Author's response to reviews

**Title:** Detection of microvascular changes in a paediatric and adolescent population with type 1 diabetes: A pilot cross-sectional study.

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**Author's response to reviews:** see over
10 July 2013

Dear Drs Arnaldi, Costacou and Tahrani,

Subject: REPLY TO REVIEWERS COMMENTS WITH MANUSCRIPT CHANGES OUTLINED

I wish to thank you for your consideration of the article “Detection of microvascular changes in a paediatric and adolescent population with type 1 diabetes: A pilot cross-sectional study” (Manuscript ID 1447754337980667), for publication in BMC Endocrine Disorders. Following this letter you will find, responses to the reviewers’ comments. In addition, I have uploaded an amended ‘clean’ copy of the manuscript, as well as one with ‘tracked’ changes.

If there is anything else you require, or any further changes sought, please do not hesitate to contact me.

Thank you once again for your consideration of this article.

Sarah Hosking
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Reviewer 1

Comment 1

“Authors need to define the different terms used in the description of capillaroscopy findings, such as: avascular areas, nailfold microhaemorrhages, microaneurisms. “

Addition to Background section with types of capillaries outlined (see pg 5 para 2)

Comment 2

“(the reviewer would like to see a classical image of microaneurisms)”

Added image to figures of nailfold capillaroscopy scoring sheet – with image of microaneurisms

Comment 3

“What about the other classical capillaroscopy findings such as bushy, tortuous, meandering and branching capillaries, capillaries width, megacapillaries, or capillary disorganization...., do the authors explore them? I suggest referring to the following article (Feasibility of Different Capillaroscopic Measures for Identifying Nailfold Microvascular Alterations. Semin Arthritis Rheum 38:289-295)

These were explored, however only the abnormal findings, which were seen in our participants were reported. An outline of these abnormal features have been added to the background and a sentence added to results specifically stating they
were not associated with diabetes control markers or the investigations (see pg 5 para 2 and pg 12 para 4)

Comment 4

"Why the authors explain only 4 fingers and no 9 fingers, previous studies disclosed that explore 8 fingers was a good approach. In any case, it should be explained in the method section"

The equipment used for capillary examination is of a static design with a stationary lens housing. This design requires the finger to be immobilised within a holding apparatus to prevent excessive movement. This can cause some discomfort and given the paediatric population it was decided to examine two fingers on each hand rather than all 8 digits. Each participant had the 2\textsuperscript{nd} and 4\textsuperscript{th} finger on each hand examined unless trauma was present. The 3\textsuperscript{rd} and 5\textsuperscript{th} finger were not examined due to considered nailfold trauma risk in the paediatric population. Thumbs are not often examined in Capillaroscopy, due to capillaries not being well visualised, in addition the positioning of thumbs within the KKtechnology utilised was likely to cause great discomfort and movement in the paediatric population. Capillaroscopy technique used in previous studies varies in regards to the number and selection of representative digits evaluated being dependent on equipment, time, finger trauma and contractures and participant focus cohort. The 4\textsuperscript{th} finger of the non-dominant hand is consistently evaluated in research and diagnostic settings as it is thought to be the most representative of the morphological features, (see pg 8 para 2)
Comment 5

“Authors states in the method section that explore the capillary density, it means the mean number of capillary per patient, but results of this analysis are lacking”

The average capillary density was found for each patient and relevant associations with other investigations were reported (see pg 12 para 4) however the average capillary density of the patient was not reported in the table stratified by clinical outcomes as there were no significant findings.

Comment 6

“Authors states that they used a panoramic video, but which was the magnification (x200, x250…?). The reviewer suggests that the authors follow the STROBE Statement (Strengthening the reporting of observational studies in epidemiology) and stated this in the method section.

Panoramic video was taken at 100x magnification – information added to methods (see pg 8 para 2).

Changes made to manuscript according to STROBE statement, also statement added to methods (see pg 8 para 1)

Reviewer 2

Comment 1

“The title should be shortened and focused on the pathogenetic aspects investigated by the procedures. Therefore a better title could be “Detection of
microvascular changes in a paediatric and adolescent population with type 1 diabetes”

Titled changed to: Detection of microvascular changes in a paediatric and adolescent population with type 1 diabetes: A pilot cross-sectional study. (see pg 1)

Comment 2

“The abstract should be completely rewritten as the methods section is incomplete and giving unnecessary details like where the study was performed etc... The section of methods should be clearly stated the criteria of enrolment and the techniques, how the population was divided or subdivided, which parameters were chosen to analyze the population.”

See marked manuscript for further details (see pg 3)

Comment 3

“The Results section should clearly summarized the data obtained and it currently is a bit confused since apparently there is no consequentiality between methods and results”

Results were summarised to each relevant investigation. When investigation results were associated with each other the results were placed in the section with the most associations. E.g. baseline perfusion associated with both capillary density and microaneurisms so reported in the laser Doppler flowmetry section.

Comment 4
“Finally, in the conclusions of the abstract it is mentioned “educating adolescent population”. Was this a purpose of the study? If not this is a comment and should be placed in the general discussion”

Sentence removed (see pg 4 para 1)

Comment 5

“The background should focused on the review of data available about investigations used to analyze early microvascular changes up to date and not to describe how laser Doppler is performed, as this should be placed in methods”

Text edited, see marked manuscript (see pg 6 para 3)

Comment 6

“The reader is usually not particularly impressed if no one has performed a similar study to yours. So avoid this kind of sentence. Usually the reader is more interested in results from authors”

Sentence removed (see pg 6 para 3)

Comment 7
“In methods it is state that patients were enrolled between Feb and Oct 2010. This is a very short and it could be a big problem. First there is no follow up therefore the prediction of “early microvascular changes” is a debating matter”

This study was of a cross-sectional nature, which aimed to find correlations between these investigation findings and poorly controlled diabetes. As stated in the discussion further prospective analysis should be performed to discover the timeline of microvascular changes. (see pg 16 para 3)

Comment 8

“The authors also mentioned that capillaroscopy was performed analysing the 2nd and 4th finger. Why did no include the V?”

The 5th finger was excluded as authors’ experience was that the 5th finger is prone to trauma, causing artifactual capillary changes, which biased interpretation of microvasculature. Also, as stated in reply to reviewer 1 comment 4, immobilisation of the 5th finger, can be uncomfortable and in a paediatric population this was thought to be unnecessary (see pg 8 para 2).

Comment 9

“How did they score the capillary abnormalities? I was unable to find any score”

Evaluation of nailfold images was based upon the EULAR system of assessing Capillary morphology, 2008. The EULAR system outlines that 4 fields of 1 linear mm are evaluated for morphological changes. In each field counts of abnormalities and density are made and averaged across fingers. The Nailfold
capillaroscopy image, assessing tool utilised in this study has been added to figures.

Comment 10

"The section of methods should also be summarized as too long"

The section of methods has been significantly revised, with reliance on previously published material for standard methods.

Comment 11

"The statistic should have an own section"

Given heading

Comment 12

"Clinical parameters should also list in a specific section."

Given heading, changed to "standard clinical parameters"

Comment 13
“In the discussion the author underlined the relationship with the length of diabetes and the findings observed but as I have already observed, it is not clearly stated in methods how patients were subdivided according to the disease length.

Disease length was divided as >8years duration of ≤ 8years duration. (See pg 11 para 1)

Comment 14

“They also mentioned autonomic disturbances. They should better explained how these alterations can induce the microvascular changes with appropriate references”

It is suggested by evidence referenced in our text that the microvascular changes occur pre-autonomic neuropathy and may be a contributing cause. This has been made clearer in the discussion (see pg 14 para 1)

Comment 15

“Furthermore they should discussed if abnormalities on retina could have different pathogenesis (is the autonomic dysfunction and explanation of these changes???)”

Retinal vascular pathogenesis has been widely discussed in other literature. See manuscript edits (see pg 15 para 2) for details.
Comment 16

“The great limitation of the study as stated by the author is the absence of a control group”

Stated in manuscript (pg 16 para 2)