than VCM and more extensive research on type of occupations at-risk of VCM exposure.

Role of the Funding Source

The Occupational Medicine department of CHU G. Montpied, Clermont-Ferrand, France funded this study. No other funding source had a role in the design, conduct, or reporting of the study.

Contributors

VL has participated as a MD student and principal investigator. FD and AC obtained research funding and generated the intellectual development of the study. FD knew potential exposed workers to VCM. VL, FD, AC and SH contributed to the conception of the protocol. VL, FD and SH made data analysis. VL, FD, MT contributed to manuscript drafting. VL recruited all participants and performed all capillaroscopies. VL and MT completed the double blind analyses of capillaroscopy. FD, AC, VL, GN and MT revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Our thanks to Dr George Mnatzaganian ~~Geraldine Naughton~~ for help with statistics~~manuscript English proof reading~~ (~~,~~ Faculty of Health Sciences, Australian Catholic University, Fitzroy, Victoria, Australia, email: George.Mnatzaganian@acu.edu.au). We also want to acknowledge the "Association of the sick from chemicals" (Association des Malades de la Chimie), 15 Av Albert Poncet 03410 Domerat, France, which helped us recruit the retired workers from VCM exposure.

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Table 1 Characteristics of participants, exposure to VCM and capillaroscopic outcomes.

	Retired from exposure to VCM (n=21)	Controls (n=40)	p-value
Age – years	74.4±2.9	76.3±3.2	NS
type of **occupation**: **blue collar** workers – "manual"	100%	100%	NS
Exposure to VCM:			
direct contact with VCM	100%	0	**<.001**
exposure time – years	29.8±1.9	0	**<.001**
time after exposure – years	15.9±2.4	-	-
Main capillaroscopic outcomes:			
1. **density**			
mean density – mm	8.6±0.4	8.8±0.3	NS
decreased capillary density <10/mm – n (%)	9 (43)	16 (46)	NS
avascular zone <7/mm – n(%)	0	0	NS
2. **length**			
mean length – µm	291±14	254±9	**.020**
augmented capillary length >300µm – n(%)	7 (33)	0	**<.001**
3. **diameter**			
mean diameter of capillaries – µm	28.9±0.9	25.7±0.6	**.006**
enlarged capillaries >25µm – n(%)	4 (19)	0	**<.007 1**
megacapillary >50µm – n(%)	0	0	NS
4. **dystrophy**			
capillary branching >15% – n(%)	6 (29)	0	**<.001**
5. **hemorrhage** – n(%)	0	0	NS
Symptoms of Raynaud:			
n(%) with Raynaud	4 (19)	0	**.007**
Participants with medications which could induce Raynaud – n(%)	2 (9)	4 (11)	NS
Other causes of Raynaud	0	0	NS
Comorbidities			
Respiratory diseases	2 (9)	6 (17)	NS
Cardiovascular diseases (except high blood pressure)	4 (19)	5 (14)	NS
Myocardial infraction	3 (14)	3(9)	NS
Routine medications – n(%) of patients treated for:			
Blood pressure	7 (33)	13 (37)	NS
Lipid lowering	4 (19)	6 (17)	NS
Smoking – n(%)	9 (43)	18 (51)	NS

Figure 1 Participant flow chart.

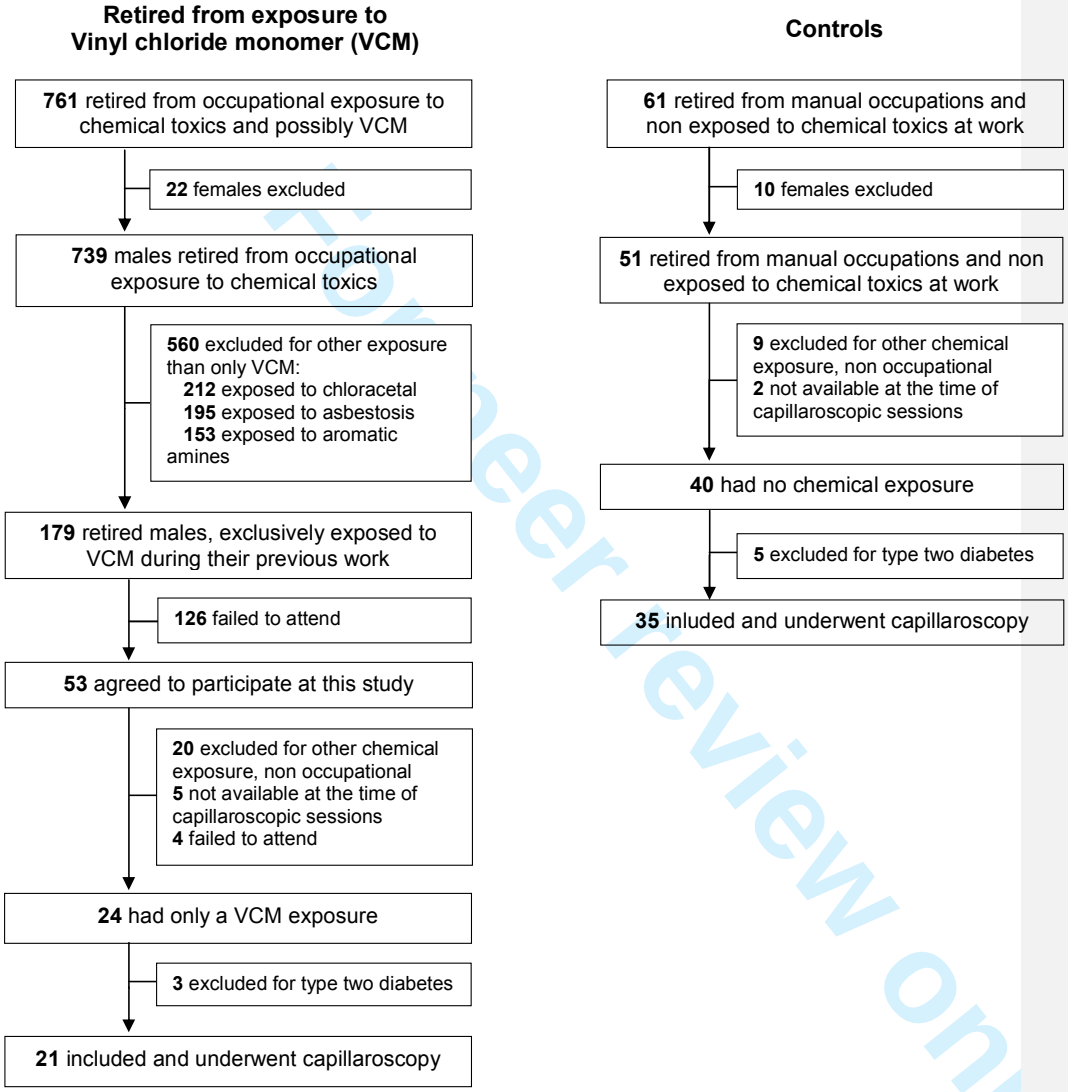
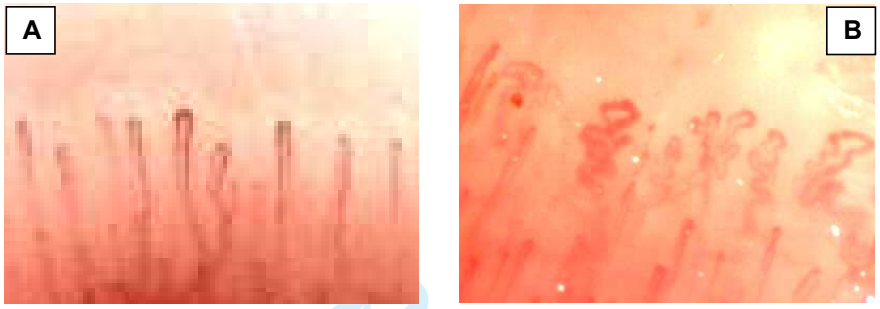


Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	19 (figure 1)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-9
		(*b*) Describe any methods used to examine subgroups and interactions	8-9
		(*c*) Explain how missing data were addressed	8-9
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8-9
		(*e*) Describe any sensitivity analyses	8-9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	19 (figure 1)
		(b) Give reasons for non-participation at each stage	19 (figure 1)
		(c) Consider use of a flow diagram	19 (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7;9; 19 (figure 1)
		(b) Indicate number of participants with missing data for each variable of interest	9
Outcome data	15*	Report numbers of outcome events or summary measures	9-10; 19 (figure 1)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10; 19 (figure 1)
		(*b*) Report category boundaries when continuous variables were categorized	9-10; 19 (figure 1)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*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.

Figure 1 Participant flow chart.

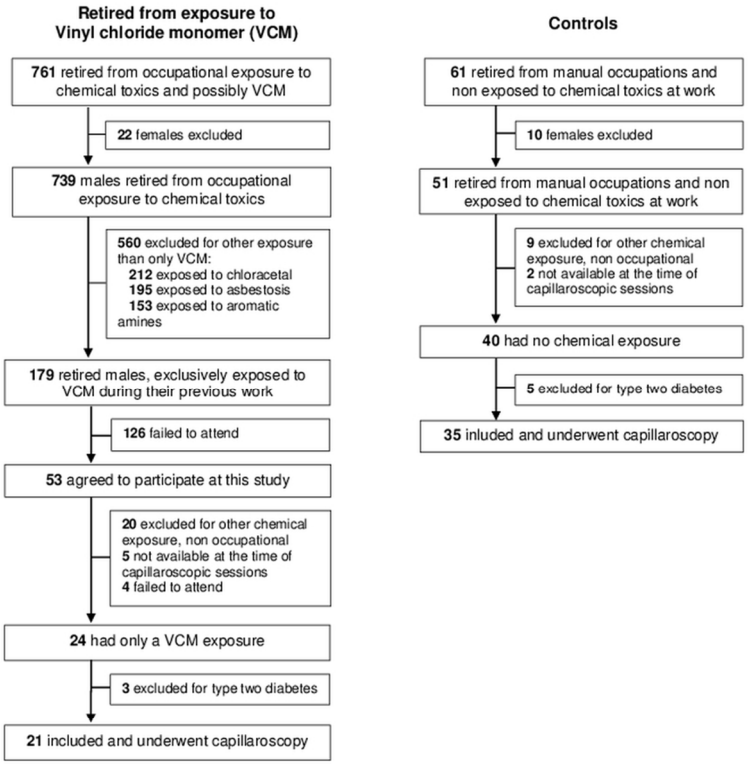


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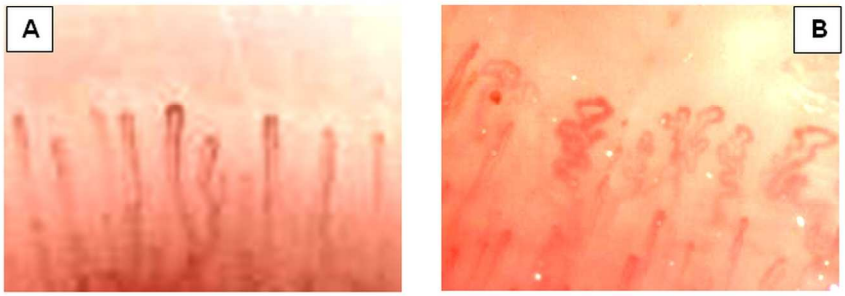
Figure 2 Normal capillaroscopy on a control participant (A) and capillaroscopy with dystrophia >15% in a retired worker exposed to VCM for 37 years, with no treatment and no comorbidity, non smoking (B).



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