

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (<http://bmjopen.bmj.com/site/about/resources/checklist.pdf>) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

|                            |  |
|----------------------------|--|
| <b>TITLE (PROVISIONAL)</b> | Validation of the Automatic image analyzer to assess retinal vessel caliber (ALTAIR).Prospective study protocol.   |
| <b>AUTHORS</b>             | GarciaOrtiz, Luis Gomez-Marcos, Manuel; Recio-Rodriguez, Jose; Maderuelo-Fernandez, Jose; Chamoso-Santos, Pablo; Rodriguez-Gonzalez, Sara; De Paz, Juan; Merchan-Cifuentes, Miguel; Corchado-Rodriguez, Juan |

### VERSION 1 - REVIEW

|                        |   |
|------------------------|---|
| <b>REVIEWER</b>        | Francisco Gude<br>Hospital Clinico Universitario de Santiago<br>Spain |
| <b>REVIEW RETURNED</b> | 11-Aug-2014   |

- The reviewer completed the checklist but made no further comments.

|                        |   |
|------------------------|---|
| <b>REVIEWER</b>        | Luca Giancardo<br>Massachusetts Institute of Technology<br>Cambridge, MA<br>USA |
| <b>REVIEW RETURNED</b> | 10-Oct-2014   |

|                         |   |
|-------------------------|---|
| <b>GENERAL COMMENTS</b> | <p>since no results are presented I have marked as N/A the sections that dealt with results.</p> <p>My review will deal with the technical aspects of the validations of the tool, since the details of the clinical aspects are outside my area of expertise.</p> <p>The aim of this clinical protocol is to evaluate a semi-automatic retina vessel calibre estimation tool (Altair) in terms of reliability and validity (or precision and accuracy) and finding a correlation with other clinical variables cross-sectionally and longitudinally.</p> <p>The need of the study is clear and well motivated.</p> <p>However, I do see a serious problem in the evaluation of the validity (accuracy) in the software validation. Here two blinded operators will measure the vessel parameters of a subset of 100 images and then the results will be compared and contrasted. This will evaluate only the reliability (precision) of the system, not the validity (accuracy). In order to evaluate this aspect another gold standard with the size of the vessels manually segmented is</p> |
|-------------------------|---|

|  |   |
|--|---|
|  | <p>required.<br/>Without it the authors cannot claim to have evaluated the validity (accuracy) of the system.</p> <p>Additionally, it is important to mention that the appearance of retinas greatly vary between ethnicity, darker pigmentations have a substantially different appearance than bright ones. This is particularly challenging for an automated tool, hence, the subsample of 50 patients should take into account this aspect.</p> <p>Since there are many other automated or semi-automated tools to calculate retinal parameters which are freely available, the study would benefit from a comparison with them, at least on a subset of patients.<br/>However, this is not essential for the publication of the study.</p> |
|--|---|

|                        |   |
|------------------------|---|
| <b>REVIEWER</b>        | Tom MacGillivray<br>University of Edinburgh, UK |
| <b>REVIEW RETURNED</b> | 22-Oct-2014                                     |

|                         |   |
|-------------------------|---|
| <b>GENERAL COMMENTS</b> | This paper describes a protocol for a yet to be conducted study. Provided that BMJ Open publishes such manuscripts, I would accept this manuscript. |
|-------------------------|---|

### VERSION 1 – AUTHOR RESPONSE

1.-Reviewer Name Francisco Gude  
Institution and Country Hospital Clinico Universitario de Santiago  
Spain  
Please state any competing interests or state 'None declared': None declared

(There are no comments.)

2.-Reviewer Name Tom MacGillivray  
Institution and Country University of Edinburgh, UK  
Please state any competing interests or state 'None declared': None declared

This paper describes a protocol for a yet to be conducted study. Provided that BMJ Open publishes such manuscripts, I would accept this manuscript.

3.-Reviewer Name Luca Giancardo  
Institution and Country Massachusetts Institute of Technology  
Cambridge, MA. USA

My review will deal with the technical aspects of the validations of the tool, since the details of the clinical aspects are outside my area of expertise.  
The aim of this clinical protocol is to evaluate a semi-automatic retina vessel calibre estimation tool (Altair) in terms of reliability and validity (or precision and accuracy) and finding a correlation with

other clinical variables cross-sectionally and longitudinally. The need of the study is clear and well motivated.

However, I do see a serious problem in the evaluation of the validity (accuracy) in the software validation. Here two blinded operators will measure the vessel parameters of a subset of 100 images and then the results will be compared and contrasted. This will evaluate only the reliability (precision) of the system, not the validity (accuracy). In order to evaluate this aspect another gold standard with the size of the vessels manually segmented is required. Without it the authors cannot claim to have evaluated the validity (accuracy) of the system.

We agree with the reviewer on the need to evaluate the two aspects of the validation of the Altair tool, the reliability (precision) and the validity (accuracy).

The validation procedure, which included both the reliability and the validity, is detailed in the section Retinal software validation. The validity assessment, comparing the results with other parameters that assess vascular structure and function, considering them as "gold standard" is detailed in the points 4 and 5. However, we think that for some errors in the in the drafting and translation was not sufficiently clear this section, and therefore we have modified the structure and wording of some paragraphs being as follows: (page 12, line 4).

#### Retinal software validation

To validate the retinal software platform, the following steps will be completed by the evaluators after previous training in imaging appreciation.

##### -Evaluation of the reliability or precision

1. Intra-observer variability: To evaluate the measurement repeatability, the operator must measure the same image of an individual on at least two occasions. To this end, an operator will measure 100 images of a random subsample of 50 patients with a 1-week difference between the two measurements. In this case, the operator and the analysed images will be the same on both days, and the information from the previous measurement will be unknown.

2. Inter-observer variability: To evaluate the reproducibility of the measurement system, a different operator than who completed the assessment in phase 1 will evaluate the same 100 images previously analysed. The information from the results obtained in the previous phase will be unknown to this operator, and both operators will have the same experience in the subject and pertaining to the use of the software. Furthermore, both operators will receive the same preparatory training.

##### -Evaluation the validity (accuracy)

3. To assess the degree of agreement between Altair and the AV Index calculator® software (1), previously validated by us, the evaluation of 100 images will be performed using both tools. In this way, we will be able to demonstrate that the new method, apart from providing the same results, is more objective and faster in elaborating the results.

4. The measurement validity will be analysed in a total sample of 386 subjects and 772 retinographies, in regards to the relationship between the results of the carotid IMT, as a measurement of vascular structure, the PWV, the gold standard measure of arterial stiffness, the CAVI, kidney function, electrocardiographic parameters, and the estimated cardiovascular risk using different scales.

5. The association between different estimated parameters of the retina with the evolution or onset of new lesions in the target organs will be analysed, as well as any cardiovascular events that occur during the 4-year follow-up of the second phase of this project.

Additionally, it is important to mention that the appearance of retinas greatly vary between ethnicity, darker pigmentations have a substantially different appearance than bright ones. This is particularly challenging for an automated tool, hence, the subsample of 50 patients should take into account this aspect.

In the second objective of the manuscript, we propose the validation of the tool in different populations, although it was not planned to include different ethnicities. However, it seems an interesting observation the inclusion of different ethnic groups in order to improve the validity of the tool. Although the environment in which the evaluation will be conducted the majority of the population is Caucasian, we will include a representation of other ethnic groups attended in the health center, primarily Hispanic, Arab and Asian.

We modified the second object being as follows (page 4, line 20):

ii) To evaluate the concurrent validity of the Altair software platform, in different populations and ethnicities, by analysing the relationship between retinal parameters and other parameters of vascular structure and function, including carotid IMT, pulse wave velocity (PWV), and the cardio-ankle vascular index (CAVI), as well as injuries in other target organs and the cardiovascular risk.

We have added the following paragraph in the study population section of Method and Analysis (page 5, line 16).

The subjects will be mostly Caucasian, majority ethnic group among patients attended in the health center, however, at least fifty ethnic minority subjects, to give more validity to the tool, will be included.

Since there are many other automated or semi-automated tools to calculate retinal parameters which are freely available, the study would benefit from a comparison with them, at least on a subset of patients. However, this is not essential for the publication of the study.

Our group developed and validated (reliability and validity) the AVIx calculator® software. The measurements of the retinal vessels made by this software have demonstrated a good relationship with other parameters of vascular structure and function considered "gold standard" of vascular injury and also with the cardiovascular risk. These data were published in the manuscript of validation (1) and in others two subsequent (2,3).

In the point 3 of Retinal software validation section, it details as will be compare the result of the new tool with the AV Index calculator® measure.

-Evaluation the validity (accuracy)

3. To assess the degree of agreement between Altair and the AV Index calculator® software (1), previously validated by us, the evaluation of 100 images will be performed using both tools. In this way, we will be able to demonstrate that the new method, apart from providing the same results, is more objective and faster in elaborating the results.

## REFERENCES

- 1.-García-Ortiz L, Recio-Rodríguez JI, Parra-Sanchez J, Elena LJG, Patino-Alonso MC, Agudo-Conde C, Rodríguez-Sánchez E, Gómez-Marcos MA: A new tool to assess retinal vessel caliber. Reliability and validity of measures and their relationship with cardiovascular risk. *Journal of Hypertension* 2012, 30(4):770-777.
- 2.-García-Ortiz L, Parra-Sanchez J, Recio-Rodríguez JI, Agudo-Conde C, González Elena LJ y Manuel A Gómez-Marcos en nombre del grupo Vaso-risk. El papel de las venas de la retina en el riesgo cardiovascular. *Hipertensión y Riesgo vascular* 2013; 30(3):92-100.
- 3.-García-Ortiz L, Recio-Rodríguez JA, Agudo-Conde C, Patino-Alonso MC, Rodríguez-Sánchez E, Maderuelo-Fernández JA and Gómez-Marcos MA for the EVIDENT Group. The role of retinal vessels caliber as a marker of vascular aging in larges arteries. *Journal Hypertension* 2014. [In press].