PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The long-term effects of occupational exposure to vinyl chloride monomer on microcirculation: a cross-sectional study 15 years after retirement
AUTHORS	Dutheil, Frederic; Lopez, Vincent; Chamoux, Alain; Tempier, Marion; Thiel, Helene; Ughetto, Sylvie; Trousselard, Marion; Naughton, Geraldine

VERSION 1 - REVIEW

REVIEWER	Froom, Paul Sackler Medical School , Tel Aviv University, Epidemiology and preventive medicine
REVIEW RETURNED	03-Mar-2013

GENERAL COMMENTS	Introduction Page 6, lines 41-10.- the known risk from exposure to VCM is for hepatocellular and angiosarcoma of the liver, and other sites are suspected. Page 6, 55-56; - leave out, unlikely that widespread identification could enable early detection and treatment. What treatment? Page 7, line 3; there is long term evidence that after cessation of exposure Raynaud's phenomena persists. Do the authors believe that these workers don't have long-term abnormalities and that at least some of them if not most of them would have positive findings on capillaroscopy. Methods Capillaroscopy Did the single investigator who took the pictures know if the patients were subjects or controls? The selection of the pictures if not blinded could introduce an observer bias. This should at least be discussed. Were the criteria for abnormalities done pre-analysis? On what basis were they defined? In the cited article I could only find the definition of abnormal length as found in the table. I could not find the definition of capillary dystrophy in the sited article (23). What is a percentage of dystrophy of 25% mean; does this mean 25% of the study group or a difference of 25% between the two groups? The power analysis therefore is unclear.

	What multivariate models were used? Even though the authors used exposure time, there should be some attempt to describe the exposure levels, at least in general. RESULTS The participation rate of 53 of 179 is low, and there is no participation rate for the controls. From the figure it appears that all the controls agreed to participate. What does no statistical difference between the two investigators mean? Were there any misclassifications when grading the pictures? What was done with cases of disagreement? This needs to be described. The presentation of the numerical differences between groups should not be redundant with the those presented in the table. It is unclear how the authors found an r2 of 53% for enlarged capillaries and 8% for capillary length with exposure time. It appears that this was done using a linear regression model. Were both variables or at least one normally distributed? Were there any outliers that lead to these results? I would like to see a scatter plot before I accepted those findings. Fisher's exact test should be used for the comparison of Raynaud's phenomena. The co-morbidities and smoking should be left out since as expected there were no differences between the two groups (as expected, and very insensitive because of very small numbers and multiple comparisons). There are no differences in the proportions of hypertension, smoking, and lipid lowering medications and therefore further analysis should not be done (groups too small for subgroup analysis). Discussion The only conclusion is that microcirculation changes persisted in those previously exposed to VCM. The time-related exposure is questionable but might be a finding. I could not find the results showing that those with Raynaud's symptoms did not have pathological capillaroscopic changes. In any case even if the 4 subjects with Raynaud's did not have such changes, that finding is not worthy of a conclusion. The one study that found no correlation with the length of occupational exposure and capillary abnormalities was not quoted (Int Arch Occup Environ Health 1983;51:337-340). What the study adds Their suggestion that a threshold exists is unfounded and not supported by the literature. Their comment that since not all develop microvascular abnormalities is suggestive of an underlying genetic susceptibility doesn't add anything since this could be said about

	nearly any vascular disease. The same can be said of the differences between male and female. Comparison with other studies I don't understand the sentence , page 12, line 12- "After 50 years? The discussion on respiratory and circulatory diseases is superficial. There are many follow-up cohort studies that overall do not support an association between exposure to VCM and diseases of the circulatory system. Their findings don't add anything to the literature on this question. This section should focus on the previous capillary studies done on subjects with SS and VCM exposures. Similar or different? What findings? What criteria? They certainly did not show that VCM was more strongly associated with compromised miocrocirculation than high blood pressure, dyslipidemia and smoking, because they did not find an association with those findings. Here they might add how their prevalence of Raynaud's differs from other studies and whether or not this is due to selection bias or exposure to VCM. I don't understand what the authors meant by the last sentence in the unanswered questions. Do they claim that the association with capillaroscopic changes and symptoms of Raynaud's in general or just for VCM exposure is unclear, based on their 4 cases (didn't see the data in the results)? How does this compare to the study in JAMA 236:1368, 1976 and other studies? Strengths and limitations Certainly the 30% attendance rate is not a strength but a weakness and possible directional bias should be discussed. The paragraph on limitations is unclear and poorly written.

REVIEWER	Matthew Cave, MD Assistant Professor Department of Medicine Department of Pharmacology and Toxicology University of Louisville Louisville, KY USA
REVIEW RETURNED	05-Mar-2013

THE STUDY	The manuscript is written by French authors and would benefit from a medical editor for assistance with English language.
GENERAL COMMENTS	This manuscript documents micro-circulatory abnormalities in vinyl chloride workers 15 years after retirement. The research study is well-done, and I have no major scientific concerns. The manuscript would, however, benefit from further editing for English language. If known, more details about the vinyl chloride exposures would be helpful (e.g. PPM-Years). Also I would be careful about concluding that the micro-circulatory abnormalities persist after retirement because these abnormalities were not documented in these specific workers prior to retirement.

REVIEWER	Enrico Davoli Environmental Health Sciences Dept. Istituto Mario Negri Milano I have no competing interests.
REVIEW RETURNED	12-Mar-2013

THE STUDY	The selection criteria is very strict. Only 3%, approximately, retired working from PVC production, exposed to VCM, have been enrolled. It is not clear why women have been excluded, first (page 12, females are more susceptible...). Also "other" exposures have been considered as an exclusion factor, even if no microcirculation specific effects are reported. It seems a selection too much stringent, and a bias, due to this fact, is possible, influencing results. The authors should discuss about this possible problem.
GENERAL COMMENTS	Page 9. Participants. We enrolled 761 male retired … Is not consistent with fig. 1 (761 retired from occupational … 22 females excluded) Page 10. Correlation between time of exposure and capillaries enlargements, being so strongly correlated, could be discussed in more details. Table 1 I am not familiar with capillaroscopic outcomes. I was wondering if parameters like 300 microns (length) 30, 50 microns diameter are standard or arbitrary for this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Paul Froom
Sackler Medical School , Tel Aviv University, Epidemiology and preventive medicine

Introduction
Page 6, lines 41-10.- the known risk from exposure to VCM is for hepatocellular and angiosarcoma of the liver, and other sites are suspected.
We have respectfully checked the references supported in this statement and we are confident that our wording is correct. In particular, even if it is taught that hepatocellular and angiosarcoma of the liver are related to VCM exposure, the evidence base behind this association is relatively weak:
- For example the latest review on hepatocellular carcinoma conclude with: “Thus, the role of inhalation exposure to VC in HCC risk remains unclear, awaiting further studies and the integration of results from epidemiological studies and animal models.” Dragani & Zocchetti. Occupational exposure to vinyl chloride and risk of hepatocellular carcinoma. Cancer Causes Control 2008;19(10):1193-1200.
- “The hypothesis that vinyl chloride causes or contributes to the development of hepatocellular carcinoma remains unproven.” Sherman M. Vinyl chloride and the liver. J Hepatol. 2009 Dec;51(6):1074-81. doi: 10.1016/j.jhep.2009.09.012.

Page 6, 55-56; - leave out, unlikely that widespread identification could enable early detection and treatment. What treatment?
We agree that treatment is without consensus. We replaced the word “treatment” with “management strategy”. For example removing the workstation could be a strategy. Although the evidence based on treatment is weak, there are emerging presentations in the literature. Even if the following reference is not on capillaroscopic abnormalities, please see: Kowal-Bielecka O, Bielecki M, Kowal K. Recent advances in the diagnosis and treatment of systemic sclerosis. Pol Arch Med Wewn. 2013 Feb 18;123(1-2):51-8. We added this reference in the manuscript.

Page 7, line 3; there is long term evidence that after cessation of exposure Raynaud's phenomena persists. Do the authors believe that these workers don't have long-term abnormalities and that at least some of them if not most of them would have positive findings on capillaroscopy.
Because of these two conditions (Raynaud’s phenomenon and capillaroscopic abnormalities) could appear independently, it is difficult to respond to this statement without further investigations.

Methods
Capillaroscopy
Did the single investigator who took the pictures know if the patients were subjects or controls? The selection of the pictures if not blinded could introduce an observer bias. This should at least be discussed.
Yes, a single investigator took the pictures from both controls and exposed workers. But the pictures were automatically recorded anonymously without the possibility of identifying the participant as either a healthy control or an exposed worker.

Were the criteria for abnormalities done pre-analysis? On what basis were they defined? In the cited article I could only find the definition of abnormal length as found in the table. I could not find the definition of capillary dystrophy in the sited article (23).
The criteria for abnormalities were defined before the analysis and are also presented in the protocol submitted to our university hospital ethics committee. These criteria were those usually used by our clinical expert in capillaroscopy. We did not originally extensively reference this section but have subsequently done so.

As you say, the definition of an augmented capillary length is cited in the article: Kabasakal Y, Elvins DM, Ring EF, McHugh NJ. Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls. Ann Rheum Dis 1996;55:507-12. We have also added the following reference using the same abnormal cut-off >300 µm of capillary length: Ingegnoli F, Gualtierotti R, Lubatti C, Zahalkova L, Meani L, Boracchi P, Zeni S, Fantini F. Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations. Semin Arthritis Rheum. 2009 Feb;38(4):289-95. doi: 10.1016/j.semarthrit.
Concerning the definition of enlarged diameter, the same reference considers that a normal width is less than 25 µm (figure 2a from Kabasakal Y, Elvins DM, Ring EF, McHugh NJ. Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls. Ann Rheum Dis 1996;55:507-12). We had written 30 µm in our protocol because of the local clinical practice of our expert. However, there were the same number of individuals with capillaries >25 or >30 in our study. The replacement of “30” with “25” in our manuscript did not alter the number of participants with enlarged diameter, and is now more consistent with the literature.
Concerning the definition of megacapillary (or giant capillary) > 50 µm, we added in the text the following references: 1) Bhakuni DS, Vasdev V, Garg MK, Narayanan K, Jain R, Mullick G. Nailfold capillaroscopy by digital microscope in an Indian population with systemic sclerosis. Int J Rheum Dis. 2012 Feb;15(1):95-101. doi: 10.1111/j.1756-185X.2011.01699.x. Please see page 99 of their publication 2) Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008 Dec;22(6):1093-108. doi: 10.1016/j.berh.2008.09.001. Please see their figure 3.
Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall (Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008 Dec;22(6):1093-108. doi: 10.1016/j.berh.2008.09.001). Please see their figure 4 explaining the definition of hemorrhage. We added this definition and reference to the manuscript.
A number of capillaries <10/mm is considered as abnormal and a number <7/mm is an advanced stage (please see their table 1) (Ingegnoli F, Gualtierotti R, Lubatti C, Zahalkova L, Meani L, Boracchi P, Zeni S, Fantini F. Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations. Semin Arthritis Rheum. 2009 Feb;38(4):289-95. doi: 10.1016/j.semarthrit). We added this reference to the manuscript.
The following reference for dystrophy >15% was added in the manuscript: The Jouanny P, Schmidt C, Feldmann L, Schmitt J. [Focus on a quick reading of nailfold capillaroscopy]. J Mal Vasc. 1994;19(3):206-9. This reference cited the definition of dystrophy in the penultimate line of their abstract.

What is a percentage of dystrophy of 25% mean; does this mean 25% of the study group or a difference of 25% between the two groups? The power analysis therefore is unclear.
It means a difference of 25% between the two groups. The confusion may come from the term dystrophy. In fact, there is no term which has consensus and some authors name this abnormality as tortuosity (for example Bhakuni DS, Vasdev V, Garg MK, Narayanan K, Jain R, Mullick G. Nailfold capillaroscopy by digital microscope in an Indian population with systemic sclerosis. Int J Rheum Dis. 2012 Feb;15(1):95-101. doi: 10.1111/j.1756-185X.2011.01699.x.) and others as disorganisation (for example Ingegnoli F, Gualtierotti R, Lubatti C, Zahalkova L, Meani L, Boracchi P, Zeni S, Fantini F. Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations. Semin Arthritis Rheum. 2009 Feb;38(4):289-95. doi: 10.1016/j.semarthrit) or sometimes also as bushy capillaries (for example Distler JH, Gay S, Distler O. Angiogenesis and vasculogenesis in systemic sclerosis. Rheumatology (Oxford). 2006 Oct;45 Suppl 3:iii26-7).

What multivariate models were used?
Multiple linear regressions were used. We added the following sentence page 10: “No multivariate models improved results from simple regressions.”

Even though the authors used exposure time, there should be some attempt to describe the exposure

levels, at least in general.
In absence of ppm-years exposure, we preferred simplifying estimates of exposure: How many years have you been exposed? However, in our present study, all our workers worked full time 40 hours a week with 4/5 weeks of leave per year.

RESULTS

The participation rate of 53 of 179 is low, and there is no participation rate for the controls.
From the figure it appears that all the controls agreed to participate.
There is no participation rate for the controls because they answered to an advertising to participate, specifying the inclusion criteria. All those who answered met the inclusion criteria.

What does no statistical difference between the two investigators mean? Were there any misclassifications when grading the pictures? What was done with cases of disagreement? This needs to be described.
There was no statistical difference between the parameters analyzed by both investigators. We added the following sentences: “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%.”
We also added in the paragraph Method – Statistics the following sentence: “Correlations were used for inter-observer reliability.”

The presentation of the numerical differences between groups should not be redundant with the those presented in the table.
We have made the following adjustments to reduce data replication: “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).”

It is unclear how the authors found an r2 of 53% for enlarged capillaries and 8% for capillary length with exposure time. It appears that this was done using a linear regression model. Were both variables or at least one normally distributed? Were there any outliers that lead to these results? I would like to see a scatter plot before I accepted those findings.
Your comments were welcomed. Enlarged capillaries (a binary variable) should have been analyzed with logistic regression. So now, we have completed two types of regression: a logistic regression for enlarged capillaries and linear regression for capillary length (a continuous variable).

Concerning the results of the logistic regression, 62% of the explained variance in the diagnosis of enlarged capillaries is linked with years of exposure. Please see the statistical output below. However, as enlarged capillaries are a binary variable, we are not able to show you a scatter plot. But to please you, please see below, even non significant, the scatter plot of diameter of capillaries related to years of exposure.

Model Summary
Step -2 Log likelihood Cox & Snell R Square Nagelkerke R Square
1 33.205a .413 .626
a. Predictors: (Constant), Durée Exposition

The results remain the same for the linked between time of exposure and capillary length (please see the scatter plot below and the statistical output).

Model Summary
Model R R Square Adjusted R Square Std. Error of the Estimate
1 .287a .082 .067 66.732
a. Predictors: (Constant), Durée Exposition

Fisher's exact test should be used for the comparison of Raynaud's phenomena.
It has been used as explained in the section on statistics.

The co-morbidities and smoking should be left out since as expected there were no differences between the two groups (as expected, and very insensitive because of very small numbers and multiple comparisons). There are no differences in the proportions of hypertension, smoking, and lipid lowering medications and therefore further analysis should not be done (groups too small for subgroup analysis).
We acknowledge statistical significance was not reached. However, some discussions on comorbidities and smoking remain relevant because of potential bias (this is also the reason why we excluded individuals with diabetes mellitus). Although our study sample is small, we reported trends, that we believe warranted discussion. We believe referring to all potential bias and confounders is a strength of this manuscript.

Discussion

The only conclusion is that microcirculation changes persisted in those previously exposed to VCM. The time-related exposure is questionable but might be a finding. I could not find the results showing that those with Raynaud's symptoms did not have pathological capillaroscopic changes.
We respectfully refer to page 10: “Symptoms of Raynaud. VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).”

In any case even if the 4 subjects with Raynaud's did not have such changes, that finding is not worthy of a conclusion.
Abnormal microcirculation and Raynaud’s phenomemon occurred independently in VCM exposed workers in the present study. However, we believe the uncertainty surrounding the pathophysiology of Raynaud’s after VCM exposure is sufficiently important to be in the conclusion.

The one study that found no correlation with the length of occupational exposure and capillary abnormalities was not quoted (Int Arch Occup Environ Health 1983;51:337-340).
We modified the sentence by the following: “The dose responsiveness of VCM exposure and compromised capillarisation is generally,13 but not always,12 reported.”

What the study adds
Their suggestion that a threshold exists is unfounded and not supported by the literature. Their comment that since not all develop microvascular abnormalities is suggestive of an underlying genetic susceptibility doesn't add anything since this could be said about nearly any vascular disease. The same can be said of the differences between male and female.
Arguments referring to genetic bases are required in comprehensive discussions of possible medical

explanations. We can confirm that the suggestion of a threshold was a postulation, a notion to stimulate further discussion.

Comparison with other studies
I don't understand the sentence, page 12, line 12- "After 50 years?
We completed the sentence by “after 50 years of age”

The discussion on respiratory and circulatory diseases is superficial. There are many follow-up cohort studies that overall do not support an association between exposure to VCM and diseases of the circulatory system. Their findings don't add anything to the literature on this question.
Once again, discussion on respiratory and circulatory related conditions remains relevant to this manuscript because they are among the serious consequences of compromised microcirculation (Laplanche, A., F. Clavel, et al. (1987). "Exposure to vinyl chloride monomer: report on a cohort study." Br J Ind Med 44(10): 711-715). Moreover, our department of occupational health frequently deals with workers who are worried about respiratory or circulatory diseases contracted under VCM exposure.

This section should focus on the previous capillary studies done on subjects with SS and VCM exposures. Similar or different? What findings? What criteria? They certainly did not show that VCM was more strongly associated with compromised miocrocirculation than high blood pressure, dyslipidemia and smoking, because they did not find an association with those findings.
Previous research into the links between SS and VCM exposure is limited by single case design (Betta A, Tommasini M, Bovenzi M, Barbone F, Versini W, Romeo L. [Scleroderma and occupational factors: a case-control study and analysis of literature]. Med Lav. 1994;85(6):496-506) and somewhat dated analyses of a population exposed to solvents (Ostlere LS, Harris D, Buckley C, Black C, Rustin MH. Atypical systemic sclerosis following exposure to vinyl chloride monomer. A case report and review of the cutaneous aspects of vinyl chloride disease. Clin Exp Dermatol. 1992 May;17(3):208-10). The broader use of term such as solvents is less specific than the VCM exposure carefully isolated for investigation in the present study.

Here they might add how their prevalence of Raynaud's differs from other studies and whether or not this is due to selection bias or exposure to VCM.
We believe we have compared the present study to others and shown similar results. We add the sentences: “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…”

I don't understand what the authors meant by the last sentence in the unanswered questions. Do they claim that the association with capillaroscopic changes and symptoms of Raynaud's in general or just for VCM exposure is unclear, based on their 4 cases (didn't see the data in the results)? How does this compare to the study in JAMA 236:1368, 1976 and other studies?
On page 10, it was stated: “VCM exposed group also had more symptoms of Raynaud (19% vs. 0%, p=.007) independent of capillaroscopic modifications (table 1).”
In the section on unanswered questions, we wanted to claim that the association with capillaroscopic changes and symptoms and patophysiology of Raynaud's following VCM exposure remains unclear and has never been described. Here we found a higher prevalence of symptoms of Raynaud but independently of capillaroscopic abnormalities. We agree that strong conclusions cannot be based on a sample of 4 individuals with Raynaud’s which reflect our statement on lack of statistical power.

Strengths and limitations
Certainly the 30% attendance rate is not a strength but a weakness and possible directional bias should be discussed. The paragraph on limitations is unclear and poorly written.

An attendance rate of 30% is not unusual in relation to other reported studies requiring far less investigations and on healthier and younger populations. These references are cited, and in this context, the 30% is perceived to be strong.

Conclusions
See above comments.

Reviewer 2: Matthew Cave, MD, Assistant Professor
Department of Medicine, Department of Pharmacology and Toxicology
University of Louisville
Louisville, KY, USA

The manuscript is written by French authors and would benefit from a medical editor for assistance with English language. This manuscript documents micro-circulatory abnormalities in vinyl chloride workers 15 years after retirement. The research study is well-done, and I have no major scientific concerns. The manuscript would, however, benefit from further editing for English language.
We are sorry that the English appears imperfect to this reviewer but the manuscript was written with the close support of native English speaking researchers (maybe it is the Australian's language that offends… !). If a medical editor wants to assist, we would not be opposed.

If known, more details about the vinyl chloride exposures would be helpful (e.g. PPM-Years).
We agree, ppm-years exposure would have significantly improved the strength of the results but regrettably we don't have this quantification. Recruitment occurred 15 years after retirement and they worked approximately from 1960 to 1995. Ppm-years is therefore not available for this population.

Also I would be careful about concluding that the micro-circulatory abnormalities persist after retirement because these abnormalities were not documented in these specific workers prior to retirement.
The point is well made. We modified the sentence in the conclusion "our study demonstrated residual long-term […]" to now state "our study demonstrated the potential for residual long-term […]"

Reviewer 3: Enrico Davoli
Environmental Health Sciences Dept.
Istituto Mario Negri
Milano, Italy

The selection criteria is very strict. Only 3%, approximately, retired working from PVC production, exposed to VCM, have been enrolled. It is not clear why women have been excluded, first (page 12, females are more susceptible...).
Female were excluded because of very low numbers among the manual workers (22/761 on the first level of recruitment – figure 1), and they potentially different responses may have confounded the results. Nevertheless, an additional study with sufficient number of women exposed to VCM may generate a lot of interest.

Also "other" exposures have been considered as an exclusion factor, even if no microcirculation specific effects are reported. It seems a selection too much stringent, and a bias, due to this fact, is possible, influencing results. The authors should discuss about this possible problem.
Rigorous inclusion criteria allowed us to isolate VCM exposure. By including other exposures conclusions about VCM exposure would be weakened. VCM is among the most common chemical exposures.

Participants. We enrolled 761 male retired … Is not consistent with fig. 1 (761 retired from occupational … 22 females excluded)
We thank the reviewer for this correction. In the abstract, we replaced the sentence “We enrolled 761 males” by “We screened 761 (97% males)”.

Correlation between time of exposure and capillaries enlargements, being so strongly correlated, could be discussed in more details.
Because exposure to VCM is not consistently reported in the literature, it is difficult to justify a lengthy discussion.

Table 1. I am not familiar with capillaroscopic outcomes. I was wondering if parameters like 300 microns (length) 30, 50 microns diameter are standard or arbitrary for this study.
We repeat from a previous response used to reviewer 1:
The criteria for abnormalities were defined before the analysis and are also presented in the protocol submitted to our university hospital ethics committee. These criteria were those usually used by our clinical expert in capillaroscopy. We did not originally extensively reference this section but have subsequently done so.
As you say, the definition of an augmented capillary length is cited in the article: Kabasakal Y, Elvins DM, Ring EF, McHugh NJ. Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls. Ann Rheum Dis 1996;55:507-12. We have also added the following reference using the same abnormal cut-off >300 µm of capillary length: Ingegnoli F, Gualtierotti R, Lubatti C, Zahalkova L, Meani L, Boracchi P, Zeni S, Fantini F. Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations. Semin Arthritis Rheum. 2009 Feb;38(4):289-95. doi: 10.1016/j.semarthrit.
Concerning the definition of enlarged diameter, the same reference considers that a normal width is less than 25 µm (figure 2a from Kabasakal Y, Elvins DM, Ring EF, McHugh NJ. Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls. Ann Rheum Dis 1996;55:507-12). We had written 30 µm in our protocol because of the local clinical practice of our expert. However, there were the same number of individuals with capillaries >25 or >30 in our study. The replacement of “30” with “25” in our manuscript did not alter the number of participants with enlarged diameter, and is now more consistent with the literature.
Concerning the definition of megacapillary (or giant capillary) > 50 µm, we added in the text the following references: 1) Bhakuni DS, Vasdev V, Garg MK, Narayanan K, Jain R, Mullick G. Nailfold capillaroscopy by digital microscope in an Indian population with systemic sclerosis. Int J Rheum Dis. 2012 Feb;15(1):95-101. doi: 10.1111/j.1756-185X.2011.01699.x. Please see page 99 of their publication 2) Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008 Dec;22(6):1093-108. doi: 10.1016/j.berh.2008.09.001. Please see their figure 3.
Hemorrhage is defined as the microvascular extravasation of the red blood cells linked to the damage of the vessel wall (Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008 Dec;22(6):1093-108. doi: 10.1016/j.berh.2008.09.001). Please see their figure 4 explaining the definition of hemorrhage. We added this definition and reference to the manuscript.
A number of capillaries <10/mm is considered as abnormal and a number <7/mm is an advanced stage (please see their table 1) (Ingegnoli F, Gualtierotti R, Lubatti C, Zahalkova L, Meani L, Boracchi P, Zeni S, Fantini F. Feasibility of different capillaroscopic measures for identifying nailfold microvascular alterations. Semin Arthritis Rheum. 2009 Feb;38(4):289-95. doi: 10.1016/j.semarthrit). We added this reference to the manuscript.
The following reference for dystrophy >15% was added in the manuscript: The Jouanny P, Schmidt C, Feldmann L, Schmitt J. [Focus on a quick reading of nailfold capillaroscopy]. J Mal Vasc. 1994;19(3):206-9. This reference cited the definition of dystrophy in the penultimate line of their abstract.

VERSION 2 – REVIEW

REVIEWER	Froom, Paul Sackler Medical School , Tel Aviv University, Epidemiology and preventive medicine I have no competing Interests
REVIEW RETURNED	07-Apr-2013

THE STUDY	It is unclear if their results can be extrapolated to other cohorts. The statistical results are often inappropriate. For example the power analysis is still unclear. The scatter plots they provides show essentially that the correlation is poor to nonexistant. How can logistic regression give you the % variance explained? You can calculated the area under the curve. Is that what is meant.
RESULTS & CONCLUSIONS	The article is poorly written, and my concerns on the first review were not entirely answered. If accepted it needs significant editing. For example; statements like not all workers develope microvascular abnormalities suggests.... should be left out. Specific findings for nearly all chronic diseases are not 100%.
GENERAL COMMENTS	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

REVIEWER	Enrico Davoli Head, Mass Spectrometry Laboratory Environmental Health Sciences Dept. Istituto di Ricerche Farmacologiche Mario Negri Milano Italy I have no competeng interest
REVIEW RETURNED	22-Apr-2013

THE STUDY	I still have some doubts about females' exclusions. The authors answered to the point, but, even if potentially different responses could confound results, I still feel that a discussion in the text is necessary.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1: Paul Froom

It is unclear if their results can be extrapolated to other cohorts.
In our article, we have stated in the introduction that: "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". Even if VCM exposure has been minimized due to improvement in occupational safety over recent decades, it remains potentially hazardous, especially in less developed countries. The authors believe that these results have the external validity to be applied to other workplaces with VCM exposure.

The statistical results are often inappropriate. For example the power analysis is still unclear.
Thank you for the opportunity to revise and clarify samples size calculations. The sentences in the "methods section – statistics" now read: "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.

The scatter plots they provides show essentially that the correlation is poor to nonexistant. How can logistic regression give you the % variance explained? You can calculated the area under the curve. Is that what is meant.
We acknowledge there is no similar analogous statistic in logistic regression to the coefficient of determination. R2 in the Model Summary Table shown in the previous response provides some approximations. Nagelkerke's R2 is part of SPSS output in the 'Model Summary' table and is the most-reported of the R-squared estimates. In our case it is 0.626, indicating a moderately strong relationship of 62.6% between the predictors and the prediction. We added in the text in the results section: "Time of exposure to VCM was strongly linked with enlarged capillaries (Nagelkerke R Square approximation of 63%, p<.0001)".
In the previous review, you requested that we complete binary regression to explore the link between enlarged capillaries and time of exposure. We have done these analyses with capillary length (Nagelkerke R Square approximation of 36%, p<.0001) and with dystrophy (Nagelkerke R Square approximation of 51%, p<.0001). Please see the output below.

Capillary length:
Récapitulatif des modèles
Etape -2log-vraisemblance R-deux de Cox & Snell R-deux de Nagelkerke
1 30,196a ,192 ,360

Dystrophy:
Récapitulatif des modèles
Etape -2log-vraisemblance R-deux de Cox & Snell R-deux de Nagelkerke
1 21,948a ,251 ,508

The sentences in the section "results – time of exposure" now read: "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 (R2 = 8%; p=.031) (table 1)." We also corrected the abstract as following: "Time exposure was linked (p<.0001) with enlarged capillaries (R2=.63), dystrophy (R2=.51), and capillary length (R2=.36)."
We chose not to report area under the curve because we thought the reported statistics had sufficiently met the study objectives.
We added in the acknowledgements: "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)."

The article is poorly written, and my concerns on the first review were not entirely answered. If accepted it needs significant editing. For example, statements like: "not all workers develope microvascular abnormalities suggests...." should be left out. Specific findings for nearly all chronic diseases are not 100%. "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"
We are confused by the reviewer's terminology of "poorly written". We respectfully believe that the reviewer's point is not as much about writing skills, as it is about omitting sentences in the discussion with which the reviewer disagrees. The strength of the sentence on genetic susceptibility lies in the citation of one of the most seminal publications in VCM exposure. We think the article would be incomplete without this reference. Moreover, it is difficult when one reviewer wants to omit discussion on females exposed to VCM and the other wants it extended. We believe the existing references and comments support the aims and results of this study.
However, we have completed some minor changes to improve the readability of the manuscript, especially the limitations paragraph.

Reviewer 2: Enrico Davoli
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Environmental Health Sciences Dept.
Istituto di Ricerche Farmacologiche Mario Negri
Milano, Italy

I still have some doubts about females' exclusions. The authors answered to the point, but, even if potentially different responses could confound results, I still feel that a discussion in the text is necessary.
It is difficult when one reviewer wants to omit discussion on females exposed to VCM and the other wants it extended. Without further gender-specific research, it is problematic to discuss mechanisms of genotoxocity previously observed in VCM-exposed females. We have added gender specificity to the limitations of the study.