Assessment of indices of conjunctival microvascular function in patients with and without obstructive coronary artery disease

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Editor-in-Chief
Cardiovascular Revascularization Medicine

16th November 2022

Dear Editor-in-Chief,

We wish to submit an original research article entitled *"Assessment of indices of conjunctival microvascular function in patients with and without obstructive coronary artery disease".*

I confirm on behalf of all authors that the article is original, not under consideration by another journal, and has not been previously published. All authors have participated in the work and have reviewed and agree with the content of the article. None of the article contents are under consideration for publication in any other journal or have been published in any journal. No portion of the text has been copied from other material in the literature (unless in quotation marks, with citation). I am aware that it is the author's responsibility to obtain permission for any figures or tables reproduced from any prior publications, and to cover fully any costs involved. Such permission must be obtained prior to final acceptance. I sign for and accept

responsibility for releasing this material on behalf of any and all co-authors. All participants in the study have provided fully informed consent.

This article presents the findings from a pilot study to evaluate the ability to non-invasively detect alterations in parameters of conjunctival microvascular function in patients with atherosclerotic coronary disease.

We have no conflicts of interest to disclose. Please address all correspondence concerning this manuscript to me at jonathan.mailey@belfasttrust.hscni.net.

Thank you for your consideration of this manuscript.

Sincerely,

Dr Jonathan A. Mailey

Highlights

- The conjunctival microvasculature can be assessed non-invasively using a combination of a smartphone and slit-lamp biomicroscope
- Hemodynamic abnormalities in microvascular function can be detected in the conjunctiva of patients presenting with myocardial infarction
- The non-invasive detection of conjunctival microvascular dysfunction may have potential utility in cardiovascular risk assessment and preventive cardiology

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jonathan Mailey reports financial support was provided by Belfast Health and Social Care Trust.

Jonathan A. Mailey: Conceptualization, Methodology, Writing, Investigation. **Julie S. Moore**: Conceptualization, Methodology, Writing- Reviewing and Editing. **Paul F. Brennan**: Conceptualization, Methodology, Writing- Reviewing and Editing. **Min Jing:** Conceptualization, Methodology. **Agnes Awuah:** Conceptualization, Methodology. **Emanuele Trucco:** Conceptualization, Methodology. **James A. D. McLaughlin:** Conceptualization, Methodology, Writing- Reviewing and Editing**. M. Andrew Nesbit**: Conceptualization, Methodology, Writing- Reviewing and Editing. **Tara C. B. Moore**: Conceptualization, Methodology, Writing- Reviewing and Editing. **Mark S. Spence**: Conceptualization, Methodology, Writing- Reviewing and Editing.

Assessment of indices of conjunctival microvascular function in patients with and without obstructive coronary artery disease

Short Title- The association of conjunctival microvascular dysfunction and coronary artery disease

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Conflict of Interest- nil

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Consent and ethical approval- All subjects provided written informed consent for participation in this study. The protocol was approved by the Research Ethics committee in the Belfast Health and Social Care Trust (BHSCT) and Ulster University (UU) and was carried out in accordance with the Declaration of Helsinki.

Funding

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Keywords

Prevention; conjunctiva; microvascular dysfunction; cardiovascular screening; coronary artery disease; cardiovascular disease

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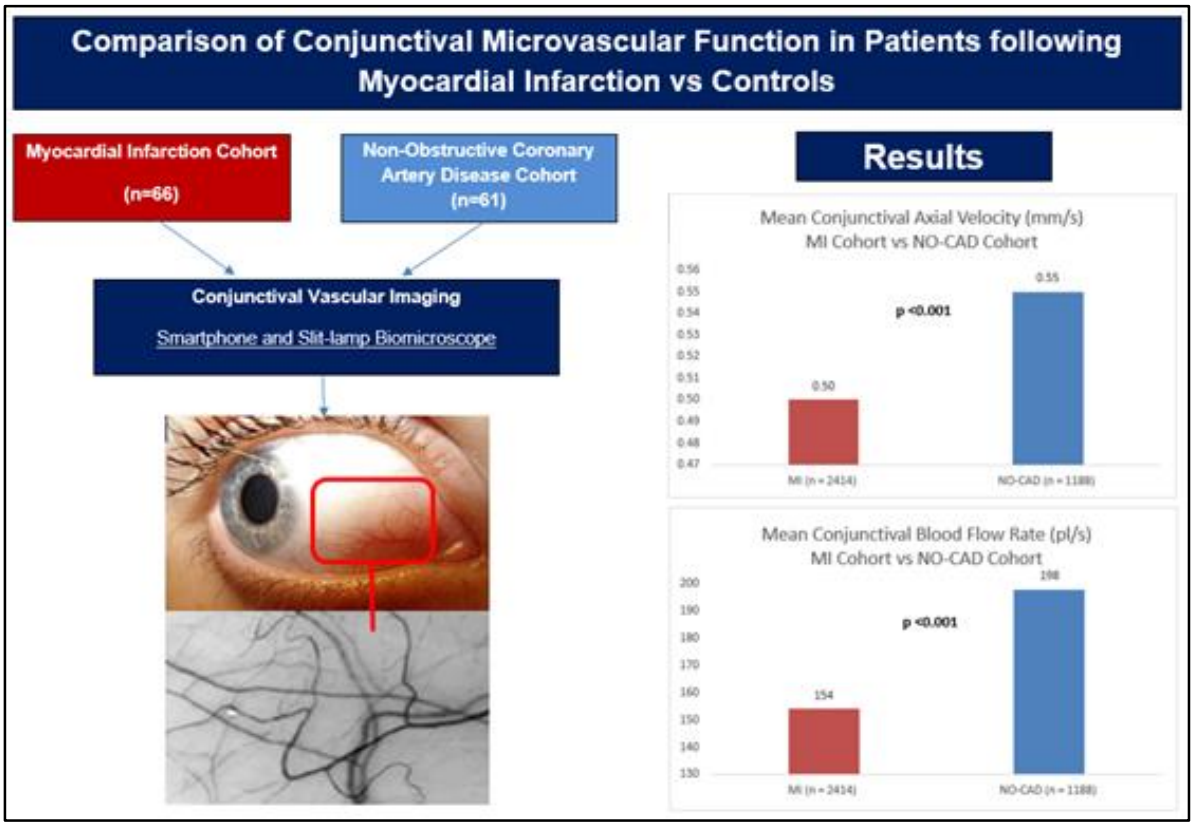
GRAPHICAL ABSTRACT



ABSTRACT

Background

Atherosclerotic heart disease often remains asymptomatic until presentation with a major adverse cardiovascular event. Primary preventive therapies improve outcomes, but conventional screening often misattributes risk. Vascular imaging can be utilised to detect atherosclerosis, but often involves ionising radiation. The conjunctiva is a readily accessible vascular network allowing non-invasive hemodynamic evaluation.

Aim

To compare conjunctival microcirculatory function in patients with and without obstructive coronary artery disease.

Methods

We compared the conjunctival microcirculation of myocardial infarction patients (MI-cohort) to controls with no obstructive coronary artery disease (NO-CAD cohort). Conjunctival imaging was performed using a smartphone and slit-lamp biomicroscope combination. Microvascular indices of axial (V_a) and cross-sectional (V_{cs}) velocity; blood flow rate (Q); and wall shear rate (WSR) were compared in all conjunctival vessels between 5 and 45 μm in diameter.

Results

A total of 127 patients were recruited (66 MI vs 61 NO-CAD) and 3602 conjunctival vessels analysed (2414 MI vs 1188 NO-CAD). Mean V_a, V_{cs} and Q were significantly lower in the MI vs NO-CAD cohort (V_a 0.50 ± 0.17 mm/s vs 0.55 ± 0.15 mm/s, **p <0.001**; V_{cs} 0.35 ± 0.12 mm/s vs 0.38 ± 0.10 mm/s, **p <0.001**; Q 154 ± 116 pl/s vs 198 ± 130 pl/s, **p < 0.001**). To correct for differences in mean vessel diameter, WSR was compared in 10 - 36 µm vessels (3268/3602 vessels) and was lower in the MI-cohort (134 ± 64s^{-1} vs 140 ± 63s^{-1}, **p=0.002**).

Conclusions

Conjunctival microcirculatory alterations can be observed in patients with obstructive coronary artery disease. The conjunctival microvasculature merits further evaluation in cardiovascular risk screening.

INTRODUCTION

Cardiovascular disease (CVD) represents a significant cause of morbidity and mortality worldwide (1). Coronary artery disease (CAD) represents the most prevalent form of CVD (2). A large proportion of patients with CAD remain asymptomatic until first presentation with a major adverse cardiac event (MACE). This observation forms the basis for guideline recommendations to perform CVD screening in asymptomatic adults (1). Several studies have highlighted that the majority of atherosclerotic plaque rupture events and resultant myocardial infarctions (MI) arise from non-obstructive plaques (3-9). The recent HUYGENS study (10) highlighted the ability for statins and PCSK9 inhibitors to positively remodel vulnerable plaques, highlighting the potential value of such evidence-based medications in CV risk reduction. Identification of individuals who will benefit from targeted primary preventative therapies remains a challenging issue, prompting research into novel CV screening modalities.

Our research group previously reported the ability to non-invasively assess the conjunctival microcirculation using an iPhone coupled with a slit-lamp biomicroscope. (11) Statistically significant differences were observed in indices of conjunctival microvascular function when comparing a cohort of patients admitted to hospital with a myocardial infarction (MI) and a cohort of age and sex-matched controls estimated to be at low CV risk using the Q-Risk 3 score. In this study mean axial velocity for the controls was 0.53 ± 0.15 mm/s compared to 0.49 ± 0.17 mm/s for the MI patients ($p < 0.001$). Wall shear rate was higher for controls than MI patients (162 ± 93 s^{-1} vs 145 ± 88 s^{-1}, $p < 0.001$). Blood volume flow did not differ significantly for the controls

and MI patients (153 ± 124 pl/s vs 154 ± 125 pl/s, p = 0.84), this however was largely due to differences in the mean vessel diameter between the groups (controls 21.41 ± 7.57 vs MI 22.32 ± 7.66 μm). This study highlighted the potential for conjunctival imaging to be utilised for CV risk assessment in asymptomatic patients. It was however limited by the absence of coronary imaging in the low-risk cohort to identify patients with asymptomatic CAD potentially confounding the results obtained.

In this study we compare indices of conjunctival microvascular function in the previously reported MI cohort to a group of patients with no obstructive epicardial coronary artery disease detected during an invasive coronary angiogram, and no personal history of either MI or percutaneous coronary intervention (PCI).

METHODS

We conducted a prospective study (Integrated Research Application System study number 166742) comparing a group of patients with a recent MI (MI cohort) to a group of patients with no obstructive coronary artery disease (NO-CAD cohort) as demonstrated by a coronary angiogram and physiological evaluation of any intermediate coronary stenoses.

All subjects provided written informed consent for participation in this study. The protocol was approved by the Research Ethics committee in the Belfast Health and

Social Care Trust (BHSCT) and Ulster University (UU) and was carried out in accordance with the Declaration of Helsinki.

Baseline clinical data and characteristics were obtained using the recruitment questionnaire, inpatient clinical notes (MI cohort), hospital cardiology database and the patient's electronic healthcare record.

MI cohort

Patients were deemed eligible for inclusion in the MI cohort if they were an inpatient with a type 1 MI as defined by the European society of cardiology (ESC) 4th universal definition of myocardial infarction (12). The MI cohort was comprised of patients presenting with both ST-elevation myocardial infarction (STEMI) and non ST-elevation myocardial infarction (NSTEMI).

NO-CAD cohort

Patients were deemed eligible for inclusion in the NO-CAD cohort if they had no past medical history of MI or previous coronary revascularisation by either PCI or coronary artery bypass grafting (CABG). All patients in this cohort were recruited following an invasive coronary angiogram that excluded obstructive epicardial coronary disease. At the time of angiography any intermediate coronary lesions were physiologically assessed by pressure wire evaluation. Patients were included in this

cohort only if fractional flow reserve (FFR) in any intermediate lesions was ≥0.80 (i.e. considered to be physiologically non-obstructive).

The majority of patients in this cohort were admitted electively (72%) for the investigation of stable symptoms of chest pain or dyspnoea. The remainder underwent inpatient coronary angiography due to presentation to the hospital emergency department with chest pain. Inpatients were only recruited to this cohort if their presenting symptoms were deemed to be non-cardiac in origin by the referring clinician, with no identifiable cardiovascular cause for admission (i.e. no elevation in serum troponin, no ECG changes and no echocardiographic findings of hemodynamically significant valvular abnormalities or impairment in biventricular function).

Exclusion criteria

Exclusion criteria for both groups included pregnancy, age less than 18 years old, inability to consent and history of recent conjunctival inflammation or current use of contact lenses.

Conjunctival Microvascular Assessment

Conjunctival imaging was performed using a commercially available Topcon SL-D4 (Topcon Medical Systems Inc., USA), an iPhone smartphone (Apple, Inc, USA) and a bespoke adapter (Zarf Enterprises Inc., USA) (see **Figure 1**). We acquired 5–10 s

videos of the conjunctival microcirculation in both nasal and temporal views, thus generating four videos per subject. All videos then underwent a process of stabilisation, image registration and then semi-automated analysis of microvascular parameters. The imaging platform is calibrated to define a pixel to millimetre (mm) conversion factor. This conversion factor is then used to estimate vessel diameter (D). Axial (V_a) and cross-sectional (V_{cs}) velocity are then estimated using this conversion factor coupled with an app that allows blood flow tracking and hence calculation of distance travelled over a 1 second stabilised video clip. Blood flow (Q) and wall shear rate (WSR) are then estimated from the results of D and V_{cs}. This technique has been described in 3 previous studies (11, 13, 14). **Figure 2** gives an example of a video frame showing the conjunctival microvascular network obtained from our imaging platform, following the process of video stabilisation. Conjunctival vessel diameter, V_a, V_{cs}, Q and WSR were assessed in vessels with observable blood flow. We analysed and report results for vessels between 5 and 45 μm in diameter.

Given the significant impact that vessel diameter creates on the parameters of Q [defined as $Q=V_{cs}(\pi r^2)$] and WSR (defined as $WSR=8V_{cs}/D$), it is important to standardise the range of vessels analysed in order to evaluate microvascular indices in vessels of comparable size. To avoid significant outliers in vessel diameter skewing the results we therefore conducted a sub-analysis of conjunctival vessels between 10 and 36 μm in diameter. This range was selected as it excluded 5% of vessels at both the upper and lower end of the diameter range (n=3268). Vessels in this diameter range were then classified into 4 distinct diameter groups (D 10 - 17μm, D 17 - 23μm, D 23 - 29μm and D 29 - 36μm).

Statistical Analysis

For statistical analysis SPSS for Apple iOS version 26 (property of IBM) was used. Continuous variables were described using the mean, standard deviation of the mean and 95% confidence intervals (CI). Kolmogorov–Smirnov testing was used to assess normality of the continuous variables. Categorical variables were expressed as a number and percentage of the total category number to which the variable belonged.

Normally distributed variables were compared between the two populations using the independent-samples t-test. Non-normally distributed continuous variables were compared using a non-parametric test (Mann–Whitney U test). Categorical comparisons were made between the two groups using Pearson Chi-Square or Fisher's exact test.

RESULTS

Baseline Characteristics

Between 31st January 2018 and 1st October 2021, 127 patients were recruited to this study. A total of 61 patients were included in the NO-CAD cohort and 66 patients were included in the MI cohort. The mean ages were 63 ± 10 years and 57 ± 11 years respectively in these cohorts (**p=0.003**). A higher proportion of patients in the MI cohort were male (78.8% vs 49.2%, **p<0.001**). There was no statistically

significant difference in the prevalence of systemic hypertension, diabetes mellitus and smoking between the groups (p=0.12, p=0.21 and 0.07, respectively). **Table 1** provides a comparative summary of the patients' baseline characteristics. Of note, the prevalence of conventional cardiovascular risk factors (hypertension, smoking and diabetes mellitus) was higher in the NO-CAD cohort than would be anticipated in the general population.

At the time of recruitment, both patient cohorts were normotensive, but the NO-CAD cohort had a higher mean systolic blood pressure (126.3 ± 13.6mmHg vs 120.4 ± 16.4mmHg, **p=0.03**).

All patients in the MI cohort underwent invasive coronary angiography. 4 (6.1%) of these patients were managed medically and the remaining 62 (93.9%) patients underwent either percutaneous or surgical revascularisation. All patients that underwent surgical revascularisation were recruited and had conjunctival imaging performed prior to surgery.

Indices of conjunctival microvascular function

Conjunctival video imaging was obtained for all patients. There were no adverse clinical events during conjunctival imaging. All image processing and subsequent quantitative microvascular assessment was performed by study investigators blinded to the clinical characteristics at the time of the analysis to prevent bias.

A total of 3602 vessel segments were analysable across the two cohorts (1188 in the NO-CAD cohort vs 2414 in the MI cohort). A mean of 28.3 vessel segments were assessable per patient. A comparison of conjunctival microcirculatory parameters is summarised in **Table 2,** and the range of results presented in **Figure 3.** Statistically significant differences were observed between cohorts for D, V_a, V_{cs} and Q.

There was no difference in wall shear rate (WSR) between cohorts; however WSR is inversely related to diameter. The lack of difference therefore related to the difference observed in mean diameter, which was significantly lower in the MI cohort (24.73 ± 7.79 vs 22.41 ± 7.46; **p < 0.001**). As described above, to compensate for this discrepancy in mean diameter, we conducted a sub-analysis of microvascular indices in vessels between 10 and 36 μm in diameter (n=3268/3602). In this sub-analysis, significant differences were observed in all conjunctival parameters (V_a 0.50 ± 0.17 mm/s vs 0.55 ± 0.15 mm/s, **p <0.001**; V_{cs} 0.35 ± 0.11 mm/s vs 0.38 ± 0.10 mm/s, **p <0.001**; Q 152 ± 100 pl/s vs 183 ± 108 pl/s, **p < 0.001;** and WSR 134 ± 64 s^{-1} vs 140 ± 63 s^{-1}, **p=0.002**).

A further analysis comparing the cohorts by 4 previously described sizing groups revealed significant reductions in V_a, V_{cs}, Q and WSR across all groups (see **Table 3**).

Figure 4 demonstrates a comparison of conjunctival vessel axial and cross-sectional velocity in patients with and without an established history of several conventional vascular risk factors (diabetes mellitus, hypertension, hypercholesterolemia and smoking history). There were no significant differences in mean axial or cross-sectional velocity in any of these groups. Given significant between group differences in some baseline characteristics (e.g. age, sex and conventional CV risk factors) a multivariate analysis was conducted, demonstrating that a reduction in conjunctival V_a below 0.50mm/s was an independent predictor of MI (**Table 4**). These findings suggest the observed differences in microvascular parameters in this

study are secondary to the underlying atherosclerotic coronary disease in the MI cohort rather than the presence of other co-morbidities that may impact microvascular function.

DISCUSSION

Opportunistic CV risk screening is recommended by international cardiology guidelines. The European Society of Cardiology (ESC) recommend the use of SCORE 2 for screening in adults aged 40-69 years of age and SCORE 2-OP in adults ≥70 years of age (1). The National Institute for Clinical Excellence (NICE) recommend the use of QRISK III (15). These CVD risk calculators rely on the identification of conventional CVD risk factors to estimate the long-term probability of either a fatal or non-fatal MACE.

Conventional screening modalities estimate a large proportion of patients to be in a low-intermediate CV risk category. It is therefore recommended to establish other modifiers of CV risk, such as the presence of diabetes mellitus, chronic kidney disease, familial dyslipidaemias or through the demonstration of established asymptomatic CVD (1). The latter can be observed through performance of computed tomography (CT) coronary artery calcium scoring to detect atherosclerotic plaques and grade total burden of CAD in comparison to individuals of a similar age and sex (16-19). A recent study evaluating CT coronary angiography (CTCA) demonstrated a high prevalence of asymptomatic atherosclerotic coronary disease in the evaluated population, with as many as 42.1% of participants having silent

atherosclerosis (20). Other modalities such as carotid ultrasound or the measurement of arterial stiffness using pulse wave velocity have been studied, but not found to be beneficial in CV risk screening to date (21, 22). CT as a screening modality; whilst being highly sensitive, is limited by availability, cost and exposure of the patient to ionizing radiation. These factors limit the widespread introduction of CT for population CV screening programmes.

The presence of coronary microvascular dysfunction not only can result in symptoms of angina, but is also prognostically adverse (23, 24). Microvascular dysfunction occurs in the initial manifestations of CVD (25). Detection of microvascular dysfunction, therefore, has the potential to augment conventional CVD screening and allow early initiation of guideline based medical therapies to reduce MACE. The diagnosis of coronary microvascular disease involves invasive coronary angiography and exposure of the patient to procedural risks. However, several previous studies have demonstrated the ability to non-invasively assess the microvasculature of the retinal, sublingual and nail-fold circulation (26-28). Recently, a novel cardiovascular disease risk stratification system using retinal photography was found to be comparable to conventional CT coronary artery calcium scoring in predicting MACE over a 5-year follow-up period using UK Biobanks and cohorts from South Korea and Singapore (n > 70,000) (29).

This study demonstrates statistically significant differences in conjunctival microcirculatory function of a group of patients with established CAD and therefore proven to be at very high CV risk in comparison to a group with no obstructive

coronary artery disease or previous major adverse cardiovascular event. The underlying pathophysiological mechanisms involved in the development of atherosclerotic vascular disease can be observed earliest in the microcirculation (25). The ability to detect microvascular dysfunction has the potential to identify asymptomatic patients who may benefit from aggressive primary preventative therapies. The conjunctiva is an easily accessible site to non-invasively assess for microvascular dysfunction without exposing patients to ionising radiation.

Coronary microvascular dysfunction can result in symptoms of angina or dyspnoea that can considerably impact quality of life. Its presence has also been shown to confer an adverse CV prognosis long-term (30 - 32). The diagnosis of coronary microvascular dysfunction is performed invasively based on demonstrating a reduction in coronary blood velocity, and hence a reduction in blood flow rate using pressure wire evaluation and thermodilution techniques. Importantly, this study demonstrates that conjunctival vessels share these same physiological alterations in patients with established atherosclerotic CAD. Further evaluation of this conjunctival microvascular screening modality in patients with invasively assessed coronary microvascular dysfunction would be of clinical interest.

A reduction in both microvascular axial/cross-sectional velocity and blood flow have previously been reported in association with CVD (33 - 36). In our study these parameters significantly differed across all vessel sizing groups, consistent with these measurements having a significant correlation with the presence of atherosclerotic disease.

The microvascular alterations observed in this study were more marked than those previously reported in a comparison of the MI cohort to patients deemed to have low CV risk (as estimated by convention CV risk calculators) in the absence coronary imaging (26). This furthers the argument that conjunctival microvascular dysfunction correlates with CAD and warrants dedicated evaluation to predict CV risk.

LIMITATIONS

The MI cohort were recruited within a few days of their clinical event. This study therefore compares microvascular indices in stable patients with individuals following an acute event, in whom both a reduction in cardiac output and systemic inflammatory response may be present. The NO-CAD cohort also encompassed a wide range of coronary pathology. Despite these patients having non-obstructive coronaries, there was a spectrum ranging from those with no coronary atheroma, to those with at least moderate coronary atheroma in the absence of physiologically significant luminal obstruction. Some included NO-CAD patients therefore might be considered not to be conventionally at low CV risk nor free from coronary atherosclerosis.

In this study we do not differentiate between conjunctival arterioles and venules, which would potentially add to the discriminatory ability of this vascular screening modality. Future research into the benefit of conjunctival vascular screening should focus on evaluation of the technique in stable symptomatic and asymptomatic

coronary disease, in order to evaluate the potential utility to augment conventional CV risk assessment.

CONCLUSION

This study highlights the ability of an iPhone coupled with slit-lamp biomicroscope to detect differences in conjunctival microcirculatory function between patients with and without obstructive coronary artery disease. The differences observed between these populations were more significant than those reported previously in a low-risk population without coronary imaging. These findings warrant further investigation in future research. Importantly, a definition of abnormal vs normal conjunctival parameters needs to be established for the purpose of CV risk categorisation. These results suggest the potential for this conjunctival imaging modality to be utilized for the detection of asymptomatic CVD to augment conventional CV screening.

REFERENCES

1. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, Benetos A, Biffi A, Boavida JM, Capodanno D, Cosyns B, Crawford C, Davos CH, Desormais I, Di Angelantonio E, Franco OH, Halvorsen S, Hobbs FDR, Hollander M, Jankowska EA, Michal M, Sacco S, Sattar N, Tokgozoglu L, Tonstad S, Tsioufis KP, van Dis I, van Gelder IC, Wanner C, Williams B; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7;42(34):3227-3337. doi: 10.1093/eurheartj/ehab484. PMID: 34458905.

2. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A, Brauer M, Brodmann M, Cahill TJ, Carapetis J, Catapano AL, Chugh SS, Cooper LT, Coresh J, Criqui M, DeCleene N, Eagle KA, Emmons-Bell S, Feigin VL, Fernández-Solà J, Fowkes G, Gakidou E, Grundy SM, He FJ, Howard G, Hu F, Inker L, Karthikeyan G, Kassebaum N, Koroshetz W, Lavie C, Lloyd-Jones D, Lu HS, Mirijello A, Temesgen AM, Mokdad A, Moran AE, Muntner P, Narula J, Neal B, Ntsekhe M, Moraes de Oliveira G, Otto C, Owolabi M, Pratt M, Rajagopalan S, Reitsma M, Ribeiro ALP, Rigotti N, Rodgers A, Sable C, Shakil S, Sliwa-Hahnle K, Stark B, Sundström J, Timpel P, Tleyjeh IM, Valgimigli M, Vos T, Whelton PK, Yacoub M, Zuhlke L, Murray C, Fuster V; GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020 Dec 22;76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010. Erratum in: J Am Coll Cardiol. 2021 Apr 20;77(15):1958-1959. PMID: 33309175; PMCID: PMC7755038.

3. Libby P, Theroux P. Pathophysiology of coronary artery disease .*Circulation* . .3488-3481:(25)111;2005PubMedGoogle ScholarCrossref

4. Little WC, Constantinescu M, Applegate RJ ,et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease ?*Circulation*78;1988 .(5 pt 1):1157-1166 .

5. Falk E, Shah PK, Fuster V. Coronary plaque disruption .*Circulation* . .671-657:(3)92;1995

6. Ambrose JA, Tannenbaum MA, Alexopoulos D ,et al. Angiographic progression of coronary artery disease and the development of myocardial infarction .*J Am Coll Cardiol* .62-56:(1)12;1988 .

7. Giroud D, Li JM, Urban P, Meier B, Rutishauer W. Relation of the site of acute myocardial infarction to the most severe coronary arterial stenosis at prior angiography .*Am J Cardiol*.732-729:(8)69;1992 .

8. Fuster V. Lewis A. Conner Memorial Lecture: Mechanisms leading to myocardial infarction: insights from studies of vascular biology .*Circulation* 21-2126:(4)90;1994 .

9. Shah PK. Molecular mechanisms of plaque instability .*Curr Opin Lipidol* . 499-492:(5)18;2007

10. Nicholls SJ, Nissen SE, Prati F, Windecker S, Kataoka Y, Puri R, Hucko T, Kassahun H, Liao J, Somaratne R, Butters J, Di Giovanni G, Jones S, Psaltis PJ. Assessing the impact of PCSK9 inhibition on coronary plaque phenotype with optical coherence tomography: rationale and design of the randomized, placebo-controlled HUYGENS study. Cardiovasc Diagn Ther. 2021 Feb;11(1):120-129. doi: 10.21037/cdt-20-684. PMID: 33708484; PMCID: PMC7944215.

11. Brennan PF, McNeil AJ, Jing M, Awuah A, Moore JS, Mailey J, Finlay DD, Blighe K, McLaughlin JAD, Nesbit MA, Trucco E, Moore TCB, Spence MS. Assessment of the conjunctival microcirculation for patients presenting with acute myocardial infarction compared to healthy controls. Sci Rep. 2021 Apr 7;11(1):7660. doi: 10.1038/s41598-021-87315-7. PMID: 33828174; PMCID: PMC8027463.

12. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD; Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018 Oct 30;72(18):2231-2264. doi: 10.1016/j.jacc.2018.08.1038. Epub 2018 Aug 25. PMID: 30153967.

13. Brennan PF, et al. Quantitative assessment of the conjunctival microcirculation using a smartphone and slit-lamp biomicroscope. *Microvasc. Res.* 2019;**126**:103907. doi: 10.1016/j.mvr.2019.103907.

14. Brennan, PF., et al. Assessment of the conjunctival microcirculation in adult patients with cyanotic congenital heart disease compared to healthy controls, Microvasc. Res. 136, 9 p., 104167.

15. Duerden M, O'Flynn N, Qureshi N. Cardiovascular risk assessment and lipid modification: NICE guideline. Br J Gen Pract. 2015 Jul;65(636):378-80. doi: 10.3399/bjgp15X685933. PMID: 26120133; PMCID: PMC4484941.

16. Lin JS, Evans CV, Johnson E, Redmond N, Coppola EL, Smith N. Nontraditional Risk Factors in Cardiovascular Disease Risk Assessment: Updated Evidence

Report and Systematic Review for the US Preventive Services Task Force. JAMA 2018;320:281297.

17. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart 2012;98:177184.

18. Vliegenthart R, Oudkerk M, Hofman A, Oei HH, van Dijck W, van Rooij FJ, Witteman JC. Coronary calcification improves cardiovascular risk prediction in the elderly. Circulation. 2005 Jul 26;112(4):572-7. doi: 10.1161/CIRCULATIONAHA.104.488916. Epub 2005 Jul 11. PMID: 16009800.

19. Hadamitzky M, Täubert S, Deseive S, Byrne RA, Martinoff S, Schömig A, Hausleiter J. Prognostic value of coronary computed tomography angiography during 5 years of follow-up in patients with suspected coronary artery disease. Eur Heart J. 2013 Nov;34(42):3277-85. doi: 10.1093/eurheartj/eht293. Epub 2013 Sep 24. PMID: 24067508.

20. Bergström G, Persson M, Adiels M, Björnson E, Bonander C, Ahlström H, Alfredsson J, Angerås O, Berglund G, Blomberg A, Brandberg J, Börjesson M, Cederlund K, de Faire U, Duvernoy O, Ekblom Ö, Engström G, Engvall JE, Fagman E, Eriksson M, Erlinge D, Fagerberg B, Flinck A, Gonçalves I, Hagström E, Hjelmgren O, Lind L, Lindberg E, Lindqvist P, Ljungberg J, Magnusson M, Mannila M, Markstad H, Mohammad MA, Nystrom FH, Ostenfeld E, Persson A, Rosengren A, Sandström A, Själander A, Sköld MC, Sundström J, Swahn E, Söderberg S, Torén K, Östgren CJ, Jernberg T. Prevalence of Subclinical Coronary Artery Atherosclerosis in the General Population. Circulation. 2021 Sep 21;144(12):916-

929. doi: 10.1161/CIRCULATIONAHA.121.055340. Epub 2021 Sep 20. PMID: 34543072; PMCID: PMC8448414

21. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, Engstrom G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intimamedia thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA 2012;308:796803.

22. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:13181327.

23. Maddox TM, Stanislawski MA, Grunwald GK, Bradley SM, Ho PM, Tsai TT, Patel MR, Sandhu A, Valle J, Magid DJ, Leon B, Bhatt DL, Fihn SD, Rumsfeld JS. Nonobstructive coronary artery disease and risk of myocardial infarction. JAMA. 2014 Nov 5;312(17):1754-63. doi: 10.1001/jama.2014.14681. PMID: 25369489; PMCID: PMC4893304.

24. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd

KG, Berry C. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. J Am Coll Cardiol. 2018 Dec 11;72(23 Pt A):2841-2855. doi: 10.1016/j.jacc.2018.09.006. Epub 2018 Sep 25. PMID: 30266608.

25. Stokes KY, Granger DN. The microcirculation: A motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolemia? *J. Physiol.* 2005;**562**:647–653. doi: 10.1113/jphysiol.2004.079640

26. Zhong Z, Petrig BL, Qi X, Burns A. In vivo measurement of erythrocyte velocity and retinal blood flow using adaptive optics scanning laser ophthalmoscopy. *Opt. Express.* 2018;**16**(17):12746–12756. doi: 10.1364/OE.16.012746.

27. Demir SU, Hakimzadeh R, Hargraves RH, Ward KR, Myer EV, Najarian K. An automated method for analysis of microcirculation videos for accurate assessment of tissue perfusion. *BMC Med. Imaging.* 2012;**12**:37. doi: 10.1186/1471-2342-12-37

28. Fagrell B, Fronek A, Intaglietta M. A microscope-television system for studying flow velocity in human skin capillaries. *Am. J. Physiol.* 1997;**233**(2):318–321

29. Rim TH, Lee CJ, Tham YC, Cheung N, Yu M, Lee G, Kim Y, Ting DSW, Chong CCY, Choi YS, Yoo TK, Ryu IH, Baik SJ, Kim YA, Kim SK, Lee SH, Lee BK, Kang SM, Wong EYM, Kim HC, Kim SS, Park S, Cheng CY, Wong TY. Deep-learning-based cardiovascular risk stratification using coronary artery calcium scores predicted from retinal photographs. Lancet Digit Health. 2021 May;3(5):e306-e316. doi: 10.1016/S2589-7500(21)00043-1. PMID: 33890578.

30. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and No Obstructive Coronary Artery Disease (INOCA): Developing Evidence-Based Therapies and

Research Agenda for the Next Decade. Circulation. 2017 Mar 14;135(11):1075-1092.

31. Ford TJ, Corcoran D, Berry C. Stable coronary syndromes: pathophysiology, diagnostic advances and therapeutic need. Heart 2018;104:284-292.

32. Kaski JC, Crea F, Gersh BJ, Camici PG. Reappraisal of Ischemic Heart Disease. Circulation. 2018;138:1463-1480.

33. Cheung, A. T. W. et al. Microvascular abnormalities in the bulbar conjunctiva of patients with type 2 diabetes mellitus. Endocr. Pract. 7, 358–363 (2001).

34. Sadr-Ameli, M. A. et al. Coronary slow flow: Benign or ominous?. Anatol. J. Cardiol. 15(7), 531–535 (2014).

35. Kord Valeshabad, A. et al. Feasibility of assessment of conjunctival microvascular hemodynamics in unilateral ischemic stroke. Microvasc. Res. 100, 4–8 (2015).

36. Khansari, et al. Assessment of conjunctival microvascular hemodynamics in stages of diabetic microvasculopathy. Sci. Rep. 7, 45916 (2017).

FIGURES

Figure 1. The iPhone 6s, TopCon SL-D4 imaging system with the Zarf bespoke adapter (red arrow) and TopCon external fixation target (green arrow)

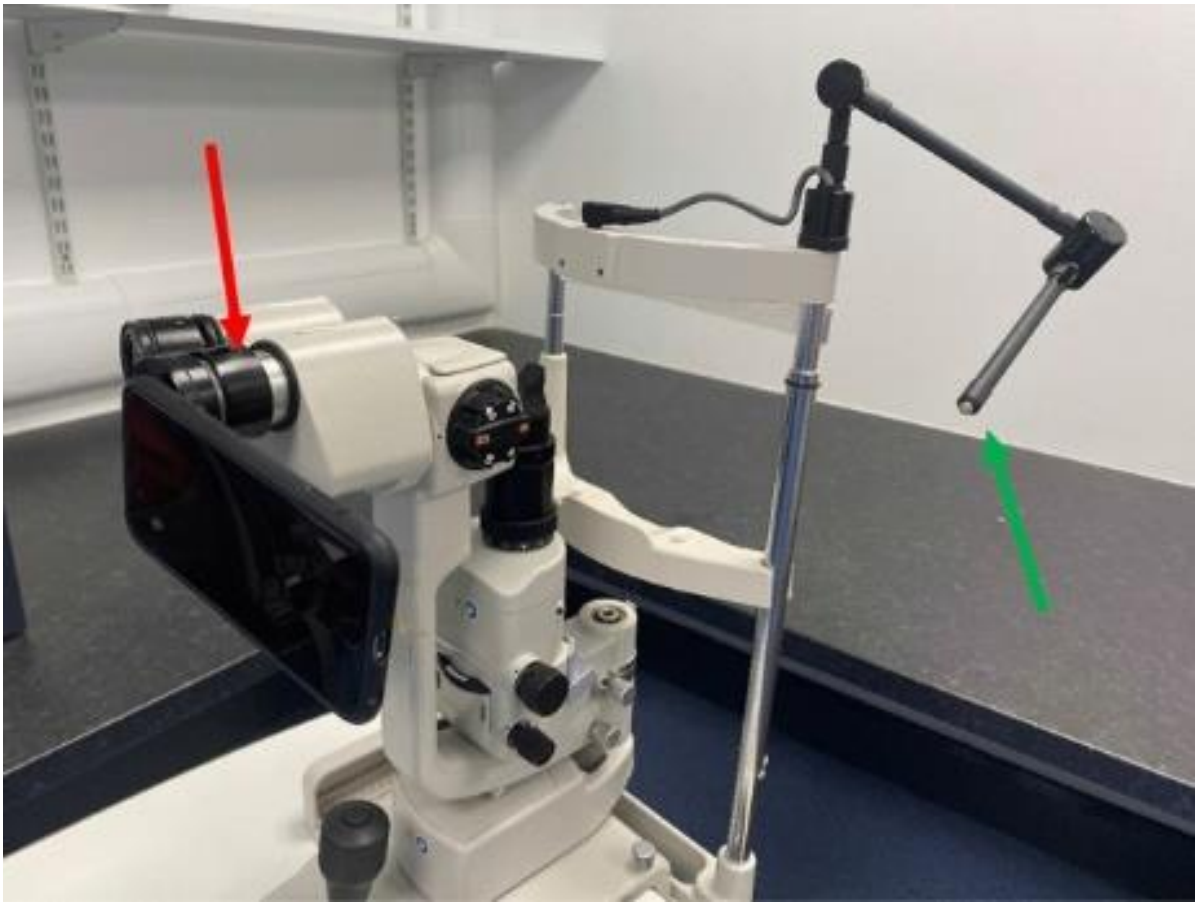


Figure 2. Stabilised video frame of conjunctival microvascular network

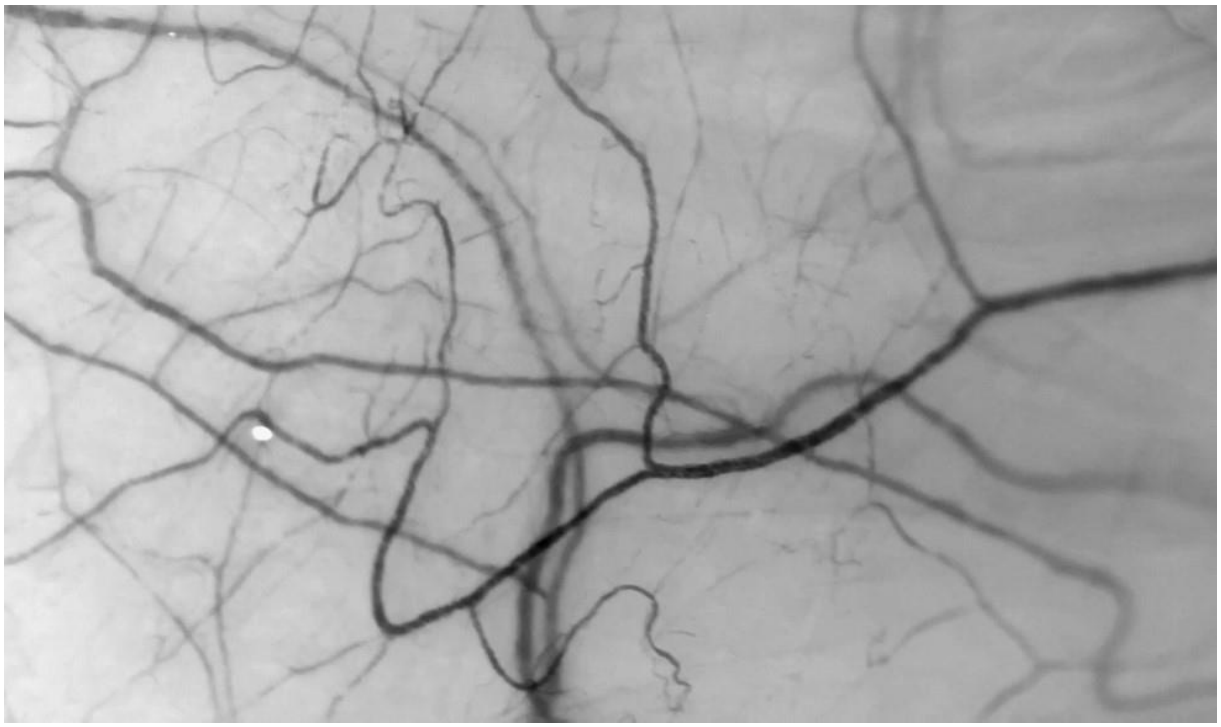


Figure 3. Boxplots comparing conjunctival haemodynamics of the MI and NO-CAD cohorts

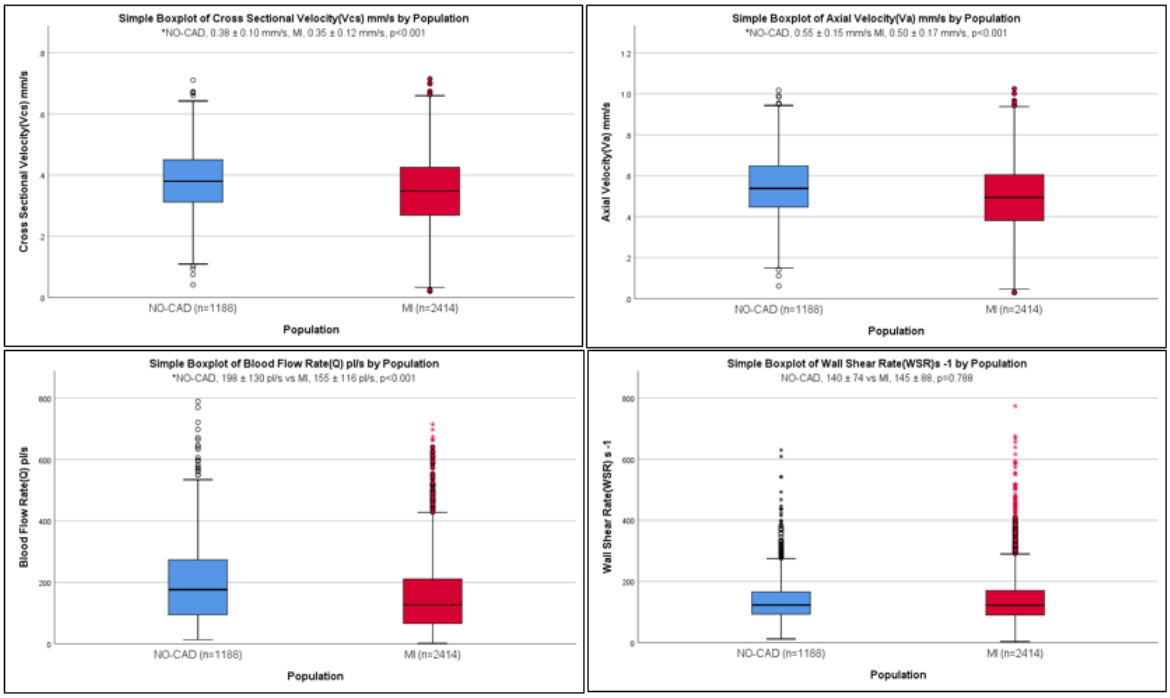
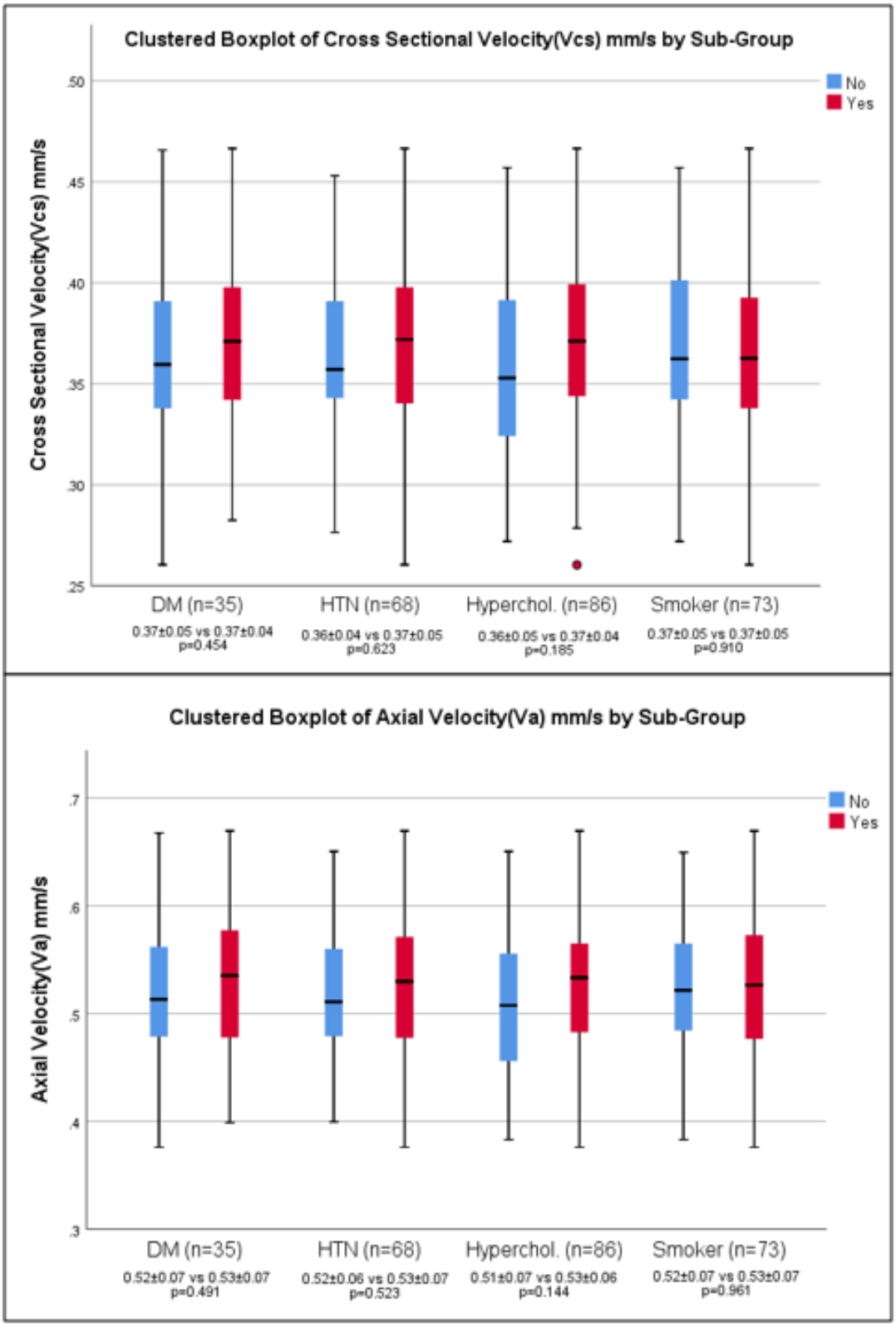


Figure 4. A comparison of axial and cross-sectional velocity in patients with and without relevant conventional vascular risk factors (diabetes mellitus, hypertension, hypercholesterolemia and smoking history)



TABLES

Table 1. Baseline characteristics

Characteristic	**NO-CAD (n = 61)**	**MI (n = 66)**	**p value**
Age- *yrs ± SD*	63 ± 10	57 ± 11	**0.003**
Male sex- *n (%)*	30 (49.2)	52 (78.8)	**<0.001**
BMI- *kg/m^2 ± SD*	30.2 ± 5.6	28.6 ± 4.7	0.10
Prior PCI- *n (%)*	0 (0.0)	9 (13.6)	**0.003**
Prior MI- *n (%)*	0 (0.0)	9 (13.6)	**0.003**
Hypertension- *n (%)*	37 (60.7)	31 (47.0)	0.12
Diabetes mellitus- *n (%)*	20 (32.8)	15 (22.7)	0.21
Smoking history- *n (%)*	30 (49.2)	43 (65.2)	0.07
HbA1c- *mmol/mol ± SD*	46.0 ± 16.0	45.4 ± 17.0	0.66
Creatinine clearance- *ml/min ± SD*	97 ± 41	104 ± 36	0.07
Haemoglobin- *g/l ± SD*	137 ± 14	144 ± 16	**0.04**
Total cholesterol- *mmol/l ± SD*	3.7 ± 0.9	4.6 ± 1.5	**<0.001**

Table 2. Comparison of conjunctival microcirculatory parameters

Parameter	**NO-CAD (n = 1188)**	**MI (n = 2414)**	**p value**
Diameter- *µm ± SD*	24.7 ± 7.8	22.4 ± 7.5	**< 0.001**
Axial velocity- *mm/s ± SD*	0.55 ± 0.15	0.50 ± 0.17	**< 0.001**
Cross-sectional velocity-*mm/s ± SD*	0.38 ± 0.10	0.35 ± 0.12	**< 0.001**
Blood flow rate- *pl/s ± SD*	198 ± 130	154 ± 116	**< 0.001**
Wall shear rate- s^{-1} *± SD*	140 ± 74	144 ± 88	0.78

Table 3. Comparison of conjunctival microcirculatory parameters based on vessel group

10 – 17 µm			
Parameter	**NO-CAD (n = 189)**	**MI (n = 466)**	**p-value**
Diameter- *µm ± SD*	14.0 ± 1.9	13.8 ± 1.9	0.26
Axial velocity- *mm/s ± SD*	0.53 ± 0.13	0.47 ± 0.15	**< 0.001**
Cross-sectional velocity-*mm/s ± SD*	0.39 ± 0.10	0.35 ± 0.12	**< 0.001**
Blood flow rate- *pl/s ± SD*	61.3 ± 22.0	52.9 ± 22.2	**< 0.001**
Wall shear rate- s^{-1} ± *SD*	230 ± 67	207 ± 78	**< 0.001**
17 – 23 µm			
Parameter	**NO-CAD (n = 282)**	**MI (n = 667)**	**p-value**
Diameter- *µm ± SD*	20.1 ± 1.8	20.2 ± 1.7	0.18
Axial velocity- *mm/s ± SD*	0.54 ± 0.14	0.48 ± 0.16	**<0.001**
Cross-sectional velocity-*mm/s ± SD*	0.38 ± 0.10	0.34 ± 0.11	**<0.001**
Blood flow rate- *pl/s ± SD*	121 ± 39	109 ± 42	**<0.001**
Wall shear rate- s^{-1} ± *SD*	151 ± 42	134 ± 47	**<0.001**
23 – 29 µm			
Parameter	**NO-CAD (n = 319)**	**MI (n = 685)**	**p-value**
Diameter- *µm ± SD*	26.0 ± 1.7	25.9 ± 1.7	0.30
Axial velocity- *mm/s ± SD*	0.55 ± 0.15	0.51 ± 0.17	**<0.001**
Cross-sectional velocity-*mm/s ± SD*	0.37 ± 0.10	0.35 ± 0.12	**<0.001**

Blood flow rate- *pl/s ± SD*	199 ± 60	285 ± 69	**<0.001**
Wall shear rate- s^{-1} ± *SD*	116 ± 32	108 ± 36	**<0.001**
29 – 36 µm			
Parameter	**NO-CAD (n = 282)**	**MI (n = 378)**	**p-value**
Diameter- *µm ± SD*	32.0 ± 1.9	31.7 ± 1.9	0.05
Axial velocity- *mm/s ± SD*	0.57 ± 0.16	0.54 ± 0.16	**0.01**
Cross-sectional velocity-*mm/s ± SD*	0.38 ± 0.11	0.36 ± 0.11	**0.01**
Blood flow rate- *pl/s ± SD*	308 ± 98	287 ± 98	**0.001**
Wall shear rate- s^{-1} ± *SD*	96 ± 28	91 ± 27	**0.03**

Table 4. Logistic regression analysis of independent predictors of myocardial infarction

Variable	Univariate		Multivariate	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Age	0.950 (0.917 – 0.984)	**0.004**	0.961 (0.922 – 1.003)	0.07
Male sex	3.838 (1.769 – 8.329)	**<0.001**	3.685 (1.538 – 8.828)	**0.003**
Smoking	1.932 (0.947 – 3.941)	0.07	2.450 (1.046 – 5.738)	**0.04**
Hypertension	0.575 (0.284 – 1.163)	0.12	0.903 (0.377 – 2.163)	0.82
Diabetes mellitus	0.603 (0.275 – 1.323)	0.21	0.755 (0.297 – 1.916)	0.55
Hypercholesterolaemia	0.264 (0.117 – 0.595)	**0.001**	0.392 (0.152 – 1.011)	0.053
Conjunctival axial velocity < 0.50mm/s	3.067 (1.439 – 6.535)	**0.004**	3.514 (1.469 – 8.406)	**0.005**

Assessment of indices of conjunctival microvascular function in patients with and without obstructive coronary artery disease

Short Title- The association of conjunctival microvascular dysfunction and coronary artery disease

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Conflict of Interest- nil

Integrated Research Application System study number= 166742

Consent and ethical approval- All subjects provided written informed consent for participation in this study. The protocol was approved by the Research Ethics committee in the Belfast Health and Social Care Trust (BHSCT) and Ulster University (UU) and was carried out in accordance with the Declaration of Helsinki.

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Keywords

Prevention; conjunctiva; microvascular dysfunction; cardiovascular screening; coronary artery disease; cardiovascular disease

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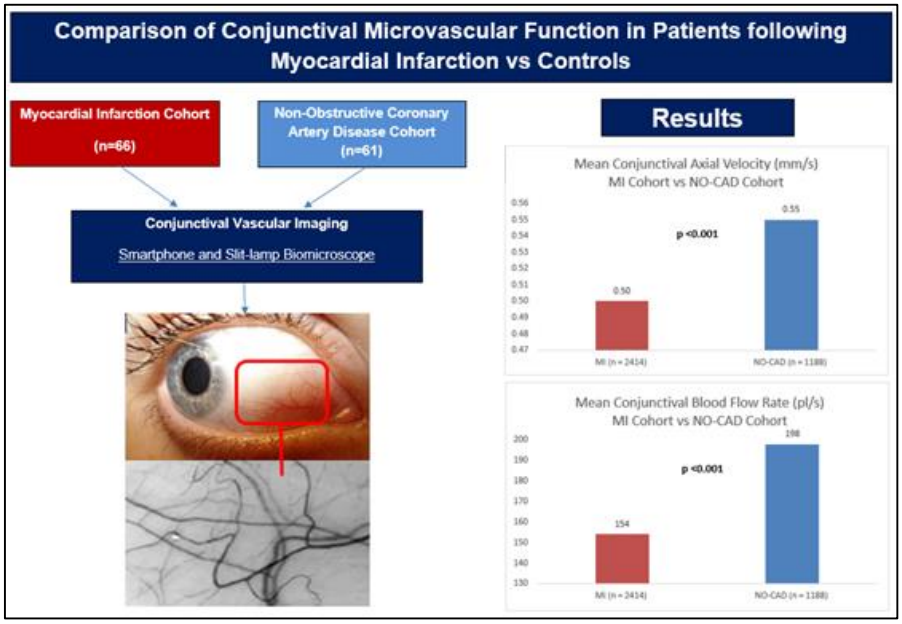
GRAPHICAL ABSTRACT



ABSTRACT

Background

Atherosclerotic heart disease often remains asymptomatic until presentation with a major adverse cardiovascular event. Primary preventive therapies improve outcomes, but conventional screening often misattributes risk. Vascular imaging can be utilised to detect atherosclerosis, but often involves ionising radiation. The conjunctiva is a readily accessible vascular network allowing non-invasive hemodynamic evaluation.

Aim

To compare conjunctival microcirculatory function in patients with and without obstructive coronary artery disease.

Methods

We compared the conjunctival microcirculation of myocardial infarction patients (MI-cohort) to controls with no obstructive coronary artery disease (NO-CAD cohort). Conjunctival imaging was performed using a smartphone and slit-lamp biomicroscope combination. Microvascular indices of axial (V_a) and cross-sectional (V_{cs}) velocity; blood flow rate (Q); and wall shear rate (WSR) were compared in all conjunctival vessels between 5 and 45 μm in diameter.

Results

A total of 127 patients were recruited (66 MI vs 61 NO-CAD) and 3602 conjunctival vessels analysed (2414 MI vs 1188 NO-CAD). Mean V_a, V_{cs} and Q were significantly lower in the MI vs NO-CAD cohort (V_a 0.50 ± 0.17 mm/s vs 0.55 ± 0.15 mm/s, **p <0.001**; V_{cs} 0.35 ± 0.12 mm/s vs 0.38 ± 0.10 mm/s, **p <0.001**; Q 154 ± 116 pl/s vs 198 ± 130 pl/s, **p < 0.001**). To correct for differences in mean vessel diameter, WSR was compared in 10 - 36 µm vessels (3268/3602 vessels) and was lower in the MI-cohort (134 ± 64s^{-1} vs 140 ± 63s^{-1}, **p=0.002**).

Conclusions

Conjunctival microcirculatory alterations can be observed in patients with obstructive coronary artery disease. The conjunctival microvasculature merits further evaluation in cardiovascular risk screening.

INTRODUCTION

Cardiovascular disease (CVD) represents a significant cause of morbidity and mortality worldwide (1). Coronary artery disease (CAD) represents the most prevalent form of CVD (2). A large proportion of patients with CAD remain asymptomatic until first presentation with a major adverse cardiac event (MACE). This observation forms the basis for guideline recommendations to perform CVD screening in asymptomatic adults (1). Several studies have highlighted that the majority of atherosclerotic plaque rupture events and resultant myocardial infarctions (MI) arise from non-obstructive plaques (3-9). The recent HUYGENS study (10) highlighted the ability for statins and PCSK9 inhibitors to positively remodel vulnerable plaques, highlighting the potential value of such evidence-based medications in CV risk reduction. Identification of individuals who will benefit from targeted primary preventative therapies remains a challenging issue, prompting research into novel CV screening modalities.

~~The European Society of Cardiology (ESC) recommend the use of SCORE 2 for screening in adults aged 40-69 years of age and SCORE 2-OP in adults ≥70 years of age (1). The National Institute for Clinical Excellence (NICE) recommend the use of QRISK III (11). These CVD risk calculators rely on the identification of conventional CVD risk factors to estimate the long-term probability of either a fatal or non-fatal MACE.~~

~~Conventional screening modalities estimate a large proportion of patients to be in a low-intermediate CV risk category. It is therefore recommended to establish other modifiers of CV risk, such as the presence of diabetes mellitus, chronic kidney disease, familial dyslipidaemias or through the demonstration of established asymptomatic CVD (1). The latter can be observed through performance of computed tomography (CT) coronary artery calcium scoring to detect atherosclerotic plaques and grade total burden of CAD in comparison to individuals of a similar age and sex (12-15). A recent study evaluating CT coronary angiography (CTCA) demonstrated a high prevalence of asymptomatic atherosclerotic coronary disease in the evaluated population, with as many as 42.1% of participants having silent atherosclerosis (16). Other modalities such as carotid ultrasound or the measurement of arterial stiffness using pulse wave velocity have been studied, but not found to be beneficial in CV risk screening to date (17, 18). CT as a screening modality; whilst being highly sensitive, is limited by availability, cost and exposure of the patient to ionizing radiation. These factors limit the widespread introduction of CT for population CV screening programmes.~~

~~The presence of coronary microvascular dysfunction not only can result in symptoms of angina, but is also prognostically adverse (19, 20). Microvascular dysfunction occurs in the initial manifestations of CVD (21). Detection of microvascular dysfunction, therefore, has the potential to augment conventional CVD screening and allow early initiation of guideline based medical therapies to reduce MACE. The diagnosis of coronary microvascular disease involves invasive coronary angiography and exposure of the patient to procedural risks. However, several previous studies have demonstrated the ability to non-invasively assess the microvasculature of the~~

~~retinal, sublingual and nail-fold circulation (22–24). Recently, a novel cardiovascular disease risk stratification system using retinal photography was found to be comparable to conventional CT coronary artery calcium scoring in predicting MACE over a 5-year follow-up period using UK Biobanks and cohorts from South Korea and Singapore (*n* > 70,000) (25).~~

Our research group previously reported the ability to non-invasively assess the conjunctival microcirculation using an iPhone coupled with a slit-lamp biomicroscope. (11~~26~~) Statistically significant differences were observed in indices of conjunctival microvascular function when comparing a cohort of patients admitted to hospital with a myocardial infarction (MI) and a cohort of age and sex-matched controls estimated to be at low CV risk using the Q-Risk 3 score. In this study mean axial velocity for the controls was 0.53 ± 0.15 mm/s compared to 0.49 ± 0.17 mm/s for the MI patients ($p < 0.001$). Wall shear rate was higher for controls than MI patients (162 ± 93 s^{-1} vs 145 ± 88 s^{-1}, $p < 0.001$). Blood volume flow did not differ significantly for the controls and MI patients (153 ± 124 pl/s vs 154 ± 125 pl/s, $p = 0.84$), this however was largely due to differences in the mean vessel diameter between the groups (controls 21.41 ± 7.57 vs MI 22.32 ± 7.66 μm). This study highlighted the potential for conjunctival imaging to be utilised for CV risk assessment in asymptomatic patients. It was however limited by the absence of coronary imaging in the low-risk cohort to identify patients with asymptomatic CAD potentially confounding the results obtained.

In this study we compare indices of conjunctival microvascular function in the previously reported MI cohort to a group of patients with no obstructive epicardial coronary artery disease detected during an invasive coronary angiogram, and no personal history of either MI or percutaneous coronary intervention (PCI).

METHODS

We conducted a prospective study (Integrated Research Application System study number 166742) comparing a group of patients with a recent MI (MI cohort) to a group of patients with no obstructive coronary artery disease (NO-CAD cohort) as demonstrated by a coronary angiogram and physiological evaluation of any intermediate coronary stenoses.

All subjects provided written informed consent for participation in this study. The protocol was approved by the Research Ethics committee in the Belfast Health and Social Care Trust (BHSCT) and Ulster University (UU) and was carried out in accordance with the Declaration of Helsinki.

Baseline clinical data and characteristics were obtained using the recruitment questionnaire, inpatient clinical notes (MI cohort), hospital cardiology database and the patient's electronic healthcare record.

MI cohort

Patients were deemed eligible for inclusion in the MI cohort if they were an inpatient with a type 1 MI as defined by the European society of cardiology (ESC) 4th universal definition of myocardial infarction (12~~27~~). The MI cohort was comprised of patients presenting with both ST-elevation myocardial infarction (STEMI) and non ST-elevation myocardial infarction (NSTEMI).

NO-CAD cohort

Patients were deemed eligible for inclusion in the NO-CAD cohort if they had no past medical history of MI or previous coronary revascularisation by either PCI or coronary artery bypass grafting (CABG). All patients in this cohort were recruited following an invasive coronary angiogram that excluded obstructive epicardial coronary disease. At the time of angiography any intermediate coronary lesions were physiologically assessed by pressure wire evaluation. Patients were included in this cohort only if fractional flow reserve (FFR) in any intermediate lesions was ≥0.80 (i.e. considered to be physiologically non-obstructive).

The majority of patients in this cohort were admitted electively (72%) for the investigation of stable symptoms of chest pain or dyspnoea. The remainder underwent inpatient coronary angiography due to presentation to the hospital emergency department with chest pain. Inpatients were only recruited to this cohort if their presenting symptoms were deemed to be non-cardiac in origin by the referring

clinician, with no identifiable cardiovascular cause for admission (i.e. no elevation in serum troponin, no ECG changes and no echocardiographic findings of hemodynamically significant valvular abnormalities or impairment in biventricular function).

Exclusion criteria

Exclusion criteria for both groups included pregnancy, age less than 18 years old, inability to consent and history of recent conjunctival inflammation or current use of contact lenses.

Conjunctival Microvascular Assessment

Conjunctival imaging was performed using a commercially available Topcon SL-D4 (Topcon Medical Systems Inc., USA), an iPhone smartphone (Apple, Inc, USA) and a bespoke adapter (Zarf Enterprises Inc., USA) (see **Figure 1**). We acquired 5–10 s videos of the conjunctival microcirculation in both nasal and temporal views, thus generating four videos per subject. All videos then underwent a process of stabilisation, image registration and then semi-automated analysis of microvascular parameters. The imaging platform is calibrated to define a pixel to millimetre (mm) conversion factor. This conversion factor is then used to estimate vessel diameter (D). Axial (V_a) and cross-sectional (V_{cs}) velocity are then estimated using this conversion factor coupled with an app that allows blood flow tracking and hence calculation of distance travelled over a 1 second stabilised video clip. Blood flow (Q) and wall shear rate (WSR) are then estimated from the results of D and V_{cs}. This

technique has been described in 3 previous studies (11~~26~~, 13~~28~~, 14~~29~~). **Figure 2** gives an example of a video frame showing the conjunctival microvascular network obtained from our imaging platform, following the process of video stabilisation. Conjunctival vessel diameter, V_a, V_{cs}, Q and WSR were assessed in vessels with observable blood flow. We analysed and report results for vessels between 5 and 45 µm in diameter.

Given the significant impact that vessel diameter creates on the parameters of Q [defined as $Q=V_{cs}(\pi r^2)$] and WSR (defined as $WSR=8V_{cs}/D$), it is important to standardise the range of vessels analysed in order to evaluate microvascular indices in vessels of comparable size. To avoid significant outliers in vessel diameter skewing the results we therefore conducted a sub-analysis of conjunctival vessels between 10 and 36 µm in diameter. This range was selected as it excluded 5% of vessels at both the upper and lower end of the diameter range (n=3268). Vessels in this diameter range were then classified into 4 distinct diameter groups (D 10 - 17µm, D 17 - 23µm, D 23 - 29µm and D 29 - 36µm).

Statistical Analysis

For statistical analysis SPSS for Apple iOS version 26 (property of IBM) was used. Continuous variables were described using the mean, standard deviation of the mean and 95% confidence intervals (CI). Kolmogorov–Smirnov testing was used to assess normality of the continuous variables. Categorical variables were expressed

as a number and percentage of the total category number to which the variable belonged.

Normally distributed variables were compared between the two populations using the independent-samples t-test. Non-normally distributed continuous variables were compared using a non-parametric test (Mann–Whitney U test). Categorical comparisons were made between the two groups using Pearson Chi-Square or Fisher's exact test.

RESULTS

Baseline Characteristics

Between 31st January 2018 and 1st October 2021, 127 patients were recruited to this study. A total of 61 patients were included in the NO-CAD cohort and 66 patients were included in the MI cohort. The mean ages were 63 ± 10 years and 57 ± 11 years respectively in these cohorts (**p=0.003**). A higher proportion of patients in the MI cohort were male (78.8% vs 49.2%, **p<0.001**). There was no statistically significant difference in the prevalence of systemic hypertension, diabetes mellitus and smoking between the groups (p=0.12, p=0.21 and 0.07, respectively). **Table 1** provides a comparative summary of the patients' baseline characteristics. Of note, the prevalence of conventional cardiovascular risk factors (hypertension, smoking and diabetes mellitus) was higher in the NO-CAD cohort than would be anticipated in the general population.

At the time of recruitment, both patient cohorts were normotensive, but the NO-CAD cohort had a higher mean systolic blood pressure (126.3 ± 13.6mmHg vs 120.4 ± 16.4mmHg, **p=0.03**).

All patients in the MI cohort underwent invasive coronary angiography. 4 (6.1%) of these patients were managed medically and the remaining 62 (93.9%) patients underwent either percutaneous or surgical revascularisation. All patients that underwent surgical revascularisation were recruited and had conjunctival imaging performed prior to surgery.

Indices of conjunctival microvascular function

Conjunctival video imaging was obtained for all patients. There were no adverse clinical events during conjunctival imaging. All image processing and subsequent quantitative microvascular assessment was performed by study investigators blinded to the clinical characteristics at the time of the analysis to prevent bias.

A total of 3602 vessel segments were analysable across the two cohorts (1188 in the NO-CAD cohort vs 2414 in the MI cohort). A mean of 28.3 vessel segments were assessable per patient. A comparison of conjunctival microcirculatory parameters is summarised in **Table 2,** and the range of results presented in **Figure 3.** Statistically significant differences were observed between cohorts for D, V_a, V_{cs} and Q.

There was no difference in wall shear rate (WSR) between cohorts; however WSR is inversely related to diameter. The lack of difference therefore related to the difference observed in mean diameter, which was significantly lower in the MI cohort (24.73 ± 7.79 vs 22.41 ± 7.46; **p < 0.001**). As described above, to compensate for this discrepancy in mean diameter, we conducted a sub-analysis of microvascular indices in vessels between 10 and 36 μm in diameter (n=3268/3602). In this sub-analysis, significant differences were observed in all conjunctival parameters (V_a 0.50 ± 0.17 mm/s vs 0.55 ± 0.15 mm/s, **p <0.001**; V_{cs} 0.35 ± 0.11 mm/s vs 0.38 ± 0.10 mm/s, **p <0.001**; Q 152 ± 100 pl/s vs 183 ± 108 pl/s, **p < 0.001;** and WSR 134 ± 64 s^{-1} vs 140 ± 63 s^{-1}, **p=0.002**).

A further analysis comparing the cohorts by 4 previously described sizing groups revealed significant reductions in V_a, V_{cs}, Q and WSR across all groups (see **Table 3**).

Figure 4 demonstrates a comparison of conjunctival vessel axial and cross-sectional velocity in patients with and without an established history of several conventional vascular risk factors (diabetes mellitus, hypertension, hypercholesterolemia and smoking history). There were no significant differences in mean axial or cross-sectional velocity in any of these groups. Given significant between group differences in some baseline characteristics (e.g. age, sex and conventional CV risk factors) a multivariate analysis was conducted, demonstrating that a reduction in conjunctival V_a below 0.50mm/s was an independent predictor of MI. These findings suggests the observed differences in microvascular parameters in this study are

secondary to the underlying atherosclerotic coronary disease in the MI cohort rather than the presence of other co-morbidities that may impact microvascular function.

DISCUSSION

Opportunistic CV risk screening is recommended by international cardiology guidelines. The European Society of Cardiology (ESC) recommend the use of SCORE 2 for screening in adults aged 40-69 years of age and SCORE 2-OP in adults ≥70 years of age (1). The National Institute for Clinical Excellence (NICE) recommend the use of QRISK III (151). These CVD risk calculators rely on the identification of conventional CVD risk factors to estimate the long-term probability of either a fatal or non-fatal MACE.

Conventional screening modalities estimate a large proportion of patients to be in a low-intermediate CV risk category. It is therefore recommended to establish other modifiers of CV risk, such as the presence of diabetes mellitus, chronic kidney disease, familial dyslipidaemias or through the demonstration of established asymptomatic CVD (1). The latter can be observed through performance of computed tomography (CT) coronary artery calcium scoring to detect atherosclerotic plaques and grade total burden of CAD in comparison to individuals of a similar age and sex (162-195). A recent study evaluating CT coronary angiography (CTCA) demonstrated a high prevalence of asymptomatic atherosclerotic coronary disease in the evaluated population, with as many as 42.1% of participants having silent atherosclerosis (2016). Other modalities such as carotid ultrasound or the measurement of arterial stiffness using pulse wave velocity have been studied, but not found to be beneficial in CV risk screening to date (2117, 2218). CT as a

screening modality; whilst being highly sensitive, is limited by availability, cost and exposure of the patient to ionizing radiation. These factors limit the widespread introduction of CT for population CV screening programmes.

The presence of coronary microvascular dysfunction not only can result in symptoms of angina, but is also prognostically adverse (23~~19~~, 24~~20~~). Microvascular dysfunction occurs in the initial manifestations of CVD (25~~21~~). Detection of microvascular dysfunction, therefore, has the potential to augment conventional CVD screening and allow early initiation of guideline based medical therapies to reduce MACE. The diagnosis of coronary microvascular disease involves invasive coronary angiography and exposure of the patient to procedural risks. However, several previous studies have demonstrated the ability to non-invasively assess the microvasculature of the retinal, sublingual and nail-fold circulation (26~~2~~-28~~4~~). Recently, a novel cardiovascular disease risk stratification system using retinal photography was found to be comparable to conventional CT coronary artery calcium scoring in predicting MACE over a 5-year follow-up period using UK Biobanks and cohorts from South Korea and Singapore (*n* > 70,000) (29~~25~~).

This study demonstrates statistically significant differences in conjunctival microcirculatory function of a group of patients with established CAD and therefore proven to be at very high CV risk in comparison to a group with no obstructive coronary artery disease or previous major adverse cardiovascular event. The underlying pathophysiological mechanisms involved in the development of atherosclerotic vascular disease can be observed earliest in the microcirculation

(251). The ability to detect microvascular dysfunction has the potential to identify asymptomatic patients who may benefit from aggressive primary preventative therapies. The conjunctiva is an easily accessible site to non-invasively assess for microvascular dysfunction without exposing patients to ionising radiation.

Coronary microvascular dysfunction can result in symptoms of angina or dyspnoea that can considerably impact quality of life. Its presence has also been shown to confer an adverse CV prognosis long-term (30 - 32). The diagnosis of coronary microvascular dysfunction is performed invasively based on demonstrating a reduction in coronary blood velocity, and hence a reduction in blood flow rate using pressure wire evaluation and thermodilution techniques. Importantly, this study demonstrates that conjunctival vessels share these same physiological alterations in patients with established atherosclerotic CAD. Further evaluation of this conjunctival microvascular screening modality in patients with invasively assessed coronary microvascular dysfunction would be of clinical interest.

A reduction in both microvascular axial/cross-sectional velocity and blood flow have previously been reported in association with CVD (33 - 36). In our study these parameters significantly differed across all vessel sizing groups, consistent with these measurements having a significant correlation with the presence of atherosclerotic disease.

The microvascular alterations observed in this study were more marked than those previously reported in a comparison of the MI cohort to patients deemed to have low CV risk (as estimated by convention CV risk calculators) in the absence coronary imaging (26). This furthers the argument that conjunctival microvascular dysfunction correlates with CAD and warrants dedicated evaluation to predict CV risk.

LIMITATIONS

The MI cohort were recruited within a few days of their clinical event. This study therefore compares microvascular indices in stable patients with individuals following an acute event, in whom both a reduction in cardiac output and systemic inflammatory response may be present. The NO-CAD cohort also encompassed a wide range of coronary pathology. Despite these patients having non-obstructive coronaries, there was a spectrum ranging from those with no coronary atheroma, to those with at least moderate coronary atheroma in the absence of physiologically significant luminal obstruction. Some included NO-CAD patients therefore might be considered not to be conventionally at low CV risk nor free from coronary atherosclerosis.

In this study we do not differentiate between conjunctival arterioles and venules, which would potentially add to the discriminatory ability of this vascular screening modality. Future research into the benefit of conjunctival vascular screening should focus on evaluation of the technique in stable symptomatic and asymptomatic

coronary disease, in order to evaluate the potential utility to augment conventional CV risk assessment.

CONCLUSION

This study highlights the ability of an iPhone coupled with slit-lamp biomicroscope to detect differences in conjunctival microcirculatory function between patients with and without obstructive coronary artery disease. The differences observed between these populations were more significant than those reported previously in a low-risk population without coronary imaging. These findings warrant further investigation in future research. Importantly, a definition of abnormal vs normal conjunctival parameters needs to be established for the purpose of CV risk categorisation. These results suggest the potential for this conjunctival imaging modality to be utilized for the detection of asymptomatic CVD to augment conventional CV screening.

REFERENCES

1. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, Benetos A, Biffi A, Boavida JM, Capodanno D, Cosyns B, Crawford C, Davos CH, Desormais I, Di Angelantonio E, Franco OH, Halvorsen S, Hobbs FDR, Hollander M, Jankowska EA, Michal M, Sacco S, Sattar N, Tokgozoglu L, Tonstad S, Tsioufis KP, van Dis I,

van Gelder IC, Wanner C, Williams B; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7;42(34):3227-3337. doi: 10.1093/eurheartj/ehab484. PMID: 34458905.

2. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A, Brauer M, Brodmann M, Cahill TJ, Carapetis J, Catapano AL, Chugh SS, Cooper LT, Coresh J, Criqui M, DeCleene N, Eagle KA, Emmons-Bell S, Feigin VL, Fernández-Solà J, Fowkes G, Gakidou E, Grundy SM, He FJ, Howard G, Hu F, Inker L, Karthikeyan G, Kassebaum N, Koroshetz W, Lavie C, Lloyd-Jones D, Lu HS, Mirijello A, Temesgen AM, Mokdad A, Moran AE, Muntner P, Narula J, Neal B, Ntsekhe M, Moraes de Oliveira G, Otto C, Owolabi M, Pratt M, Rajagopalan S, Reitsma M, Ribeiro ALP, Rigotti N, Rodgers A, Sable C, Shakil S, Sliwa-Hahnle K, Stark B, Sundström J, Timpel P, Tleyjeh IM, Valgimigli M, Vos T, Whelton PK, Yacoub M, Zuhlke L, Murray C, Fuster V; GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020 Dec 22;76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010. Erratum in: J Am Coll Cardiol. 2021 Apr 20;77(15):1958-1959. PMID: 33309175; PMCID: PMC7755038.

3. Libby P, Theroux P. Pathophysiology of coronary artery disease .*Circulation* . .3488-3481:(25)111;2005PubMedGoogle ScholarCrossref

4. Little WC, Constantinescu M, Applegate RJ ,et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease ?*Circulation*78;1988 .(5 pt 1):1157-1166 .

5. Falk E, Shah PK, Fuster V. Coronary plaque disruption .*Circulation* . .671-657:(3)92;1995

6. Ambrose JA, Tannenbaum MA, Alexopoulos D ,et al. Angiographic progression of coronary artery disease and the development of myocardial infarction .*J Am Coll Cardiol* .62-56:(1)12;1988 .

7. Giroud D, Li JM, Urban P, Meier B, Rutishauer W. Relation of the site of acute myocardial infarction to the most severe coronary arterial stenosis at prior angiography .*Am J Cardiol*.732-729:(8)69;1992 .

8. Fuster V. Lewis A. Conner Memorial Lecture: Mechanisms leading to myocardial infarction: insights from studies of vascular biology .*Circulation* 21-2126:(4)90;1994 .

9. Shah PK. Molecular mechanisms of plaque instability .*Curr Opin Lipidol* . 499-492:(5)18;2007

10. Nicholls SJ, Nissen SE, Prati F, Windecker S, Kataoka Y, Puri R, Hucko T, Kassahun H, Liao J, Somaratne R, Butters J, Di Giovanni G, Jones S, Psaltis PJ. Assessing the impact of PCSK9 inhibition on coronary plaque phenotype with optical coherence tomography: rationale and design of the randomized, placebo-controlled HUYGENS study. Cardiovasc Diagn Ther. 2021 Feb;11(1):120-129. doi: 10.21037/cdt-20-684. PMID: 33708484; PMCID: PMC7944215.

11. Brennan PF, McNeil AJ, Jing M, Awuah A, Moore JS, Mailey J, Finlay DD, Blighe K, McLaughlin JAD, Nesbit MA, Trucco E, Moore TCB, Spence MS. Assessment of the conjunctival microcirculation for patients presenting with acute myocardial infarction compared to healthy controls. Sci Rep. 2021 Apr 7;11(1):7660. doi: 10.1038/s41598-021-87315-7. PMID: 33828174; PMCID: PMC8027463.

12. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD; Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018 Oct 30;72(18):2231-2264. doi: 10.1016/j.jacc.2018.08.1038. Epub 2018 Aug 25. PMID: 30153967.

13. Brennan PF, et al. Quantitative assessment of the conjunctival microcirculation using a smartphone and slit-lamp biomicroscope. *Microvasc. Res.* 2019;**126**:103907. doi: 10.1016/j.mvr.2019.103907.

14. Brennan, PF., et al. Assessment of the conjunctival microcirculation in adult patients with cyanotic congenital heart disease compared to healthy controls, Microvasc. Res. 136, 9 p., 104167.

15. Duerden M, O'Flynn N, Qureshi N. Cardiovascular risk assessment and lipid modification: NICE guideline. Br J Gen Pract. 2015 Jul;65(636):378-80. doi: 10.3399/bjgp15X685933. PMID: 26120133; PMCID: PMC4484941.

16. Lin JS, Evans CV, Johnson E, Redmond N, Coppola EL, Smith N. Nontraditional Risk Factors in Cardiovascular Disease Risk Assessment: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA 2018;320:281297.

17. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart 2012;98:177184.

18. Vliegenthart R, Oudkerk M, Hofman A, Oei HH, van Dijck W, van Rooij FJ, Witteman JC. Coronary calcification improves cardiovascular risk prediction in the elderly. Circulation. 2005 Jul 26;112(4):572-7. doi: 10.1161/CIRCULATIONAHA.104.488916. Epub 2005 Jul 11. PMID: 16009800.

19. Hadamitzky M, Täubert S, Deseive S, Byrne RA, Martinoff S, Schömig A, Hausleiter J. Prognostic value of coronary computed tomography angiography during 5 years of follow-up in patients with suspected coronary artery disease. Eur Heart J. 2013 Nov;34(42):3277-85. doi: 10.1093/eurheartj/eht293. Epub 2013 Sep 24. PMID: 24067508.

20. Bergström G, Persson M, Adiels M, Björnson E, Bonander C, Ahlström H, Alfredsson J, Angerås O, Berglund G, Blomberg A, Brandberg J, Börjesson M, Cederlund K, de Faire U, Duvernoy O, Ekblom Ö, Engström G, Engvall JE, Fagman E, Eriksson M, Erlinge D, Fagerberg B, Flinck A, Gonçalves I, Hagström E, Hjelmgren O, Lind L, Lindberg E, Lindqvist P, Ljungberg J, Magnusson M, Mannila M, Markstad H, Mohammad MA, Nystrom FH, Ostenfeld E, Persson A, Rosengren A, Sandström A, Själander A, Sköld MC, Sundström J, Swahn E, Söderberg S, Torén K, Östgren CJ, Jernberg T. Prevalence of Subclinical Coronary Artery Atherosclerosis in the General Population. Circulation. 2021 Sep 21;144(12):916-929. doi: 10.1161/CIRCULATIONAHA.121.055340. Epub 2021 Sep 20. PMID: 34543072; PMCID: PMC8448414

21. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ,

Engstrom G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intimamedia thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA 2012;308:796803.

22. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:13181327.

23. Maddox TM, Stanislawski MA, Grunwald GK, Bradley SM, Ho PM, Tsai TT, Patel MR, Sandhu A, Valle J, Magid DJ, Leon B, Bhatt DL, Fihn SD, Rumsfeld JS. Nonobstructive coronary artery disease and risk of myocardial infarction. JAMA. 2014 Nov 5;312(17):1754-63. doi: 10.1001/jama.2014.14681. PMID: 25369489; PMCID: PMC4893304.

24. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd KG, Berry C. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. J Am Coll Cardiol. 2018 Dec 11;72(23 Pt A):2841-2855. doi: 10.1016/j.jacc.2018.09.006. Epub 2018 Sep 25. PMID: 30266608.

25. Stokes KY, Granger DN. The microcirculation: A motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolemia? *J. Physiol.* 2005;**562**:647–653. doi: 10.1113/jphysiol.2004.079640

26. Zhong Z, Petrig BL, Qi X, Burns A. In vivo measurement of erythrocyte velocity and retinal blood flow using adaptive optics scanning laser ophthalmoscopy. *Opt. Express.* 2018;**16**(17):12746–12756. doi: 10.1364/OE.16.012746.

27. Demir SU, Hakimzadeh R, Hargraves RH, Ward KR, Myer EV, Najarian K. An automated method for analysis of microcirculation videos for accurate assessment of tissue perfusion. *BMC Med. Imaging.* 2012;**12**:37. doi: 10.1186/1471-2342-12-37

28. Fagrell B, Fronek A, Intaglietta M. A microscope-television system for studying flow velocity in human skin capillaries. *Am. J. Physiol.* 1997;**233**(2):318–321

29. Rim TH, Lee CJ, Tham YC, Cheung N, Yu M, Lee G, Kim Y, Ting DSW, Chong CCY, Choi YS, Yoo TK, Ryu IH, Baik SJ, Kim YA, Kim SK, Lee SH, Lee BK, Kang SM, Wong EYM, Kim HC, Kim SS, Park S, Cheng CY, Wong TY. Deep-learning-based cardiovascular risk stratification using coronary artery calcium scores predicted from retinal photographs. Lancet Digit Health. 2021 May;3(5):e306-e316. doi: 10.1016/S2589-7500(21)00043-1. PMID: 33890578.

30. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and No Obstructive Coronary Artery Disease (INOCA): Developing Evidence-Based Therapies and Research Agenda for the Next Decade. Circulation. 2017 Mar 14;135(11):1075-1092.

31. Ford TJ, Corcoran D, Berry C. Stable coronary syndromes: pathophysiology, diagnostic advances and therapeutic need. Heart 2018;104:284-292.

32. Kaski JC, Crea F, Gersh BJ, Camici PG. Reappraisal of Ischemic Heart Disease. Circulation. 2018;138:1463-1480.

33. Cheung, A. T. W. et al. Microvascular abnormalities in the bulbar conjunctiva of patients with type 2 diabetes mellitus. Endocr. Pract. 7, 358–363 (2001).

34. Sadr-Ameli, M. A. et al. Coronary slow flow: Benign or ominous?. Anatol. J. Cardiol. 15(7), 531–535 (2014).

35. Kord Valeshabad, A. et al. Feasibility of assessment of conjunctival microvascular hemodynamics in unilateral ischemic stroke. Microvasc. Res. 100, 4–8 (2015).

36. Khansari, et al. Assessment of conjunctival microvascular hemodynamics in stages of diabetic microvasculopathy. Sci. Rep. 7, 45916 (2017).

~~1. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, Benetos A, Biffi A, Boavida JM, Capodanno D, Cosyns B, Crawford C, Davos CH, Desormais I, Di Angelantonio E, Franco OH, Halvorsen S, Hobbs FDR, Hollander M, Jankowska EA, Michal M, Sacco S, Sattar N, Tokgozoglu L, Tonstad S, Tsioufis KP, van Dis I, van Gelder IC, Wanner C, Williams B; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep 7;42(34):3227-3337. doi: 10.1093/eurheartj/ehab484. PMID: 34458905.~~

~~2. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A, Brauer M, Brodmann M, Cahill TJ, Carapetis J, Catapano AL, Chugh SS, Cooper LT, Coresh J, Criqui M,~~

DeCleene N, Eagle KA, Emmons-Bell S, Feigin VL, Fernández-Solà J, Fowkes G, Gakidou E, Grundy SM, He FJ, Howard G, Hu F, Inker L, Karthikeyan G, Kassebaum N, Koroshetz W, Lavie C, Lloyd-Jones D, Lu HS, Mirijello A, Temesgen AM, Mokdad A, Moran AE, Muntner P, Narula J, Neal B, Ntsekhe M, Moraes de Oliveira G, Otto C, Owolabi M, Pratt M, Rajagopalan S, Reitsma M, Ribeiro ALP, Rigotti N, Rodgers A, Sable C, Shakil S, Sliwa-Hahnle K, Stark B, Sundström J, Timpel P, Tleyjeh IM, Valgimigli M, Vos T, Whelton PK, Yacoub M, Zuhlke L, Murray C, Fuster V; GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020 Dec 22;76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010. Erratum in: J Am Coll Cardiol. 2021 Apr 20;77(15):1958-1959. PMID: 33309175; PMCID: PMC7755038.

3. Libby P, Theroux P. Pathophysiology of coronary artery disease .*Circulation* . .3488-3481:(25)111;2005PubMedGoogle ScholarCrossref

4. Little WC, Constantinescu M, Applegate RJ ,et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease ?*Circulation*78;1988 .(5 pt 1):1157-1166 .

5. Falk E, Shah PK, Fuster V. Coronary plaque disruption .*Circulation* . .671-657:(3)92;1995

6. Ambrose JA, Tannenbaum MA, Alexopoulos D ,et al. Angiographic progression of coronary artery disease and the development of myocardial infarction .*J Am Coll Cardiol* .62-56:(1)12;1988 .

7. Giroud D, Li JM, Urban P, Meier B, Rutishauer W. Relation of the site of acute myocardial infarction to the most severe coronary arterial stenosis at prior angiography .*Am J Cardiol*.732-729:(8)69;1992 .

8. Fuster V. Lewis A. Conner Memorial Lecture: Mechanisms leading to myocardial infarction: insights from studies of vascular biology .*Circulation* 21-2126:(4)90;1994 .

9. Shah PK. Molecular mechanisms of plaque instability .*Curr Opin Lipidol* . 499-492:(5)18;2007

10. Nicholls SJ, Nissen SE, Prati F, Windecker S, Kataoka Y, Puri R, Hucko T, Kassahun H, Liao J, Somaratne R, Butters J, Di Giovanni G, Jones S, Psaltis PJ. Assessing the impact of PCSK9 inhibition on coronary plaque phenotype with optical coherence tomography: rationale and design of the randomized, placebo-controlled HUYGENS study. Cardiovasc Diagn Ther. 2021 Feb;11(1):120-129. doi: 10.21037/cdt-20-684. PMID: 33708484; PMCID: PMC7944215.

11. Duerden M, O'Flynn N, Qureshi N. Cardiovascular risk assessment and lipid modification: NICE guideline. Br J Gen Pract. 2015 Jul;65(636):378-80. doi: 10.3399/bjgp15X685933. PMID: 26120133; PMCID: PMC4484941.

12. Lin JS, Evans CV, Johnson E, Redmond N, Coppola EL, Smith N. Nontraditional Risk Factors in Cardiovascular Disease Risk Assessment: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA 2018;320:281297.

13. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart 2012;98:177184.

14. Vliegenthart R, Oudkerk M, Hofman A, Oei HH, van Dijck W, van Rooij FJ, Witteman JC. Coronary calcification improves cardiovascular risk prediction in the elderly. Circulation. 2005 Jul 26;112(4):572-7. doi: 10.1161/CIRCULATIONAHA.104.488916. Epub 2005 Jul 11. PMID: 16009800.

15. Hadamitzky M, Täubert S, Deseive S, Byrne RA, Martinoff S, Schömig A, Hausleiter J. Prognostic value of coronary computed tomography angiography during 5 years of follow-up in patients with suspected coronary artery disease. Eur Heart J. 2013 Nov;34(42):3277-85. doi: 10.1093/eurheartj/eht293. Epub 2013 Sep 24. PMID: 24067508.

16. Bergström G, Persson M, Adiels M, Björnson E, Bonander C, Ahlström H, Alfredsson J, Angerås O, Berglund G, Blomberg A, Brandberg J, Börjesson M, Cederlund K, de Faire U, Duvernoy O, Ekblom Ö, Engström G, Engvall JE, Fagman E, Eriksson M, Erlinge D, Fagerberg B, Flinck A, Gonçalves I, Hagström E, Hjelmgren O, Lind L, Lindberg E, Lindqvist P, Ljungberg J, Magnusson M, Mannila M, Markstad H, Mohammad MA, Nystrom FH, Ostenfeld E, Persson A, Rosengren A, Sandström A, Själander A, Sköld MC, Sundström J, Swahn E, Söderberg S, Torén K, Östgren CJ, Jernberg T. Prevalence of Subclinical Coronary Artery Atherosclerosis in the General Population. Circulation. 2021 Sep 21;144(12):916-929. doi: 10.1161/CIRCULATIONAHA.121.055340. Epub 2021 Sep 20. PMID: 34543072; PMCID: PMC8448414

17. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ,

Engstrom G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, Robertson C, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. Common carotid intimamedia thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA 2012;308:796803.

18. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:13181327.

19. Maddox TM, Stanislawski MA, Grunwald GK, Bradley SM, Ho PM, Tsai TT, Patel MR, Sandhu A, Valle J, Magid DJ, Leon B, Bhatt DL, Fihn SD, Rumsfeld JS. Nonobstructive coronary artery disease and risk of myocardial infarction. JAMA. 2014 Nov 5;312(17):1754-63. doi: 10.1001/jama.2014.14681. PMID: 25369489; PMCID: PMC4893304.

20. Ford TJ, Stanley B, Good R, Rocchiccioli P, McEntegart M, Watkins S, Eteiba H, Shaukat A, Lindsay M, Robertson K, Hood S, McGeoch R, McDade R, Yii E, Sidik N, McCartney P, Corcoran D, Collison D, Rush C, McConnachie A, Touyz RM, Oldroyd KG, Berry C. Stratified Medical Therapy Using Invasive Coronary Function Testing in Angina: The CorMicA Trial. J Am Coll Cardiol. 2018 Dec 11;72(23 Pt A):2841-2855. doi: 10.1016/j.jacc.2018.09.006. Epub 2018 Sep 25. PMID: 30266608.

21. Stokes KY, Granger DN. The microcirculation: A motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolemia? *J. Physiol.* 2005;**562**:647–653. doi: 10.1113/jphysiol.2004.079640

22. Zhong Z, Petrig BL, Qi X, Burns A. In vivo measurement of erythrocyte velocity and retinal blood flow using adaptive optics scanning laser ophthalmoscopy. *Opt. Express.* 2018;**16**(17):12746–12756. doi: 10.1364/OE.16.012746.

23. Demir SU, Hakimzadeh R, Hargraves RH, Ward KR, Myer EV, Najarian K. An automated method for analysis of microcirculation videos for accurate assessment of tissue perfusion. *BMC Med. Imaging.* 2012;**12**:37. doi: 10.1186/1471-2342-12-37

24. Fagrell B, Fronek A, Intaglietta M. A microscope-television system for studying flow velocity in human skin capillaries. *Am. J. Physiol.* 1997;**233**(2):318–321

25. Rim TH, Lee CJ, Tham YC, Cheung N, Yu M, Lee G, Kim Y, Ting DSW, Chong CCY, Choi YS, Yoo TK, Ryu IH, Baik SJ, Kim YA, Kim SK, Lee SH, Lee BK, Kang SM, Wong EYM, Kim HC, Kim SS, Park S, Cheng CY, Wong TY. Deep-learning-based cardiovascular risk stratification using coronary artery calcium scores predicted from retinal photographs. Lancet Digit Health. 2021 May;3(5):e306-e316. doi: 10.1016/S2589-7500(21)00043-1. PMID: 33890578.

26. Brennan PF, McNeil AJ, Jing M, Awuah A, Moore JS, Mailey J, Finlay DD, Blighe K, McLaughlin JAD, Nesbit MA, Trucco E, Moore TCB, Spence MS. Assessment of the conjunctival microcirculation for patients presenting with acute myocardial infarction compared to healthy controls. Sci Rep. 2021 Apr 7;11(1):7660. doi: 10.1038/s41598-021-87315-7. PMID: 33828174; PMCID: PMC8027463.

27. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD; Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018 Oct 30;72(18):2231-2264. doi: 10.1016/j.jacc.2018.08.1038. Epub 2018 Aug 25. PMID: 30153967.

28. Brennan PF, et al. Quantitative assessment of the conjunctival microcirculation using a smartphone and slit-lamp biomicroscope. *Microvasc. Res.* 2019;**126**:103907. doi: 10.1016/j.mvr.2019.103907.

29. Brennan, PF., et al. Assessment of the conjunctival microcirculation in adult patients with cyanotic congenital heart disease compared to healthy controls, Microvasc. Res. 136, 9 p., 104167.

30. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and No Obstructive Coronary Artery Disease (INOCA): Developing Evidence-Based Therapies and Research Agenda for the Next Decade. Circulation. 2017 Mar 14;135(11):1075-1092.

31. Ford TJ, Corcoran D, Berry C. Stable coronary syndromes: pathophysiology, diagnostic advances and therapeutic need. Heart 2018;104:284-292.

32. Kaski JC, Crea F, Gersh BJ, Camici PG. Reappraisal of Ischemic Heart Disease. Circulation. 2018;138:1463-1480.

33. Cheung, A. T. W. et al. Microvascular abnormalities in the bulbar conjunctiva of patients with type 2 diabetes mellitus. Endocr. Pract. 7, 358–363 (2001).

34. Sadr-Ameli, M. A. et al. Coronary slow flow: Benign or ominous?. Anatol. J. Cardiol. 15(7), 531–535 (2014).

35. Kord Valeshabad, A. et al. Feasibility of assessment of conjunctival microvascular hemodynamics in unilateral ischemic stroke. Microvasc. Res. 100, 4–8 (2015).

36. Khansari, et al. Assessment of conjunctival microvascular hemodynamics in stages of diabetic microvasculopathy. Sci. Rep. 7, 45916 (2017).

FIGURES

Figure 1. The iPhone 6s, TopCon SL-D4 imaging system with the Zarf bespoke adapter (red arrow) and TopCon external fixation target (green arrow)

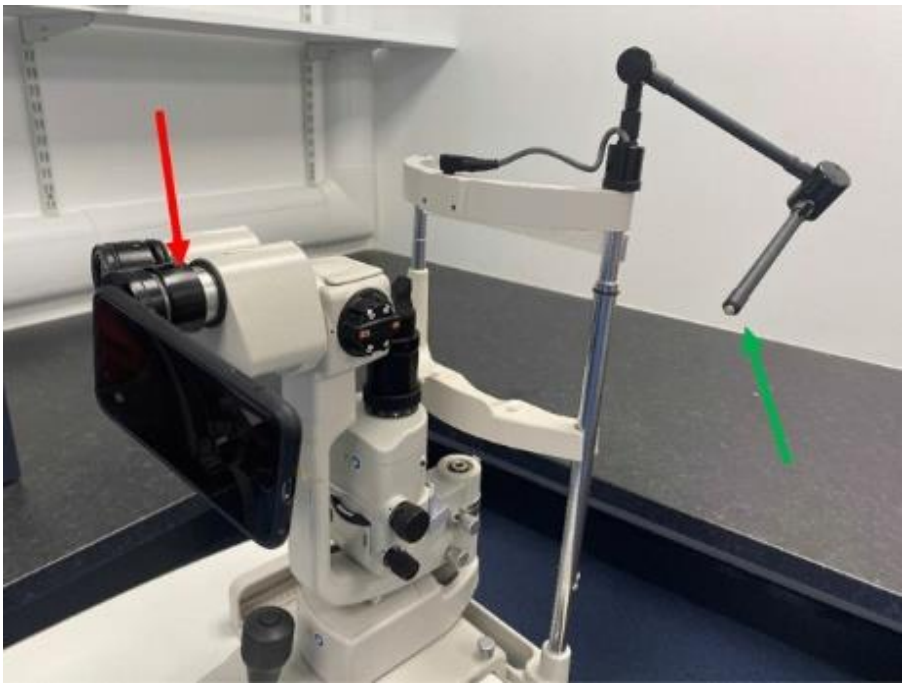


Figure 2. Stabilised video frame of conjunctival microvascular network

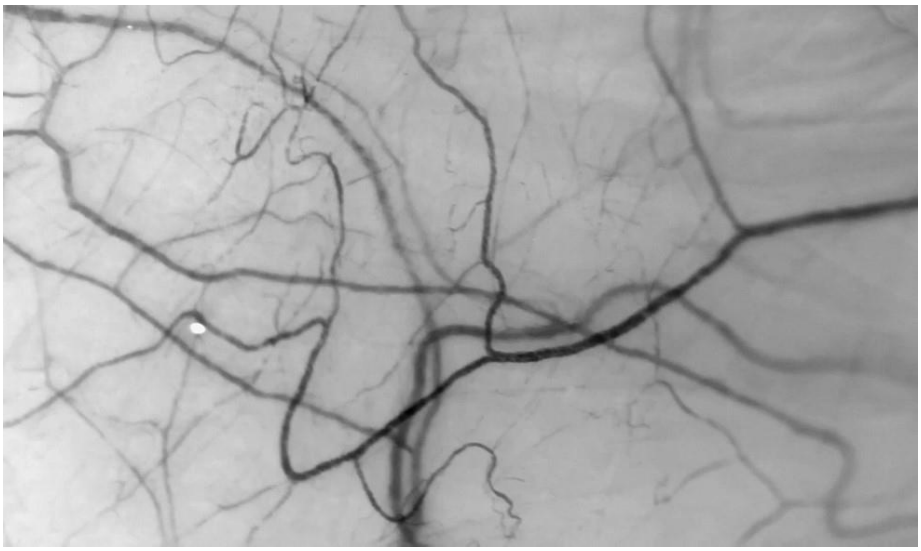


Figure 3. Boxplots comparing conjunctival haemodynamics of the MI and NO-CAD cohorts

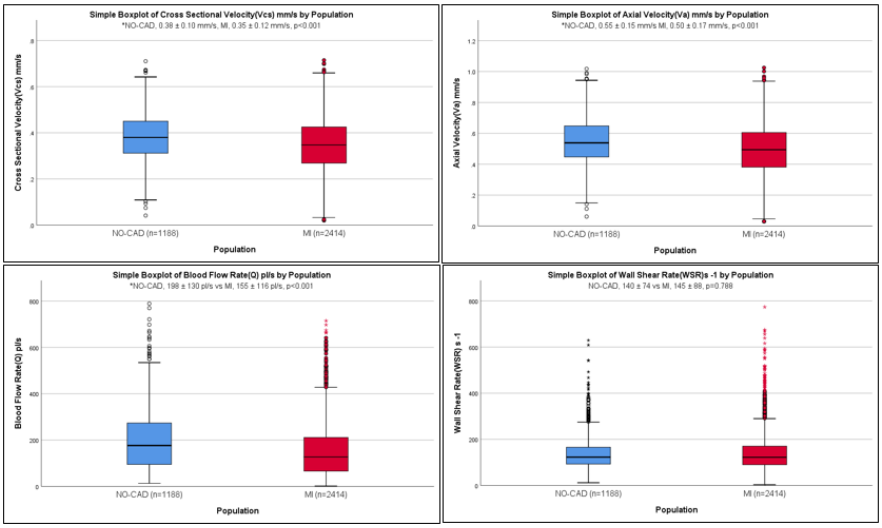
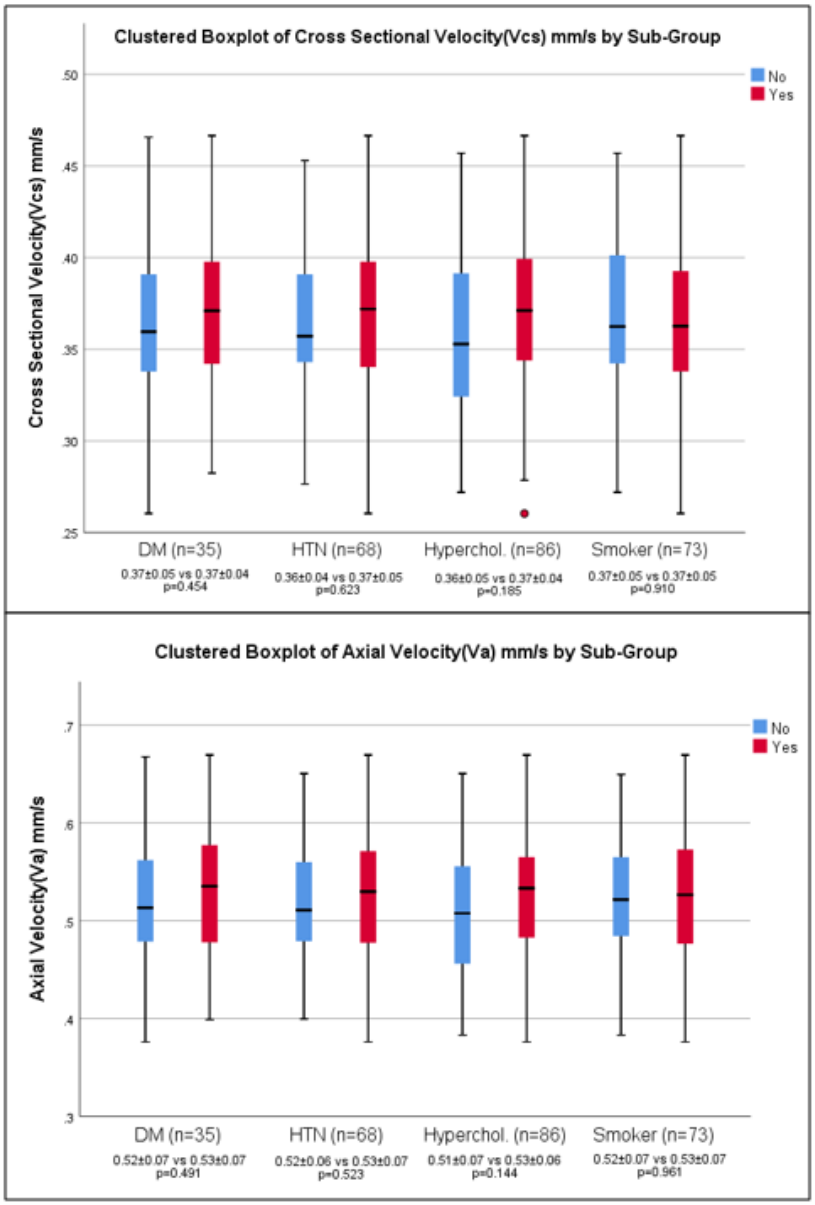


Figure 4. A comparison of axial and cross-sectional velocity in patients with and without relevant conventional vascular risk factors (diabetes mellitus, hypertension, hypercholesterolemia and smoking history)



TABLES

Table 1. Baseline characteristics

Characteristic	NO-CAD (n = 61)	MI (n = 66)	p value
Age- *yrs ± SD*	63 ± 10	57 ± 11	**0.003**
Male sex- *n (%)*	30 (49.2)	52 (78.8)	**<0.001**
BMI- *kg/m^2 ± SD*	30.2 ± 5.6	28.6 ± 4.7	0.10
Prior PCI- *n (%)*	0 (0.0)	9 (13.6)	**0.003**
Prior MI- *n (%)*	0 (0.0)	9 (13.6)	**0.003**
Hypertension- *n (%)*	37 (60.7)	31 (47.0)	0.12
Diabetes mellitus- *n (%)*	20 (32.8)	15 (22.7)	0.21
Smoking history- *n (%)*	30 (49.2)	43 (65.2)	0.07
HbA1c- *mmol/mol ± SD*	46.0 ± 16.0	45.4 ± 17.0	0.66
Creatinine clearance- *ml/min ± SD*	97 ± 41	104 ± 36	0.07
Haemoglobin- *g/l ± SD*	137 ± 14	144 ± 16	**0.04**
Total cholesterol- *mmol/l ± SD*	3.7 ± 0.9	4.6 ± 1.5	**<0.001**

Table 2. Comparison of conjunctival microcirculatory parameters

Parameter	NO-CAD (n = 1188)	MI (n = 2414)	p value
Diameter- *µm ± SD*	24.7 ± 7.8	22.4 ± 7.5	**< 0.001**
Axial velocity- *mm/s ± SD*	0.55 ± 0.15	0.50 ± 0.17	**< 0.001**
Cross-sectional velocity-*mm/s ± SD*	0.38 ± 0.10	0.35 ± 0.12	**< 0.001**
Blood flow rate- *pl/s ± SD*	198 ± 130	154 ± 116	**< 0.001**
Wall shear rate- s^{-1} *± SD*	140 ± 74	144 ± 88	0.78

Table 3. Comparison of conjunctival microcirculatory parameters based on vessel group

10 – 17 µm			
Parameter	**NO-CAD (n = 189)**	**MI (n = 466)**	**p-value**
Diameter- *µm ± SD*	14.0 ± 1.9	13.8 ± 1.9	0.26
Axial velocity- *mm/s ± SD*	0.53 ± 0.13	0.47 ± 0.15	**< 0.001**
Cross-sectional velocity-*mm/s ± SD*	0.39 ± 0.10	0.35 ± 0.12	**< 0.001**
Blood flow rate- *pl/s ± SD*	61.3 ± 22.0	52.9 ± 22.2	**< 0.001**
Wall shear rate- s^{-1} *± SD*	230 ± 67	207 ± 78	**< 0.001**
17 – 23 µm			
Parameter	**NO-CAD (n = 282)**	**MI (n = 667)**	**p-value**
Diameter- *µm ± SD*	20.1 ± 1.8	20.2 ± 1.7	0.18
Axial velocity- *mm/s ± SD*	0.54 ± 0.14	0.48 ± 0.16	**<0.001**
Cross-sectional velocity-*mm/s ± SD*	0.38 ± 0.10	0.34 ± 0.11	**<0.001**
Blood flow rate- *pl/s ± SD*	121 ± 39	109 ± 42	**<0.001**
Wall shear rate- s^{-1} *± SD*	151 ± 42	134 ± 47	**<0.001**
23 – 29 µm			
Parameter	**NO-CAD (n = 319)**	**MI (n = 685)**	**p-value**
Diameter- *µm ± SD*	26.0 ± 1.7	25.9 ± 1.7	0.30
Axial velocity- *mm/s ± SD*	0.55 ± 0.15	0.51 ± 0.17	**<0.001**
Cross-sectional velocity-*mm/s ± SD*	0.37 ± 0.10	0.35 ± 0.12	**<0.001**
Blood flow rate- *pl/s ± SD*	199 ± 60	285 ± 69	**<0.001**
Wall shear rate- s^{-1} *± SD*	116 ± 32	108 ± 36	**<0.001**
29 – 36 µm			

Parameter	NO-CAD (n = 282)	MI (n = 378)	p-value
Diameter- *μm* ± *SD*	32.0 ± 1.9	31.7 ± 1.9	0.05
Axial velocity- *mm/s* ± *SD*	0.57 ± 0.16	0.54 ± 0.16	**0.01**
Cross-sectional velocity-*mm/s* ± *SD*	0.38 ± 0.11	0.36 ± 0.11	**0.01**
Blood flow rate- *pl/s* ± *SD*	308 ± 98	287 ± 98	**0.001**
Wall shear rate- s^{-1} ± *SD*	96 ± 28	91 ± 27	**0.03**

Table 4. Logistic regression analysis of independent predictors of myocardial infarction

Variable	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	0.950 (0.917 – 0.984)	**0.004**	0.961 (0.922 – 1.003)	0.07
Male sex	3.838 (1.769 – 8.329)	**<0.001**	3.685 (1.538 – 8.828)	**0.003**
Smoking	1.932 (0.947 – 3.941)	0.07	2.450 (1.046 – 5.738)	**0.04**
Hypertension	0.575 (0.284 – 1.163)	0.12	0.903 (0.377 – 2.163)	0.82
Diabetes mellitus	0.603 (0.275 – 1.323)	0.21	0.755 (0.297 – 1.916)	0.55
Hypercholesterolaemia	0.264 (0.117 – 0.595)	**0.001**	0.392 (0.152 – 1.011)	0.053

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Conjunctival axial velocity < 0.50mm/s	3.067 (1.439 – 6.535)	**0.004**	3.514 (1.469 – 8.406)	**0.005**

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Reviewer #2:

Perhaps I did not make my prior concern clear. While the authors find an association between conjunctival hemodynamics and CAD, and as they explain there was not association between DM and smoking with conjunctival hemodynamics, Figure 4 and Table 1 do not compare the predictive value of the conjunctival hemodynamics with that of other baseline risk factors that were significantly different between the No-CAD and MI populations (cholesterol, male sex, age). I would think that a multivariate analysis with an odds ratio, etc, would be necessary to demonstrate that conjunctival hemodynamics is not just a covariate of hypercholesterolemia, age, etc, AND that preferably conjunctival hemodynamics have independent predictive value.

Many thanks for clarifying this point. This was an excellent suggestion and we agree additive to the manuscript. We have conducted a multivariate analysis including baseline characteristics and conventional CV risk factors. A reduction in conjunctival axial velocity to below 0.5mm/s was an independent predictor of MI. This has been included in the results section and the analysis can be found in the additional Table 4.