Atherosclerosis

Can fast wall shear stress computation predict adverse cardiac events in patients with intermediate non-flow limiting stenoses?

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Abstract:	Background and aims: Coronary angiography-derived wall shear stress (WSS) may enable identification of vulnerable plaques and patients. A new recently introduced software allows seamless three-dimensional quantitative coronary angiography (3D-QCA) reconstruction and WSS computation within a single user-friendly platform carrying promise for clinical applications. This study examines for the first time the efficacy of this software in detecting vulnerable lesions in patients with intermediate non-flow limiting stenoses. Methods: This multicentre retrospective study included patients who had coronary angiography showing at least one lesion with borderline negative fractional flow reserve (FFR: 0.81-0.85). In these lesions, 3D-QCA reconstruction and blood flow simulation were performed using the CAAS Workstation WSS prototype (Pie Medical Imaging, Maastricht, Netherlands). Time averaged and multidirectional WSS were extracted across the lesion at every 3mm segments. The primary endpoint of the study

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	was lesion-oriented clinical events (LOCE), defined as the composite of cardiac death, target lesion related myocardial infarction (MI) or clinically indicated target lesion revascularization. Results: 352 patients (355 lesions) were included in the analysis. Over a median follow-up of 4.1 years, 57 LOCE were recorded. Lesions causing events had a larger area stenosis (AS) [59.4 (54.6-67.7)% vs 52.8 (43.8–60.1)%, p<0.001], maximum time averaged WSS (TAWSS) [11.56 (8.25-13.64)Pa vs 7.73 (5.41-11.51)Pa, p<0.001], mean TAWSS at the minimum lumen area (MLA) [9.30 (5.44-11.94)Pa vs 6.19 (3.96-9.00)Pa, p<0.001] and maximum transverse WSS [0.30 (0.21-0.45)Pa vs 0.23 (0.17-0.32)Pa, p=0.002] than those remaining quiescent. In multivariable models, AS was the only independent predictor of LOCE. Kaplan-Meier curves demonstrated that lesions with elevated maximum TAWSS and AS had a higher rate of LOCE than those with low TAWSS and AS values (26% vs 7%, p <0.001). Conclusions: For non-flow limiting lesions with borderline negative FFR, fast WSS computation using a dedicated software is feasible and holds potential for cardiovascular risk stratification.

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HIGHLIGHTS

What is known

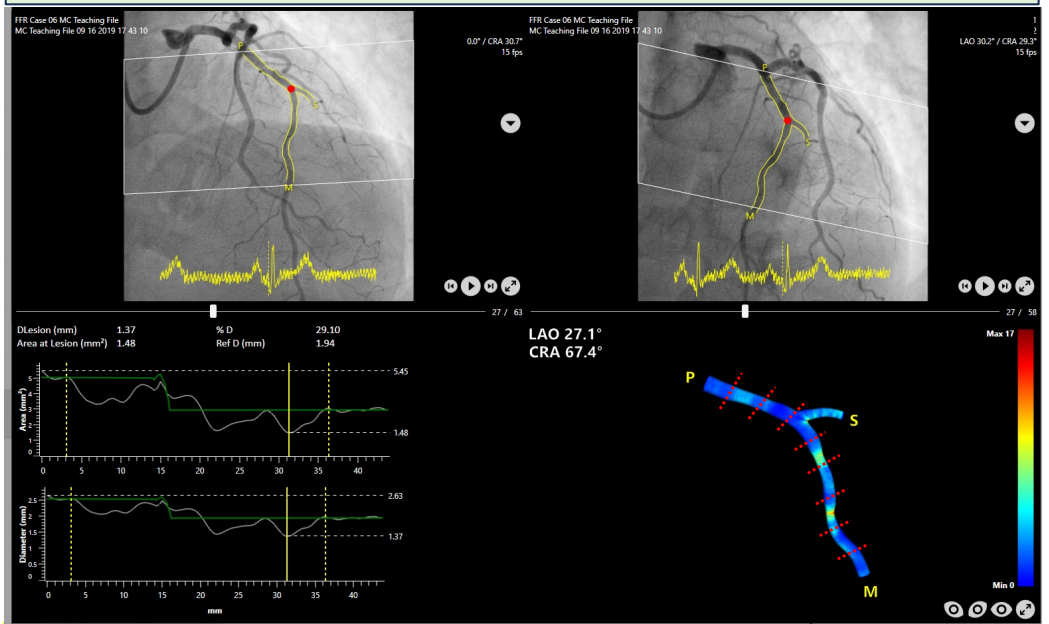
- Wall shear stress (WSS) estimated in three-dimensional quantitative coronary angiography (3D-QCA) models provides prognostic information in patients with coronary artery disease.
- Computational fluid dynamic analysis to estimate WSS is a time-consuming process which does not allow its clinical application.
- The novel "CAAS workstation WSS prototype" is fast enabling 3D-QCA analysis and WSS computation in <10 minutes.

What the study adds

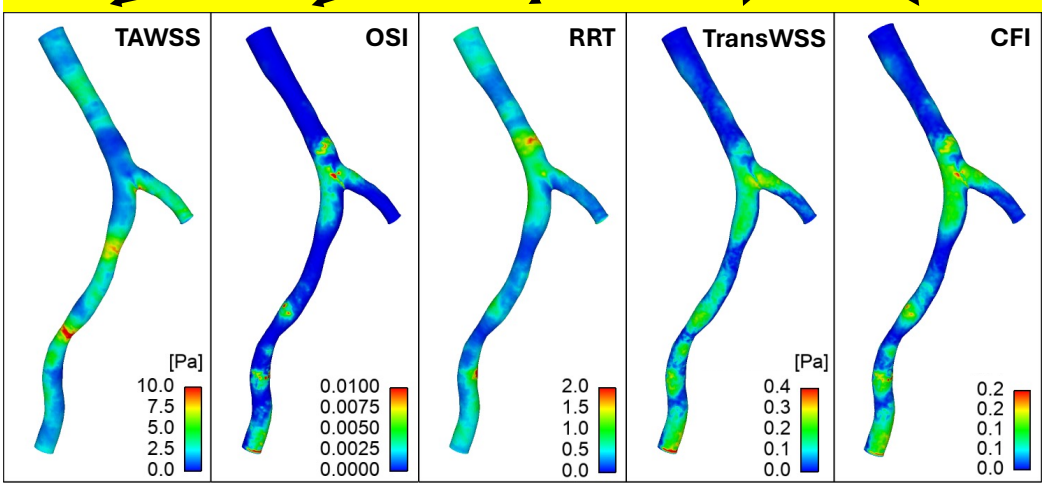
- This study examines for the first time the value of this software to detect high-risk plaques in patients with non-flow limiting lesions.
- Use of the CAAS Workstation WSS prototype was found able to detect vulnerable plaques and may have a value for cardiovascular risk stratification.

Graphical abstract

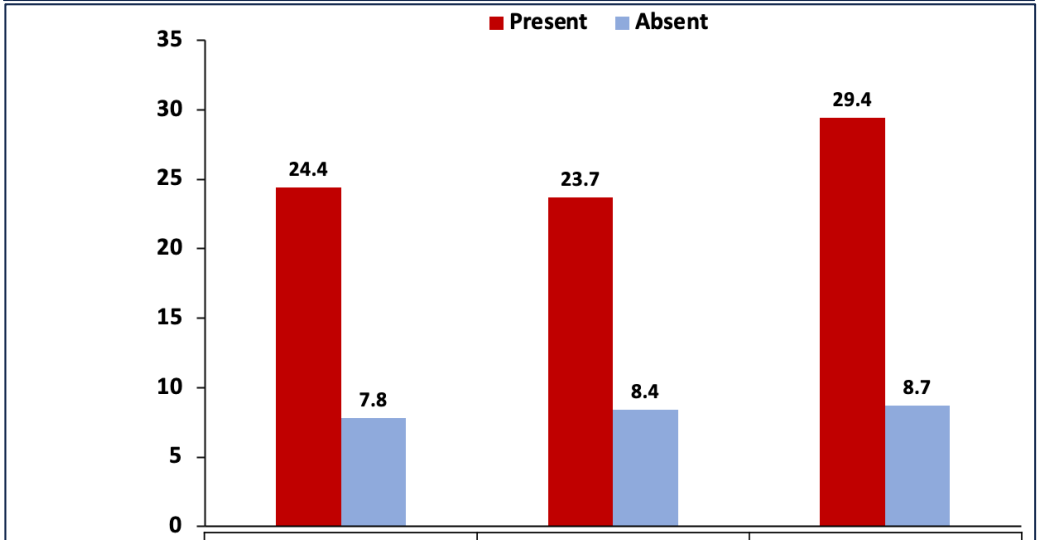
Real-time TAWSS and multidirectional WSS performed in 355 lesions with borderline negative fractional flow reserve (0.80<FFR≤0.85) using a dedicated software



WSS computation



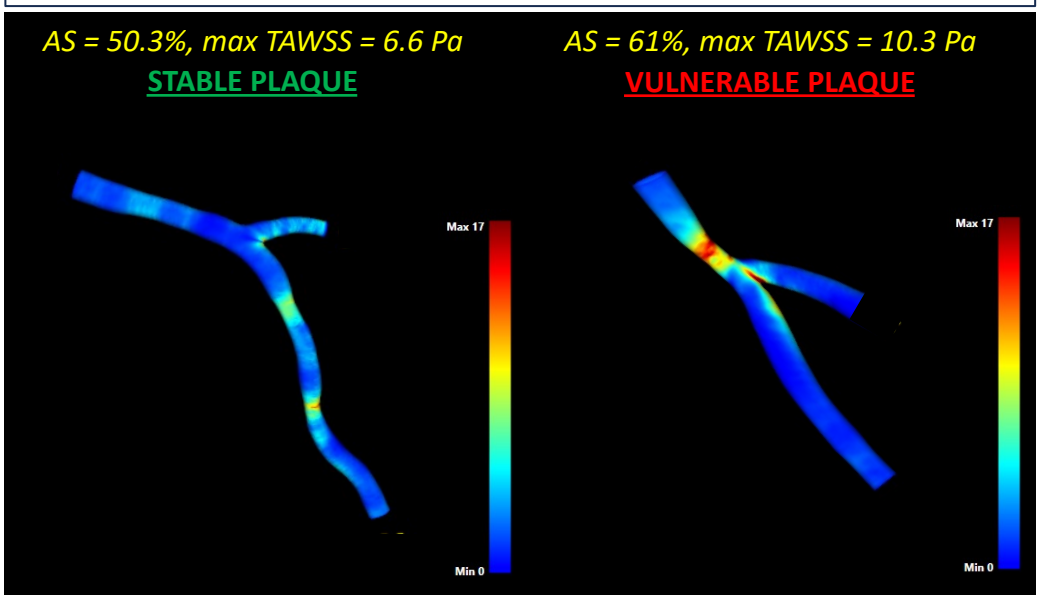
57 LOCE recorded at 4-year follow-up
Area stenosis and max TAWSS were predictors of events



	AS >54.2%	WSS >8.3 Pa	AS >54.2% + WSS >8.3 Pa
HR (95% CI)	3.43 (1.87-6.27)	3.06 (1.70-5.23)	3.70 (2.15-6.37)
P value	<0.001	<0.001	<0.001
Prevalence (%)	176 (49.6%)	177 (49.9%)	126 (35.5%)

AS = 50.3%, max TAWSS = 6.6 Pa
STABLE PLAQUE

AS = 61%, max TAWSS = 10.3 Pa
VULNERABLE PLAQUE



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Abstract

Background and aims: Coronary angiography-derived wall shear stress (WSS) may enable identification of vulnerable plaques and patients. A new recently introduced software allows seamless three-dimensional quantitative coronary angiography (3D-QCA) reconstruction and WSS computation within a single user-friendly platform carrying promise for clinical applications. This study examines for the first time the efficacy of this software in detecting vulnerable lesions in patients with intermediate non-flow limiting stenoses.

Methods: This multicentre retrospective study included patients who had coronary angiography showing at least one lesion with borderline negative fractional flow reserve (FFR: 0.81-0.85). In these lesions, 3D-QCA reconstruction and blood flow simulation were performed using the CAAS Workstation WSS prototype (Pie Medical Imaging, Maastricht, Netherlands). Time averaged and multidirectional WSS were extracted across the lesion at every 3mm segments. The primary endpoint of the study was lesion-oriented clinical events (LOCE), defined as the composite of cardiac death, target lesion related myocardial infarction (MI) or clinically indicated target lesion revascularization.

Results: 352 patients (355 lesions) were included in the analysis. Over a median follow-up of 4.1 years, 57 LOCE were recorded. Lesions causing events had a larger area stenosis (AS) [59.4 (54.6-67.7)% vs 52.8 (43.8–60.1)%, $p<0.001$], maximum time averaged WSS (TAWSS) [11.56 (8.25-13.64)Pa vs 7.73 (5.41-11.51)Pa, $p<0.001$], mean TAWSS at the minimum lumen area (MLA) [9.30 (5.44-11.94)Pa vs 6.19 (3.96-9.00)Pa, $p<0.001$] and maximum transverse WSS [0.30 (0.21-0.45)Pa vs 0.23 (0.17-0.32)Pa, $p=0.002$] than those remaining quiescent. In multivariable models, AS was the only independent predictor of LOCE. Kaplan-Meier curves demonstrated that lesions with elevated maximum TAWSS and AS had a higher rate of LOCE than those with low TAWSS and AS values (26% vs 7%, p <0.001).

Conclusions: For non-flow limiting lesions with borderline negative FFR, fast WSS computation using a dedicated software is feasible and holds potential for cardiovascular risk stratification.

1. Introduction

Coronary artery disease is a leading cause death and has been associated with increased morbidity worldwide.[1] Surgical revascularization, percutaneous coronary intervention and novel pharmacotherapies appear to improve outcomes in patients with ischaemic heart disease. Despite these therapies, the event rate remains high with up to 20% of patients who had a revascularization experiencing an event at 2 years.[2] Over the recent years, efforts have been made to better understand atherosclerotic evolution and predict lesions that will progress and cause events. Today, it is known that lesion severity, its composition but also the local hemodynamic forces distribution provides useful prognostic information and can be used to stratify cardiovascular risk.[3-5]

Local hemodynamic forces distribution is traditionally assessed in high-resolution coronary artery models reconstructed from coronary angiography and intravascular imaging data fusion. However, intravascular imaging is not routinely performed in the entire coronary artery tree and the fusion of the X-ray and intravascular imaging is a tedious process that requires expertise.[6] Alternative modalities for coronary artery reconstruction include computed tomography coronary angiography and coronary angiography. Computational fluid dynamics (CFD) studies showed a high agreement between the wall shear stress (WSS) measured by computed tomography and by intravascular imaging-based models, and that computed tomography-derived WSS can predict atherosclerotic evolution but it has limited efficacy in predicting lesions causing events.[7,8] Conversely, there is convincing evidence that WSS estimated in three-dimensional quantitative coronary angiography (3D-QCA) models can identify vulnerable plaques and stratify cardiovascular risk.[9-11] However, in all these reports model reconstruction and CFD analysis was performed offline with a stepwise approach that used several dedicated software and required 20-25 minutes per model, which does not allow clinical application. The CAAS workstation WSS prototype (Pie Medical Imaging, Maastricht, Netherlands) has been recently introduced to overcome these limitations, enabling quick 3D-QCA and WSS computation in <10 minutes on a user-friendly platform. The efficacy of this software to measure WSS patterns and its reproducibility has been evaluated using conventional CFD analysis as reference standard.[12] However, the value of this platform to detect vulnerable plaques and patients has not been tested yet. The current

study aims to examine the prognostic value of different WSS metrics estimated by the CAAS workstation in a large cohort of patients with intermediate non-flow limiting lesions.

2. Patients and methods

2.1 Studied patients

We retrospectively screened patients who underwent a coronary angiography in four different Cardiology Departments (Barts Heart Centre, London, UK; Essex Cardiothoracic Centre, Basildon, UK; Royal Free Hospital, London, UK; Seoul National University Hospital, Seoul, Korea) from January 2012 to December 2017. Patients eligible for recruitment were those admitted with either acute or chronic coronary syndrome, who had at least one non-obstructive coronary lesion with a borderline negative fractional flow reserve (FFR: 0.81-0.85) that was treated conservatively in accordance with international guidelines. All the included patients had complete follow-up until December 2019.

We excluded from the study: i) patients admitted with an acute coronary syndrome who had an ambiguous culprit lesion, or a borderline negative FFR on a possible culprit lesion; ii) patients with left main stem disease, ostial left anterior descending, circumflex or right coronary artery lesions, coronary bypass grafts lesions, or lesion located in a stented segment (in-stent restenosis) or within 5mm from the edge of a stent (stent-edge restenosis); iii) and patients with inadequate angiographic data for 3D-QCA analysis (poor angiographic image quality, insufficient DICOM information, or lack of two angiographic projections ≥30° apart).

For the recruited patients, baseline demographics, cause of admission, medications at the time of discharge, angiogram and report of the index procedure were collected from hospital electronic patient records. In patients who had an adverse event, cause of re-admission was recorded, repeat angiography was reviewed blindly to baseline demographics and events were adjudicated and attributed to the intermediate non-flow limiting lesions assessed by FFR or to other lesions. Death certificates were also collected, and cause of death was recorded. This study was conducted as part of a local audit investigating long-term cardiovascular outcomes in patients with borderline negative FFR. Patients' identifiable information was deleted prior to merging of datasets and angiographic analysis. Formal ethical approval was not required based on local ethics committee advice.

2.2 Clinical endpoints

The primary endpoint of the study was the incidence of lesion-oriented clinical events (LOCE) defined as the composite of cardiac death, target lesion related myocardial infarction (MI) or clinically indicated target lesion revascularizations attributed to disease progression of the lesions with borderline negative FFR. Cardiac death included sudden death or death attributed to MI or heart failure. MI diagnosis was based on evidence of myocardial necrosis (i.e., dynamic troponin rise) in addition to clinical symptoms, electrocardiographic findings and coronary angiography. Clinically indicated revascularizations included revascularizations performed in patients with increasing angina symptoms despite maximum tolerated medical therapy who had a repeat angiogram showing coronary artery disease progression by visual assessment, with or without evidence of ischemia or coronary physiology assessment. The secondary endpoint of the study was the combined endpoint of target lesion related MI and/or clinically indicated revascularisations.

2.3 3D-QCA analysis

Coronary artery reconstruction was performed using the CAAS Workstation WSS prototype software at the Coronary Imaging Core lab Queen Mary University London. An experienced analyst identified the intermediate stenosis assessed by FFR, blinded to baseline demographics, clinical outcomes and FFR results, and used side branches located proximally and distally to this lesion to define the segment of interest (SOI).

Reconstruction was performed from two angiographic projections that were at least 30° apart and portrayed the SOI with minimal foreshortening or overlapping. Two end-diastolic projections showing the SOI filled with contrast were selected and co-registration was performed by defining a corresponding point in these projections. The CAAS workstation enables reconstruction of a single side branch, and this was included in the 3D geometry when it had a reference diameter ≥1mm; in case of ≥2 side branches arising from the SOI, only the largest one was reconstructed. The information located in the DICOM files were used to define the location of the two projections in 3D space, and estimate pixel size, and then an edge detection algorithm was applied to detect the lumen borders of the SOI and its side branch when present. Corrections of the detected borders were made by the expert analyst if this was deemed necessary. The identified borders were then used to generate the 3D geometry of the SOI

and its branch, if applicable, assuming an elliptical cross-sectional shape.[13] In the obtained 3D models, the model length, lesion length, proximal and distal reference area, the % area stenosis (AS) and minimum lumen area (MLA) were estimated.

2.4 WSS computation

Following 3D-QCA reconstruction, fast CFD analysis was performed by the software to compute the WSS across the SOI (Online Supplementary Material). The software assumes a pulsatile flow profile that allows not only measurements of the WSS, but also evaluation of the changes in the flow direction during the cardiac cycle that can been quantified with the computation of multidirectional shear stress indices (Supplementary Figure 1).[14] In pulsatile flow, the WSS was averaged over the entire phase of the cardiac cycle to estimate the time-averaged WSS (TAWSS). The TAWSS values correlate well with the WSS estimated under steady flow conditions which have been found in numerous studies to be predictors of cardiovascular events.[4,5,9,15] The multidirectional WSS metrics that have been found to affect plaque biology and determine its evolution include:

- The oscillatory shear index (OSI), which is used to quantify oscillations in coronary flow and is computed by the ratio of forward WSS to the total WSS variation (value of 0.5 being always-forward oscillation, whilst 0.0 being back-and-forth oscillation).
- The relative residence time (RRT), a parameter that combines TAWSS and OSI and reflects the time that a blood particle spends at a certain location on the vessel wall.
- The transverse WSS (transWSS), which corresponds to the WSS component that is perpendicular to the TAWSS vector.
- Crossflow index (CFI), which is defined as the transWSS normalized by the TAWSS and provides an estimate of the changes in the direction of the WSS throughout the cardiac cycle.

Each SOI was split in 3mm segments and in each segment the mean TAWSS as well as the maximum predominant and minimum predominant TAWSS were computed. The maximum and minimum predominant TAWSS were defined as the maximum and minimum values of the TAWSS computed in each 3mm segment in all surface data located in a 90° arc window across the circumference of the vessel wall.[4,10,11] The highest maximum predominant and the lowest minimum predominant TAWSS values in the 3mm segments encompassing the lesion corresponded to the “maximum” and “minimum” TAWSS,

respectively. In addition, the highest mean TAWSS in the 3mm segments of the lesion, the mean TAWSS at the MLA and the mean TAWSS proximally to the MLA were recorded. The maximum predominant multidirectional WSS parameters such as OSI, RRT, transWSS and CFI were also computed at every 3mm segment; for each of these variables, the highest value within the lesion was recorded.

In diffusely diseased segments and in lesions with borderline negative FFR along the vessel, 3D-QCA and CFD analysis was performed in the entire segment. In tandem lesions we extracted the WSS only in the lesion with the higher AS – as this was the most powerful predictor of future events in previous studies[11,16] – and this lesion was included in the analysis. An event was censored in this setting only if there was disease progression in the lesion with the largest area stenosis.

2.5 Statistical analysis

Continuous variables are presented as medians (1st and 3rd quartiles), while categorical variables as frequencies (n) and relational proportion (%).

Proximal and distal reference area, mean reference area, lesion length, MLA, AS, minimum and maximum TAWSS, maximum mean TAWSS, mean TAWSS at the MLA and mean TAWSS proximally to the MLA, maximum OSI, RRT, transWSS and CFI were considered candidate predictors. Differences in baseline clinical, angiographic, and CFD metrics between patients with and without LOCE were assessed using Mann-Whitney test or Chi-square test, as appropriate. Variables with a p-value <0.25 in the univariable Cox model and those biologically plausible were taken as adjustment variables in the model.

The relationship between LOCE and candidate predictors were evaluated using multivariable Cox proportional hazard regression. In the multivariable models, a base model was created using only adjustment variables through the Cox proportional hazard method. Then, a single candidate predictor was sequentially added to the model. Each variable incorporated into the model underwent a 3-knot restricted cubic spline transformation to capture non-linearities. Therefore, the hazard ratio (HR) and 95% confidence interval (CI) for interquartile change in candidate predictors have been provided for the relationship between the outcome and candidate predictors. Subsequently, each model was evaluated based on the following metrics:

1. Likelihood ratio Chi-square (x2).
2. R-squared.
3. Area under the curve (AUC); overall and time dependent.
4. HR and 95% CI, and p-value for each candidate predictor.

In partial effect plots, the relationship between each candidate predictor and the HR was plotted. The point where the candidate predictor crosses the HR=1 line was considered the best cut-off, and accordingly, Kaplan-Meier plots were drawn and differences between categories were analysed with the log-rank test. Moreover, to reduce the risk of overfitting, the survival model was refitted using the ridge regularization method. AUC results for ridge regression and standard Cox proportional hazard regression were given pairwise. A p-value of <0.05 was considered statistically significant.

All statistical analyses were performed with R-software v.4.2.2 (R statistical software, Institute for Statistics and Mathematics, Vienna, Austria) using the "rms", "ggplot2", "survival", "survivalROC", and "mice" packages, and Python 3.10.12 (Python Software Foundation) using the forestplot 0.4.0 package.

3. Results

3.1 Studied population

In total, 806 patients with borderline negative FFR lesions were screened; 355 lesions (352 patients) were deemed suitable for 3D-QCA analysis and were included in the study (Supplementary Figure 2). Median age was 64 years, 81.3% were male and 82.9% of the patients were admitted with a chronic coronary syndrome. During a median follow-up of 1502 days, 6 (1.7%) patients died from cardiac causes, 16 (4.5%) suffered a target lesion related MI and 35 (9.9%) underwent target lesion clinically indicated revascularization due to progressive angina.

Baseline demographics of the patients who had a LOCE and those that did not have an event are showed in Table 1; there were no significant differences between the two groups apart from the incidence of hypercholesterolemia that was higher in the LOCE group.

3.2 Morphological and physiological characteristics of culprit and non-culprit lesions

Stenosis location was similar between lesions causing LOCE and those remaining quiescent; FFR values were also similar and there were no differences in blood flood velocity between groups (Supplementary Table 1). The AS was significantly larger in lesions causing events, whereas other QCA parameters were similar in the two groups (Table 2). Additionally, table 2 shows that the maximum TAWSS, the highest mean TAWSS, the mean TAWSS at the MLA and proximally to the MLA, and the maximum transWSS were significantly higher in lesions causing LOCE than those remaining silent.

3.3 Predictors of LOCE

The baseline demographics associated with LOCE in univariable Cox regression analysis were age, clinical presentation, diabetes mellitus and dual antiplatelet therapy; these variables were used to create a "*base model*". Subsequently, the morphological and physiological characteristics of the studied lesions from Table 2 were added to the base model to build 15 additional prediction models. Figure 1 showed that AS, maximum TAWSS, the highest mean TAWSS, mean TAWSS at the MLA and proximally to MLA, and maximum transWSS were independently associated with LOCE ($p < 0.05$). WSS metrics were not a predictor of LOCE when they were added to a model including baseline demographics and AS. Conversely, AS provided incremental prognostic information as a predictor of LOCE when this was added to models built from baseline demographics and WSS metrics (Figure 2). Performance metrics for all models are displayed in Supplementary Figure 3. When comparing the Likelihood Ratio Chi Square/R Square of the models, AS was the strongest predictors of events followed by mean TAWSS at the MLA, the highest mean TAWSS and maximum TAWSS. In addition, AS was the only predictor with an AUC >0.7. Additional discriminative metrics obtained from the ridge regression model were similar to standard models (Supplementary Figure 4).

Analysis of time dependent AUC showed that the discrimination increased in the first 3 years for the AS and then decreased. Conversely, for the TAWSS variables there was a slight increase in discrimination in the first 3 years which did not significantly change in the remaining years (Supplementary Figure 5).

The partial effect plots displaying the relationship between AS and the WSS metrics that are predictors of LOCE are showed in Figure 3. The higher is the variable, the greater is the risk of LOCE. Based on

partial effect plots, the best cut-offs for predicting adverse events were 54.2% for AS, 8.3Pa for maximum TAWSS, 6.5Pa for TAWSS at the MLA and 7.5Pa for the highest mean TAWSS. The Kaplan-Meier plots and log-rank test p-values based on these cut-off values are represented in Figure 4. Patients exceeding the AS and maximum TAWSS thresholds exhibited a cumulative event rate of 26% (95% CI: 17-33%) vs. 7% (95% CI: 2-11%) in those with low AS and TAWSS ($p < 0.001$) at 4-year follow-up (Figure 5); conversely, there was no discernible difference in outcomes between patients solely presenting elevated AS or maximum TAWSS and those with low AS or maximum TAWSS.

Results for secondary endpoint were similar and are presented in Supplementary Material including tables 2-4 and figures 6-8.

4. Discussion

In the present study, we investigated for the first time the prognostic value of WSS metrics derived by the CAAS Workstation WSS prototype in a large cohort of patients with borderline non-flow limiting lesions. We found that: 1) these lesions were associated with a high event frequency of 16.2% at 4-year follow-up; 2) AS and CFD metrics provided additional prognostic information to baseline demographics enabling more accurate risk stratification; 3) TAWSS and transWSS did not have an incremental value to AS in detecting lesions that will cause events in multivariable analysis; however, 4) in the Kaplan-Meier analysis, lesions with high maximum TAWSS and AS were more likely to cause an event than those with low maximum TAWSS and AS.

It is well known that WSS plays a key role in atherosclerotic disease progression, plaque destabilisation and rupture.[6] The PREDICTION[5] and a sub-analysis of the PROSPECT study[4] revealed that WSS computed in models reconstructed from intravascular ultrasound and coronary angiography enables more accurate risk-stratification than intravascular imaging alone. In these studies, vessel reconstruction and CFD analysis was time-consuming and could not be performed within a clinically practical timeframe. To overcome this limitation, angiography-derived WSS has been proposed and validation studies have showed a good correlation with WSS computed in intravascular imaging-based models.[17] Recent reports using CFD analysis in models reconstructed from 3D-QCA data have shown that WSS provides useful prognostic information and can help identifying lesions causing events. A post-hoc

analysis of the FAME-2 study that included flow-limiting lesions (FFR<0.80)[10], a post-hoc analysis of the IBIS 4 and PROSPECT studies focusing on lipid-rich lesions[9], and a study that included intermediate stenoses with borderline negative FFR[11] have shown that high WSS at the lesion location can be an independent predictor of future events.[11] The above analyses, however, required the use of multiple software to perform 3D-QCA and then run CFD analysis with a computation time of at least 20 minutes per vessel.

To streamline workflows and expedite analysis, Pie Medical Imaging has developed the CAAS workstation WSS prototype. This allows 3D-QCA reconstruction of the main vessel and its side branch and then CFD analysis and computation of the TAWSS and multidirectional WSS within <10minutes. The estimations of this software are highly reproducible, while its efficacy to assess WSS distribution has been tested against the conventional approach – which uses a series of commercially available tools – showing a high agreement between the two approaches for the CFD-derived variables.[12,18] The CAAS workstation prototype has been used to evaluate to predict events in 80 patients who had a coronary angiogram and were then re-admitted with acute MI; in this study, the culprit lesions had increased TAWSS and topological shear variation index compared to those that did not cause events.[16] However, the small number of patients included, the lack of a control group (patients with no events) and significant differences in the computed FFR and AS which can also affect WSS were limitations of this analysis.

The present study overcomes these limitations and examines for the first time the prognostic value of the CAAS workstation WSS prototype in a large cohort of patients. In this study we included lesions with FFR 0.81-85 which are more likely to exhibit vulnerable plaque features[19-21] and are at increased risk of causing adverse events compared to lesions with a higher FFR.[22-25] In line with our findings from a previous analysis conducted on a similar group of patients,[11] this study showed that AS and WSS metrics were predictors of LOCE and of the combined end-point target lesion MI and revascularizations. The WSS cut-offs predicting events were similar and >7Pa, which in experimental studies was found to have an unfavourable effects on vessel wall biology.[6] Moreover, the AS cut-off associated with adverse events was similar to previous reports.[11,16] With regards to transWSS - which was the only predictor among multidirectional WSS metrics - there is no established cut-off associated

with adverse effects on vessel biology. Studies in animal models showed that high transWSS may be associated to plaque growth[14], whereas a recent study demonstrated that transWSS did not affect plaque progression but influences plaque composition with segments exposed to high transWSS showing a larger necrotic core area than those exposed to low values.[26]

Unlike previous findings[11], WSS metrics did not demonstrate an additive prognostic value over baseline demographics and AS for the primary endpoint. A possible explanation is the different set of patients included in this analysis and the use of dedicate software for fast coronary artery reconstruction, quantification of lesion severity and WSS computation. The AS estimations with the CAAS workstation may have a higher prognostic value than those computed in previous studies[9,11] using a different software, while the different mesh generator and blood flow simulation for CFD analysis may have impacted the prognostic efficacy of the WSS metrics. Moreover, the CAAS Workstation WSS prototype allows reconstruction of a single side branch and in cases with multiple side branches only the largest was included in the model. This approximation might have an impact on WSS computation, but results were not different when segments with multiple side branches were excluded from our analysis (Supplementary Figure 9). Nevertheless, following the identification of optimal AS and WSS cut-offs for event prediction, Kaplan-Meier curves showed that patients with elevated AS and maximum TAWSS exhibit an increased risk of experiencing events. This finding suggests that local hemodynamic forces computed in 3D-QCA based models might offer supplementary prognostic insights beyond lesion geometrical characteristics (Fig. 6) and underscore the need to conduct large prospective studies to explore their incremental value in predicting events. In addition, validation studies that examine the performance of this software in measuring WSS using intravascular imaging as reference standard are currently ongoing and are anticipated to fully explore its potential in assessing local vessel physiology.

4.1 Limitations

Although this analysis is the largest in the field assessing the prognostic value of CAAS workstation WSS prototype, it has limitations that should be acknowledged. The retrospective design resulted in the exclusion of 56.5% of lesions, which has likely introduced bias. A prospective study will overcome this limitation and allow evaluation of the CAAS workstation performance in optimal angiographic data

specifically collected for this purpose. The lesion subset selected for this study (FFR 0.85-0.81) was very specific; thus, it is unclear whether these findings apply to other non-flow limiting lesions. Future studies should be conducted to explore the prognostic value of 3D-QCA-derived WSS in this setting. The number of LOCE recorded may not have been sufficient to fully explore the additive prognostic value of WSS metrics over AS and baseline demographics in multivariable analysis. Moreover, given the small number of cardiac death (6) and MI (16), there was not enough power to assess the additive value of WSS in predicting hard clinical events.

We have included in our analysis patients admitted with a chronic or an acute coronary syndrome as FFR has a value in guiding revascularization in both cohorts; studies have shown significant differences in lesion morphology and worse outcomes in patients with a borderline negative FFR in patients with an acute coronary syndrome.[27] To account for these differences, patient's clinical presentation was included in the Cox regression analysis. Of note, the results were not different when we repeated the analysis only in patients with a chronic coronary syndrome. As previously stated, the CAAS Workstation WSS prototype is unable to include multiple side branches in the 3D model and this may have influenced WSS computation. Finally, blood flow velocity was manually estimated using the conventional TIMI frame count approach which is operator dependent. The inflow velocity has a critical effect on WSS computation and the use of a dedicated software to calculate this velocity may allow a more reproducible and accurate computation than TIMI frame count approach and reduce risk of bias.

4.2 Conclusions

In patients with borderline negative FFR lesions, WSS metrics calculated using the CAAS Workstation WSS prototype did not provide additional prognostic value beyond patients' clinical characteristics and AS. However, Kaplan-Meier analysis revealed promising indications that the flow pattern distribution measured in 3D-QCA models could be useful in predicting adverse events. Further studies, involving a larger number of patients, is warranted to validate these observations and explore the full potential of this software in risk stratification.

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Declaration of interests

Jean-Paul Aben is an employee of Pie Medical Imaging. The remaining authors have no conflicts of interest to disclose.

CRediT author contributions

Vincenzo Tufaro: formal analysis, investigation, writing - original draft, writing - review & editing, visualization. **Ryo Torii**: software, writing - review & editing, supervision. **Jean-Paul Aben**: software, writing - review & editing. **Ramya Parasa, Bon-Kwon Koo, Roby Rakhit and Grigoris V. Karamasis**: investigation, writing - review & editing. **Ibrahim H. Tanboga**: formal analysis, writing - review & editing. **Ameer HA Khan, Michael McKenna, Murat Cap, Mazen A Gamrah**: investigation. **Patrick W. Serruys and Yoshi Onuma**: conceptualization, writing - review & editing. **Giulio G Stefanini, Daniel A Jones, Krishna Rathod and Anthony Mathur**: writing - review & editing. **Andreas Baumbach**: writing - review & editing, supervision. **Christos V. Bourantas**: conceptualization, writing - original draft, writing - review & editing, supervision.

References

1. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. *Nat Rev Dis Primers.* 2019;5(1):56.
2. Waksman R, Di Mario C, Torguson R, et al. Identification of patients and plaques vulnerable to future coronary events with near-infrared spectroscopy intravascular ultrasound imaging: a prospective, cohort study. *Lancet.* 2019;394(10209):1629-1637.
3. Bourantas CV, Raber L, Sakellarios A, et al. Utility of Multimodality Intravascular Imaging and the Local Hemodynamic Forces to Predict Atherosclerotic Disease Progression. *JACC Cardiovasc Imaging.* 2020;13(4):1021-1032.
4. Stone PH, Maehara A, Coskun AU, et al. Role of Low Endothelial Shear Stress and Plaque Characteristics in the Prediction of Nonculprit Major Adverse Cardiac Events: The PROSPECT Study. *JACC Cardiovasc Imaging.* 2018;11(3):462-471.
5. Stone PH, Saito S, Takahashi S, et al. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation.* 2012;126(2):172-181.
6. Gijsen F, Katagiri Y, Barlis P, et al. Expert recommendations on the assessment of wall shear stress in human coronary arteries: existing methodologies, technical considerations, and clinical applications. *Eur Heart J.* 2019;40(41):3421-3433.
7. Hakim D, Coskun AU, Maynard C, et al. Endothelial shear stress computed from coronary computed tomography angiography: A direct comparison to intravascular ultrasound. *J Cardiovasc Comput Tomogr.* 2023;17(3):201-210.
8. Lee JM, Choi G, Koo BK, et al. Identification of High-Risk Plaques Destined to Cause Acute Coronary Syndrome Using Coronary Computed Tomographic Angiography and Computational Fluid Dynamics. *JACC Cardiovasc Imaging.* 2019;12(6):1032-1043.
9. Bourantas CV, Zanchin T, Torii R, et al. Shear Stress Estimated by Quantitative Coronary Angiography Predicts Plaques Prone to Progress and Cause Events. *JACC Cardiovasc Imaging.* 2020;13(10):2206-2219.
10. Kumar A, Thompson EW, Lefieux A, et al. High Coronary Shear Stress in Patients With Coronary Artery Disease Predicts Myocardial Infarction. *J Am Coll Cardiol.* 2018;72(16):1926-1935.
11. Tufaro V, Safi H, Torii R, et al. Wall shear stress estimated by 3D-QCA can predict cardiovascular events in lesions with borderline negative fractional flow reserve. *Atherosclerosis.* 2021;322:24-30.
12. Tufaro V, Torii R, Erdogan E, et al. An automated software for real-time quantification of wall shear stress distribution in quantitative coronary angiography data. *Int J Cardiol.* 2022;357:14-19.
13. Girasis C, Schuurbiers JC, Muramatsu T, et al. Advanced three-dimensional quantitative coronary angiographic assessment of bifurcation lesions: methodology and phantom validation. *EuroIntervention.* 2013;8(12):1451-1460.
14. Hoogendoorn A, Kok AM, Hartman EMJ, et al. Multidirectional wall shear stress promotes advanced coronary plaque development: comparing five shear stress metrics. *Cardiovasc Res.* 2020;116(6):1136-1146.
15. Feldman CL, Ilegbusi OJ, Hu Z, Nesto R, Waxman S, Stone PH. Determination of in vivo velocity and endothelial shear stress patterns with phasic flow in human coronary arteries: a methodology to predict progression of coronary atherosclerosis. *Am Heart J.* 2002;143(6):931-939.

16. Candreva A, Pagnoni M, Rizzini ML, et al. Risk of myocardial infarction based on endothelial shear stress analysis using coronary angiography. *Atherosclerosis.* 2022;342:28-35.
17. Bourantas CV, Ramasamy A, Karagiannis A, et al. Angiographic derived endothelial shear stress: a new predictor of atherosclerotic disease progression. *Eur Heart J Cardiovasc Imaging.* 2019;20(3):314-322.
18. Kageyama S, Tufaro V, Torii R, et al. Agreement of wall shear stress distribution between two core laboratories using three-dimensional quantitative coronary angiography. *Int J Cardiovasc Imaging.* 2023;39(8):1581-1592.
19. Chen SL, Xu B, Chen JB, et al. Diagnostic accuracy of quantitative angiographic and intravascular ultrasound parameters predicting the functional significance of single de novo lesions. *Int J Cardiol.* 2013;168(2):1364-1369.
20. Tian J, Dauerman H, Toma C, et al. Prevalence and characteristics of TCFA and degree of coronary artery stenosis: an OCT, IVUS, and angiographic study. *J Am Coll Cardiol.* 2014;64(7):672-680.
21. Pinilla-Echeverri N, Mehta SR, Wang J, et al. Nonculprit Lesion Plaque Morphology in Patients With ST-Segment-Elevation Myocardial Infarction: Results From the COMPLETE Trial Optical Coherence Tomography Substudys. *Circ Cardiovasc Interv.* 2020;13(7):e008768.
22. Ahn J-M, Park D-W, Shin E-S, et al. Fractional Flow Reserve and Cardiac Events in Coronary Artery Disease. *Circulation.* 2017;135(23):2241-2251.
23. Johnson NP, Toth GG, Lai D, et al. Prognostic value of fractional flow reserve: linking physiologic severity to clinical outcomes. *J Am Coll Cardiol.* 2014;64(16):1641-1654.
24. Kuramitsu S, Matsuo H, Shinozaki T, et al. Five-Year Outcomes After Fractional Flow Reserve-Based Deferral of Revascularization in Chronic Coronary Syndrome: Final Results From the J-CONFIRM Registry. *Circ Cardiovasc Interv.* 2022;15(2):e011387.
25. Weerts J, Pustjens T, Amin E, et al. Long-term outcome after deferred revascularization due to negative fractional flow reserve in intermediate coronary lesions. *Catheter Cardiovasc Interv.* 2021;97(2):247-256.
26. Kok AM, Molony DS, Timmins LH, et al. The influence of multidirectional shear stress on plaque progression and composition changes in human coronary arteries. *EuroIntervention.* 2019;15(8):692-699.
27. Kato K, Yonetsu T, Kim SJ, et al. Nonculprit plaques in patients with acute coronary syndromes have more vulnerable features compared with those with non-acute coronary syndromes: a 3-vessel optical coherence tomography study. *Circ Cardiovasc Imaging.* 2012;5(4):433-440.

Figure legends

Figure 1. Multivariable models testing the additive prognostic value of 3D-QCA and CFD variables over baseline characteristics ('base model') for the primary endpoint.

Figure footnote: 3D-QCA, three-dimensional quantitative coronary angiography; CFD, computational fluid dynamics; CFI, coronary flow index; Conf. Inter., Confidence Interval; MLA, minimum lumen area; OSI, oscillatory shear index; RRT, relative residence time; TAWSS, time averaged wall shear stress; transWSS, transverse wall shear stress.

*Hazard ratio represents interquartile range.

Figure 2. Additive predictive value of 3D-QCA or WSS variables over the models including baseline demographics and WSS parameters or 3D-QCA metrics, respectively.

Figure footnote: 3D-QCA, three-dimensional quantitative coronary angiography; AS, area stenosis; AUC, area under the curve; CFD, computational fluid dynamics; CFI, coronary flow index; Conf. Inter., Confidence Interval; MLA, minimum lumen area; OSI, oscillatory shear index; RRT, relative residence time; TAWSS, time averaged wall shear stress; transWSS, transverse wall shear stress.

*Hazard ratio represents interquartile range.

Figure 3. Partial effect plots for the independent predictors of LOCE.

Figure footnote: Partial effects plots allow identification of the best cut-off for each variable to predict the primary endpoint. The relationship between each candidate predictor and the hazard ratio (HR) is plotted. The point where the candidate predictor crosses HR=1 line was considered the best cut-off. MLA, minimum lumen area; LOCE, lesion-oriented clinical events; TAWSS, time averaged wall shear stress; transWSS, transverse wall shear stress.

Figure 4. Kaplan-Meier curves display time to LOCE at a lesion level analysis for four individual variables.

Figure footnote: AS, area stenosis; LOCE, lesion-oriented clinical events; MLA, minimum lumen area; TAWSS, time averaged wall shear stress.

Figure 5. Kaplan-Meier curves display time to LOCE at a lesion-level analysis for lesions with low/high AS exposed to low/high maximum TAWSS.

Figure footnote: Overall, log-rank p-value <0.001; however, when taking low AS and low maximum TAWSS as a reference group, only lesion with AS and maximum TAWSS above their cut-offs had a significantly higher cumulative event rate at 4-year follow-up (26% v. 7%, p <0.001).

AS, area stenosis; LOCE, lesion-oriented clinical events; TAWSS, time averaged wall shear stress.

Figure 6 Graphical abstract. Use of CAAS Workstation WSS prototype (Pie Medical Imaging, Maastricht, Netherlands) in non-flow limiting lesions to identify vulnerable plaques.

Figure footnote: AS, area stenosis; CFI, coronary flow index; LOCE, lesion-oriented clinical events; OSI, oscillatory shear index; RRT, relative residence time; TAWSS, time averaged wall shear stress; ; transWSS, transverse wall shear stress.

Table 1. Baseline demographics in the entire population, differences between LOCE vs non-LOCE group and univariable Cox regression analysis.

	Population (n=352)	LOCE (n=57)	Non-LOCE (n=295)	*p*	Unadjusted HR	95%CI	*p*
Clinical characteristics							
Age	64 (55.9-71.1)	63 (54-70)	64 (56.7-72)	0.217	0.98	0.96-1.01	0.193
Male (%)	286 (81.3)	49 (86)	237 (80.3)	0.319	1.36	0.65-2.88	0.417
ACS presentation (%)	58 (16.5)	14 (24.6)	44 (14.9)	0.076	1.76	0.96-3.22	0.066
Co-morbidities							
Hypertension (%)	226 (67.5)	37 (64.9)	189 (68)	0.652	0.87	0.51-1.51	0.625
Hypercholesterolemia (%)	207 (60.5)	42 (73.7)	165 (57.9)	0.026	1.94	1.07-3.50	0.028
Diabetes mellitus (%)	114 (33)	23 (41.1)	91 (31.5)	0.163	1.41	0.83-2.40	0.205
Smoking history[a] (%)	156 (44.7)	26 (46.4)	130 (44.4)	0.776	1.05	0.62-1.77	0.869
Reduced LVEF[b] (%)	29 (9.6)	5 (10.9)	24 (9.4)	0.758	1.34	0.53-3.41	0.535
CKD[c] (%)	50 (14.8)	11 (19.6)	39 (13.9)	0.268	1.46	0.76-2.84	0.262
Previous MI (%)	74 (21.2)	10 (17.5)	64 (21.9)	0.460	0.81	0.41-1.60	0.546
Previous PCI (%)	108 (30.9)	18 (31.6)	90 (30.8)	0.910	1.04	0.59-1.81	0.904
Previous CABG (%)	4 (1.1)	2 (3.5)	2 (0.7)	0.126	4.03	0.98-16.5	0.053

Medical therapy							
Aspirin	308 (88.8)	54 (94.7)	254 (87.6)	0.118	2.57	0.80-8.22	0.112
Thienopyridine	189 (55.6)	34 (61.8)	155 (54.4)	0.310	1.32	0.76-2.27	0.322
DAPT	171 (50.3)	33 (60)	138 (48.4)	0.116	1.56	0.91-2.67	0.108
Beta-blocker	130 (50.8)	28 (58.3)	102 (49)	0.246	1.57	0.88-2.80	0.123
RASS	142 (56.8)	27 (58.7)	115 (56.4)	0.774	1.08	0.60-1.95	0.793
Statin	229 (87.4)	43 (87.8)	186 (87.3)	0.935	1.23	0.52-2.89	0.639

Table footnote: ACS, acute coronary syndrome; CABG, coronary artery bypass graft; CKD, chronic kidney disease; DAPT, dual antiplatelet therapy; LVEF, left ventricular ejection fraction; LOCE, lesion-oriented clinical events; MI, myocardial infarction; PCI, percutaneous coronary intervention; RAAS, renin-angiotensin-aldosterone system inhibitor.

[a] Smoking history (active or ex-smoker) at the time of baseline angiography

[b] Reduced LVEF was defined as ejection fraction <50%.

[c] CKD defined as estimated glomerular filtration rate <60mL/min/1.73m^2

Table 2. QCA and CFD-derived variables of lesions associated and those not associated with LOCE, with univariable Cox regression analysis.

	Studied lesions (n=355)	LOCE (n=57)	Non-LOCE (n=298)	*p*	Unadjusted HR[a]	95%CI[a]	*p*
3D-QCA variables							
Prox reference area (mm^2)	6.55 (5.19-7.98)	7.10 (5.68-8.92)	6.50 (5.10-7.88)	0.089	1.35	0.85-2.14	0.233
Distal reference area (mm^2)	4.82 (3.81-6.25)	5.16 (4.21-6.62)	4.77 (3.75-6.11)	0.159	1.38	0.87-2.19	0.305
Mean reference area (mm^2)	5.77 (4.62-7.26)	6.07 (5.06-7.74)	5.66 (4.53-7.16)	0.081	1.46	0.92-2.34	0.203
Lesion length (mm)	22.5 (16.8-32.2)	23.4 (15.0-32.2)	22.3 (17.1-32.3)	0.921	0.92	0.62-1.36	0.917
MLA (mm^2)	2.21 (1.78-2.96)	2.12 (1.58-2.70)	2.24 (1.80-2.98)	0.184	0.76	0.52-1.11	0.347
AS (%)	54.0 (45.3-61.7)	59.4 (54.6-67.7)	52.8 (43.8-60.1)	<0.001	2.40	1.51-3.81	<0.001
CFD-derived variables							
Min TAWSS (Pa)	0.67 (0.46-0.98)	0.72 (0.48-1.02)	0.66 (0.45-0.97)	0.385	1.35	0.82-2.24	0.481
Max TAWSS (Pa)	8.27 (5.58-12.40)	11.56 (8.25-13.64)	7.73 (5.41-11.51)	<0.001	2.76	1.49-5.11	0.002
Highest Mean TAWSS (Pa)	7.50 (4.92-10.80)	10.36 (7.12-12.40)	6.93 (4.76-10.40)	<0.001	2.72	1.48-5.0	0.001
Mean TAWSS at the MLA (Pa)	6.55 (4.17-10.02)	9.33 (5.44-11.94)	6.19 (3.96-9.00)	<0.001	2.65	1.44-4.88	0.001

Mean TAWSS prox to MLA (Pa)	4.76 (2.85-7.51)	6.16 (3.84-10.00)	4.35 (2.65-7.14)	0.003	1.98	1.13-3.47	0.021
Max OSI	0.07 (0.03-0.12)	0.09 (0.04-0.12)	0.07 (0.03-0.12)	0.293	1.45	0.88-2.37	0.210
Max RRT	2.55 (1.61-4.38)	2.77 (1.68-3.70)	2.50 (1.55-4.47)	0.733	1.16	0.71-1.91	0.145
Max transWSS (Pa)	0.24 (0.17-0.36)	0.30 (0.21-0.45)	0.23 (0.17-0.32)	0.002	2.33	1.29-4.21	0.014
Max CFI	0.21 (0.16-0.26)	0.23 (0.17-0.26)	0.21 (0.15-0.25)	0.099	1.35	0.94-1.95	0.255

Table footnote: 3D-QCA, three-dimensional quantitative coronary angiography; AS, area stenosis; CFI, coronary flow index; CFD, computational fluid dynamic; CI, confidence interval; FFR, fractional flow reserve; HR, hazard ratio; LOCE, lesion-oriented clinical events; MLA, minimum lumen area; OSI, oscillatory shear index; RRT, relative residence time; transWSS, transverse wall shear stress; TAWSS, time averaged wall shear stress.

[a]HR represents interquartile range.

Responses to the reviewers' comments

Reviewer #1

The authors in their manuscript present the results of a retrospective study including patients with borderline negative FFR and suitable angiographic images for 3D QCA reconstructions. In these patients 3d QCA was performed using the baseline angiogram and wall shear stress (WSS) metrics were calculated using a software able to calculate these variables in <10 minutes. Their main findings were that %AS was an independent predictor of adverse events at follow-up, while WSS metrics were not; however, a high maximum TAWSS was an important discriminator in patients with high %AS better identifying patients at risk for future events.

It is an interesting study, despite the caveats of retrospective studies, that shows a potential value of hemodynamic measurements in risk stratification of non-culprit lesions using coronary angiography alone. There are however several important issues that need to be addressed.

We are grateful to the reviewer for the complementary feedback, the thorough review and the insightful comments that we have tried to address in the revised manuscript.

We agree with the reviewer that this analysis is important in the field as it underscores for the first time the prognostic value of a user-friendly software that is capable to assess the distribution of the local hemodynamic forces – including the multidirectional shear stress – in few minutes. For this reason, we believe that this work is an important step toward the widespread use of the shear stress to stratify cardiovascular risk and predict plaques that are prone to progress and cause events. Despite its retrospective design, we are confident that this analysis, that is one of the largest in the field, will attract attention and will stimulate further research.

In the revised manuscript, we have tried to clarify the important points raised by the reviewers and enhanced the quality of the manuscript – we hope that the revised draft is improved and meets the expectations of the reviewers and editors for publication.

Specific comments:

- The lesion subset is very specific and it is hard to tell how results from lesions with a borderline negative FFR may translate to other non-significant lesions.

The point raised by the reviewer is valid. Indeed, in this analysis we included only intermediate stenosis with borderline negative FFR (0.81-0.85) for the following reasons:

- FFR is currently the gold standard for the functional assessment of coronary lesions in patients with undergoing coronary angiography. A cutoff of ≤0.80 is recommended to detect flow limiting lesions which require revascularization. (Vrints et al., 2024)

- Cumulative data have shown that some of the lesions that cause non-flow limiting stenosis (FFR >0.80) are likely to progress and cause events. These lesions have a vulnerable plaque phenotype and in particular increased plaque burden, small lumen area and a large necrotic core component that is covered by a thin fibrous cap. (Kedhi et al., 2021; Mol et al., 2023) Intravascular imaging can detect these lesions, but this is not practically feasible in routine practice. Computed tomography coronary angiography (CTCA) provides an attractive alternative for vulnerable plaque detection; however, its accuracy is limited. In addition, only patients with stable angina have a CTCA before coronary angiography, as the patients with an ACS - that have more advanced atherosclerotic lesions and constitute 2/3 of the patients having an invasive angiogram – are listed directly for this procedure. Hence, it is essential to introduce alternative approaches to detect vulnerable plaques.
- Numerous physiology and imaging studies have shown that plaque vulnerability is associated with lesion severity. Therefore, it is more likely the lesions that have a borderline negative FFR to have a high-risk phenotype than the lesions with FFR close to one. This is supported by several reports which showed that the lesions with an FFR of 0.81-0.85 are at higher risk to progress and cause events than lesions with an FFR between 0.86-0.90 or >0.91. (Adjedj et al., 2016; Hakeem et al., 2016; Masrani Mehta et al., 2015) These lesions are seen often in patients undergoing an FFR study as they comprise roughly one-third of all negative FFR lesions. (Johnson et al., 2014; Masrani Mehta et al., 2015)

Summarizing the above, it is apparent that there is an unmet need to stratify more accurately cardiovascular risk in patients with a borderline negative FFR before considering their pre-emptive treatment. This analysis was conducted to address this need, and we believe that is clinically relevant as it introduces an alternative approach in the search of vulnerable plaques.

It should be acknowledged, however, that it is unclear whether our findings apply to mild stenoses. There is however published literature supporting the prognostic value of 3D-QCA-derived WSS in other lesion types; more specifically:

- Kumar et al. (Kumar et al., 2018) showed that in patients with stable CAD and hemodynamically significant lesions, high WSS in the proximal lesion segments was a predictor of myocardial infarction.
- We have also conducted a post-hoc analysis of the IBIS 4 and PROSPECT studies (Bourantas et al., 2020), which included non-flow limiting lesions with a lipid-rich phenotype (thin or thick-cap fibroatheroma) and showed that high WSS at the lesion site was associated with an increased risk of adverse cardiovascular events.
- Candreva et al. (Candreva et al., 2022) analysed data from patients that had an angiogram before suffering a myocardial infarction and showed that WSS metrics at the lesion site predicted the lesions that caused myocardial infarction.

All these studies are important in the field as they provide robust evidence about the prognostic value of the WSS computed in 3D-QCA models. However, they all have significant limitations as some focused on a subset of lesions with a specific phenotype (i.e. lipid rich, or lesions with severe stenosis) (Bourantas et al., 2020; Kumar et al., 2018), other used two propensity matched groups (one group that consisted of patients that had an event and the other a control group) (Candreva et al., 2022) and others included only patients that had an event. (P. H. Stone et al., 2018; P. H. Stone et al., 2012)

An all-comers study including lesions with mild stenosis will require analysis of angiographic data collected from a large number of patients as the event rate is expected to be low in this cohort; for example, in the PROSPECT II study (Erlinge et al., 2021) only 112 out of the 898 patients that were assessed by NIRS-IVUS had an event at 4 years follow-up.

Before embarking in this challenging analysis, it is therefore essential to further optimize the CAAS Workstation WSS prototype, improve vessel meshing, reduce analysis time, streamline WSS computation and data post-processing. We are working with Pie Medical towards this direction, and we hope that we will be able to conduct this study in the foreseeable future.

In the revised manuscript, text has been added in the limitation section (line 396-398) where we discuss these points:

"*The lesion subset selected for this study (FFR 0.85-0.81) was very specific; thus, it is unclear whether these findings apply to other non-flow limiting lesions. Future studies should be conducted to explore the prognostic value of 3D-QCA-derived WSS in this setting.*"

- More details should be added regarding the 3d QCA method, while exclusion criteria should be moved to the main manuscript, and reasons for exclusion of the majority of the patients specifically mentioned.

Thank you for raising this important point. In the originally submitted manuscript, we have provided the relevant references rather than a detailed description of the 3D-QCA method. We agree with the reviewer that this is an important argument that should have been described with more clarity. In the revised draft, we have modified the relevant section and added a paragraph where we summarize the methodology of our 3D-QCA analysis (line 185-203):

"*3D-QCA analysis*

Coronary artery reconstruction was performed using the CAAS Workstation WSS prototype software at the Coronary Imaging Core lab Queen Mary University London. An experienced analyst identified the intermediate stenosis assessed by FFR, blinded to baseline demographics, clinical outcomes and FFR results, and used side branches located proximally and distally to this lesion to define the segment of interest (SOI).

Reconstruction was performed from two angiographic projections that were at least 30° apart and portrayed the SOI with minimal foreshortening or overlapping. Two end-diastolic projections showing

the SOI filled with contrast were selected and co-registration was performed by defining a corresponding point in these projections. The CAAS workstation enables reconstruction of a single side branch, and this was included in the 3D geometry when it had a reference diameter ≥1mm; in case of ≥2 side branches arising from the SOI, only the largest one was reconstructed. The information located in the DICOM files were used to define the location of the two projections in 3D space, and estimate pixel size, and then an edge detection algorithm was applied to detect the lumen borders of the SOI and its side branch when present. Corrections of the detected borders were made by the expert analyst if this was deemed necessary. The identified borders were then used to generate the 3D geometry of the SOI and its branch, if applicable, assuming an elliptical cross-sectional shape.[13] *In the obtained 3D models, the model length, lesion length, proximal and distal reference area, the % area stenosis (AS) and minimum lumen area (MLA) were estimated."*

In the originally submitted manuscript the exclusion criteria were described in the supplementary file. These have now been moved to the main manuscript (line 157-163). In the revised draft, we have also added a supplementary figure (Supplementary Material, page 11) displaying the study flowchart:
"*We excluded from the study: i) patients admitted with an acute coronary syndrome who had an ambiguous culprit lesion, or a borderline negative FFR on a possible culprit lesion; ii) patients with left main stem disease, ostial left anterior descending, circumflex or right coronary artery lesions, coronary bypass grafts lesions, or lesion located in a stented segment (in-stent restenosis) or within 5mm from the edge of a stent (stent-edge restenosis); iii) and patients with inadequate angiographic data for 3D-QCA analysis (poor angiographic image quality, insufficient DICOM information, or lack of two angiographic projections ≥30° apart).*"

"Supplementary Figure 2. Flowchart of the patients and lesions included in the final analysis.

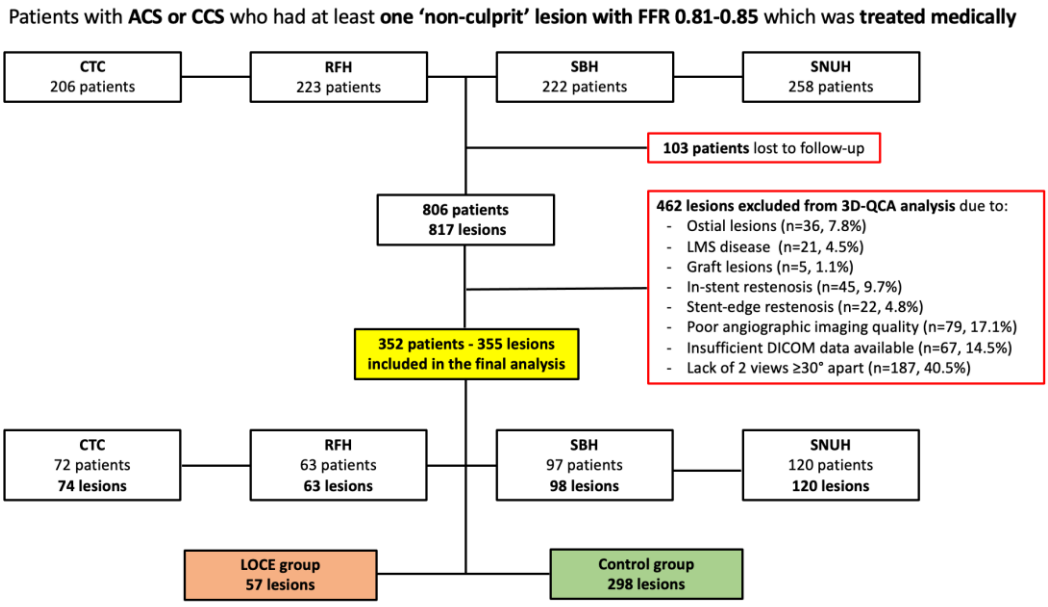


Figure footnote: ACS, acute coronary syndrome; CCS, chronic coronary syndrome; 3D-QCA, three-dimensional quantitative coronary angiography; CTC, Essex CardioThoracic Centre; DICOM, Digital Imaging and Communications in Medicine; FFR, fractional flow reserve; LMS, left main stem; LOCE, lesion-oriented clinical events; RFH, Royal Free Hospital; SBH, St Bartholomew's Hospital; SNUH, Seoul National University Hospital."

- Were the outcomes of the excluded patients similar to those included?

Thank you for this relevant comment.
In our study, the primary endpoint was the incidence of lesion-oriented clinical events (LOCE) defined as the composite of cardiac death, target lesion related myocardial infarction (MI) or clinically indicated target lesion revascularization (TLR) attributed to disease progression of the lesions with a borderline negative FFR. The cause of death was confirmed based on death certificate reports.
For the lesions included in the final analysis (n=355), we recorded 6 cardiac deaths (1.7%) and 51 target lesion MI or clinically indicated TLR (14.4%).
For the patients excluded from the final analysis (n=454; 462 lesions), death certificates were not collected; thus, data on cardiac death are not available. Therefore, we can only compare the incidence of target lesion MI and clinically indicated TLR between included and excluded patients. In total, 61 events were reported in 462 intermediate lesions that were excluded from the analysis; the event rate in this cohort was 13.2% that was very similar to the event rate reported in our study (14.4%).

- What was the follow-up procedure? A complete follow-up of at least 2 years is mentioned in the methods section, however a median follow-up of >4 years is reported.

We apologise for the lack of clarity with regards to the follow-up period. It is important to stress that none of the patients had a follow-up procedure as part of this study. A repeat angiogram and revascularization were considered only for clinical purposes in patients that had angina symptoms or a cardiovascular event. As stated in the methods sections we have included in this study patients that had an angiogram between Jan 2012 and December 2017. All the patients had complete follow-up data till the 31st of December 2019; thus, all patients had at least 2 years of follow-up. For patients who underwent angiogram prior to December 2017, the follow-up was >2 years and up to >7 years for patients who had their angiograms in 2012. Therefore, the median follow-up in this study was 4.2 years. This study was conducted as part of a local audit (reference number: 10630, 1/2/2020) investigating the long-term cardiovascular outcomes in patients with borderline negative FFR.
To clarify this point, we have amended the relevant text that now reads (line 150-156):
"*We retrospectively screened patients who underwent a coronary angiography in four different Cardiology Departments (Barts Heart Centre, London, UK; Essex Cardiothoracic Centre, Basildon,*

UK; Royal Free Hospital, London, UK; Seoul National University Hospital, Seoul, Korea) from January 2012 to December 2017. Patients eligible for recruitment were those admitted with either acute or chronic coronary syndrome, who had at least one non-obstructive coronary lesion with a borderline negative fractional flow reserve (FFR: 0.81-0.85) that was treated conservatively in accordance with international guidelines. All the included patients had complete follow-up until December 2019."

- An important issue with including lesions post FFR measurement is that it is unclear whether the FFR is due to the lesion per se or upstream or downstream disease

This is another important point raised by the reviewer. There are indeed different pathophysiological patterns of atherosclerotic disease; recently the pull-back pressure gradient has been introduced to classify disease as focal or diffuse. A diffuse pattern of disease is defined as a gradual pressure loss across a long vessel segment (≥20 mm) without a significant abrupt pressure drop at any level. (Collet et al., 2019)

Studies combining imaging and physiology have provided conflicting results with some showing that the presence of focal disease is associated with increased plaque vulnerability and others showing no difference in plaque morphology in lesions with focal or diffuse disease. (Kotoku et al., 2023; Sakai et al., 2023) The effect of pathophysiological patterns of atherosclerotic disease on shear stress distribution has been recently assessed in a study published by Candreva et al that showed higher WSS in patients with focal disease. (Candreva et al., 2024) Nevertheless, this study had significant limitations, it included a small number of lesions, it did not incorporate the side branches in the reconstructed vessel geometry, and it was unable to examine the prognostic implications of WSS distribution.

In the present study, we included all lesion types including focal disease, diffuse with a single long lesion and diffuse disease with tandem lesions. In case of tandem lesions that are defined as multiple focal stenoses separated by segments without a significant pressure drop the FFR can estimate the overall hemodynamic effect of these lesions on coronary circulation, but it is unable to accurately estimate the effect of each lesion of myocardial blood supply. Non-hyperaemic indices seem to outperform to FFR in this setting. Under non-hyperaemic conditions, the crosstalk phenomenon is minimized since coronary flow is maintained at a constant level by microcirculatory self-regulation mechanisms at rest. Therefore, NHPR-based pressure wire pullback may have a theoretical advantage over FFR until the compensatory reduction in microvascular reserve is depleted. (Scarsini et al., 2022) However, published studies have also underscored their limitations in predicting vessel physiology following treatment of one of the detected tandem lesions. (Jeremias et al., 2019) Additionally, angiographic derived FFR solutions may also provide complementary data and predict the individual contribution of each of the tandem lesion on the FFR however recent reports have shown that these have a limited efficacy in detecting flow limiting disease and an accuracy at the range of 0.75. (Ninomiya et al., 2023)

In the present study, we have included all the cases with focal and diffuse disease with pressure wire advanced distally to the most distal lesion. In cases of tandem lesions, the SOI included all the lesions detected on coronary angiography; however, the pull-back pressure gradient information was not available to classify the pathophysiological patterns of atherosclerotic disease. In tandem lesions, our analysis focused on the lesion with the higher area stenosis as this has been found to be the stronger predictor of events. (Candreva et al., 2022; Tufaro et al., 2021) We reported only the WSS distribution measured in this lesion and events were censored only if there was a disease progression in the lesion with the smaller area stenosis. This point has been clarified in the revised manuscript (line 234-238) that now reads:

"In diffusely diseased segments and in tandem lesions with borderline negative FFR along the vessel, 3D-QCA and CFD analysis was performed in the entire segment. In tandem lesions we extracted the WSS only in the lesion with the higher AS – as this was the most powerful predictor of future events in previous studies[11,16] – and this lesion was included in the analysis. An event was censored in this setting only if there was disease progression in the lesion with the largest area stenosis."

- Moreover the inclusion of ACS patients raises further questions regarding the validity of the measurements and the subset of lesions where these results apply

The comment of the reviewer is insightful. Indeed, there is evidence that in patients with an ACS the lesions with a borderline negative FFR are more likely to progress and cause events than lesions with a borderline negative FFR in patients with a chronic coronary syndrome (CCS). This can be attributed to several reasons. Firstly, FFR is often used to guide revascularization in patients with multi-vessel disease admitted with an ACS when there is ambiguity about the culprit lesion. This approach is likely to lead to stent implantation in a non-culprit lesion and leave the culprit lesion untreated (Heitner et al., 2019; Kerensky et al., 2002; Opolski et al., 2019; Reynolds et al., 2011) as in culprit lesions the FFR tends to underestimate lesions severity after an ACS because of the increase peripheral resistance due to distal embolization. (Montone, Meucci, & Niccoli, 2020) In addition, the culprit lesions that were left untreated are more likely to progress and cause events than the non-culprit lesions. (L. Chen, Chester, Crook, & Kaski, 1996)

In our study, we have tried to overcome this limitation (line 157-158):

"*We excluded from the study: i) patients admitted with an acute coronary syndrome who had an ambiguous culprit lesion, or a borderline negative FFR on a possible culprit lesion;".*

Moreover, it has been recognized that the non-culprit plaques in patients with an ACS have a more vulnerable phenotype than the plaques in patients with a CCS. (Kato et al., 2012) However, FFR guided revascularisation is currently recommended to guide therapy in patients with a CCS and in patients with an ACS when there is multivessel disease and the culprit lesion is known. (Engstrom et al., 2015; Sels

et al., 2011; Smits et al., 2017) The FFR cutoff used to guide revascularization in both cohorts has been set at 0.80 as this strategy aims at the identification of flow limiting lesions rather than the detection of high-risk lesions.

Considering the above, we have decided to include in the study both patients with a CCS and an ACS. To account for the differences in the prognosis noted in the two groups we entered the clinical presentation in the Cox regression analysis. We have demonstrated that ACS presentation was not a predictor of worse outcomes in univariable analysis (Table 1). Despite that the clinical presentation was taken as adjustment variable for the base model and used in the multivariable analysis as its p-value was <0.25 in the univariable Cox model. We have found that the WSS provided incremental prognostic information to the base model and enabled better prediction of LOCE.

To exclude a potential pitfall due to the inclusion of ACS patients in the reported results, we repeated the analysis only in the subgroup of patients presenting with a CCS (n=292 lesions). We found no significant differences in the results; as before, the AS, the TAWSS at the MLA, the highest mean TAWSS, the maximum TAWSS and the maximum tranWSS were predictors for the primary endpoint.

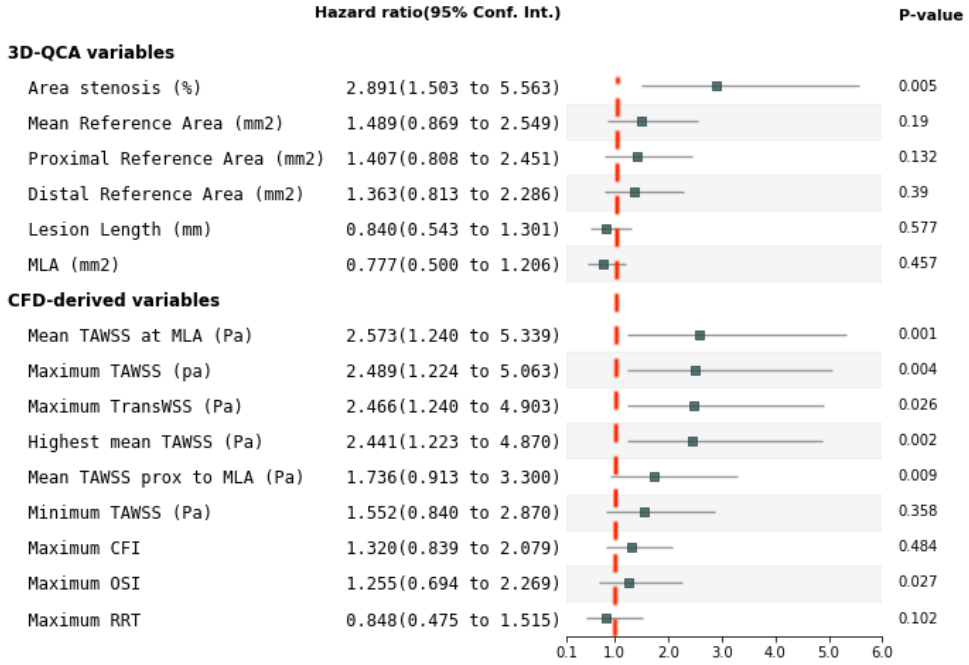


Figure. Multivariable models testing the additive prognostic value of 3D-QCA and CFD variables for lesion-oriented clinical events (LOCE).

When we examined the additive predictive value of the 3D-QCA derived variables over the models including the baseline demographics and CFD variables and of the CFD-variables over the model including the baseline demographics and 3D-QCA metrics we found that the AS but not the WSS metrics was an independent predictor of LOCE.

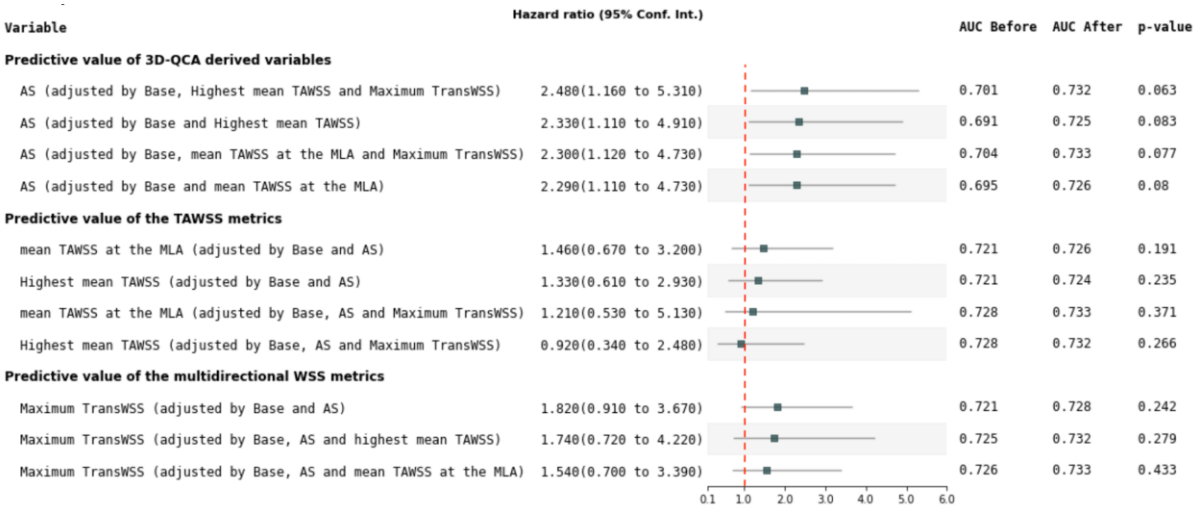


Figure. Additive predictive value of 3D-QCA variables over the models including baseline demographics and CFD variables, and of CFD-variables over the model including baseline demographics and 3D-QCA metrics.

To address the reviewer's comment, text has been added in the limitations section which now reads (line 403-408):

"We have included in our analysis patients admitted with a chronic or an acute coronary syndrome as FFR has a value in guiding revascularization in both cohorts; studies have shown significant differences in lesion morphology and worse outcomes in patients with a borderline negative FFR in patients with an acute coronary syndrome.[27] To account for these differences, patient's clinical presentation was included in the Cox regression analysis. Of note, the results were not different when we repeated the analysis only in patients with a chronic coronary syndrome."

- The statistics section regarding the multivariable Cox proportional hazard regression is complex, and it is not clear whether these results are prone to errors due to multiple comparisons

Thank you for highlighting this important point.

We would like to stress that the analysis was performed by Professor Halil Tanboga who has expertise in Biostatistics. We acknowledge that the conduction of multiple comparisons and statistical tests may increase the risk of Type I errors. However, we believe that the methodology that we have applied mitigates, at least partially, these risks. The rationale for using this specific analysis protocol is summarized below:

1. The present research is an observational and exploratory study that aims to identify potential associations and generate hypotheses for future studies. In exploratory analyses, examining multiple variables is essential to provide novel insights, although we acknowledge that this can increase the risk of false positive results.

2. The subgroup analyses - in randomized controlled trials (RCTs), where multiple comparisons are often made to explore potential effects in different subsets of the recruited patients and in retrospective analyses including our study - aim to uncover meaningful relationships between metrics that may warrant further investigation. While we are aware of the risks associated with multiple comparisons, such exploratory analyses are valuable as they can help us to better understand the association between baseline clinical anatomical and physiological characteristics and cardiovascular events.
3. Rather than focusing solely on p-values, we applied comprehensive model evaluation and included a range of performance metrics that allow us to better assess the robustness of our models. These include the likelihood ratio Chi-square (χ^2), R-squared values and the overall and time-dependent area under the curve (AUC) for each candidate predictor. This multifaceted approach provides a more thorough evaluation of model performance and allow us to better examine the strength of associations.
4. We performed adjustment for multiple variables based on biological plausibility and the univariate analysis results (variables with p-value < 0.25). This method helps to control for potential confounders and reduces the likelihood of spurious associations due to multiple comparisons.
5. To address the risk of overfitting associated with multiple comparisons, we refitted our survival models using the ridge regularization method. Regularization techniques penalize model complexity, which can help to prevent overfitting and improve the generalizability of the model.
6. Finally, we employed restricted cubic spline transformations with three knots for each variable to capture potential non-linear relationships.
While we believe these steps address the concern of multiple comparisons, we acknowledge that the risk cannot be eliminated in exploratory studies.

- It is not clear how references 23, 26, and 27 provide evidence that 'borderline non-flow limiting lesions on functional assessment are at risk to progress and cause events due to an increased plaque burden and high incidence of intravascular imaging features associated with plaque vulnerability' when discordant results between FFR and plaque morphology are not rare and have implications for risk stratification (doi: 10.1093/eurheartj/ehab433.)

Thank you for raising this point. The papers cited in the first draft of the manuscript were:

- **Ref 23**, Barbato et al. JACC 2016 (Barbato et al., 2016), this study showed that in patients with a CCS the relationship between FFR and 2-year MACE is a continuous function rather than a dichotomic pattern (events in FFR <0.8, no events in FFR $\geq$0.8). Authors reported a significant decrease in MACE per 0.05-unit increase in FFR which was statistically significant even after adjustment for all clinical and angiographic features. These findings suggests that lesions with a borderline negative FFR are more likely to progress and cause an event than those with an FFR close to 1.

- **Ref 26**, Chen et al. Int J Card 2013 (S. L. Chen et al., 2013), this study demonstrated a correlation between IVUS metrics and FFR values in patients with single vessel coronary artery disease. The authors in this study showed that lesions with FFR≤0.8 were located more often in the proximal LAD, and had a higher plaque burden, %DS, and smaller MLA than those with an FFR ≥0.8. In that study the MLA, the PB, the lesion length and the located of the lesion in the LAD were predictors of FFR≤0.8.
- **Ref 27** is the paper of Tian et al. JACC 2014 (Tian et al., 2014) which showed that plaque rupture is primarily influenced by FCT, and the combination of significant plaque burden and luminal narrowing contributes to acute coronary syndrome.

We agree with the reviewer that the study of Barbato et al. and Tian et al. do not strongly support our statement, thus they have been removed from the revised manuscript. Conversely, we kept the study of Chen et al. in our references as it demonstrates an association between plaque morphology and lesion hemodynamic severity. In line with these findings are the results of the OCT sub-study of the COMPLETE trial that showed that flow limiting lesions were more likely to have high-risk characteristics than those that did not cause an obstruction. (Pinilla-Echeverri et al., 2020) Therefore, it is not a surprise that stenotic lesions or lesions that have a borderline hemodynamic severity are more likely to cause events than lesions with no significant stenosis. This has been shown in several reports including:

- A Meta-analysis by Johnson et al. (Johnson et al., 2014) which found that lesions with FFR values between 0.80-0.85 were associated with higher event rates compared to lesions with higher FFR values.
- The IRIS-FFR Registry (Ahn et al., 2017) which showed that lesions with FFR values between 0.81-0.85 were associated with a higher risk of future coronary events compared to lesions with FFR >0.85.
- A retrospective study (Weerts et al., 2021) including patients with an FFR>0.80 that showed that low FFR values (FFR 0.81–0.85), multivessel disease, distal lesions and the presence of disease in a vein graft were associated with a higher risk of target lesion failure in multivariable analysis.
- The J-CONFIRM Registry (Kuramitsu et al., 2022) which showed that the cumulative 5-year incidence of target vessel failure was similar in patients with FFR 0.75-0.80 and 0.81-0.85, and that these two groups had significantly higher incidence of events than patients with FFR 0.86-1.00.

As the reviewer correctly pointed out, it is likely that also plaques mild in severity have a vulnerable phenotype. For example, in the PROSPECT (G. W. Stone et al., 2011) and PROSPECT II (Erlinge et al., 2021) studies most of the vulnerable plaques that caused an event had a mild stenosis. Similarly, as the reviewer has stated the COMBINE OCT-FFR study (Kedhi et al., 2021) that included only non-

flow limiting lesions (FFR >0.80) showed that the presence of TCFAs detected by OCT was a strong predictors of future adverse cardiac events in diabetic patients. Similar were the results of the PECTUS study (Mol et al., 2023) that included patients with a myocardial infarction that had FFR-negative non-culprit lesions and demonstrated that the presence of high-risk plaque features on OCT was associated with worse outcome. From the above, it is apparent that although the plaque morphology is not always associated with lesion severity it is more likely plaques that cause a hemodynamically significant stenosis to have high-risk plaque characteristics.

In the revised manuscript (line 358-360), we have modified the sentence and added different references to address your comment:

"*In this study, we included lesions with FFR 0.81-85 which are more likely to exhibit vulnerable plaque features*[19-21] *and are at increased risk of causing adverse events compared to lesions with a higher FFR.*[22-25] ,,

Reviewer #3: Thank you for the opportunity to review this manuscript that explore the clinical implications of wall shear stress in coronary lesions (352 patients, 355 lesions) that were deemed borderline on physiological assessment (FFR 0.81-0.85).

1. I think the use of "real time" in this context is misleading. It is a retrospective cohort, with wall shear stress being calculated retrospectively and not in "real time" during usual clinical practice. It may have the ability to be used in real time, but it was not in this setting.

Thank you for your valuable feedback and the constructive comment. We agree that the term "real-time" was used to emphasise the potential of this software to have clinical applications as it allows for the first time measurement of the WSS in a user-friendly environment and within a few minutes. However, as the reviewer has correctly pointed out in this study the WSS was calculated retrospectively and not in "real-time" and therefore this statement is not supported by the paper.
In the revised manuscript, the expression “real-time” has been removed from the title, abstract and discussion section and has been replaced by the term fast in the title and abstract.

Title
“*Can fast wall shear stress computation predict adverse cardiac events in patients with intermediate non-flow limiting stenoses?*”
Abstract (line 67-68)
“For non-flow limiting lesions with borderline negative FFR, fast WSS computation using a dedicated software is feasible and holds potential for cardiovascular risk stratification.”
Discussion (line 322-324)
“In the present study, we investigated for the first time the prognostic value of WSS metrics derived by the CAAS Workstation WSS prototype in a large cohort of patients with borderline non-flow limiting lesions.”

2. Exclusions have to be described in greater detail, and it is insufficient to bury them in the supplementary materials. For each exclusion criterion I would want to see the number of patients excluded for each result. This manuscript is evaluating the potential / applicability of this software in usual clinical practice, if >50% of patients are excluded because the software can't be used in those settings, or images are inadequate, it says a lot about the applicability of the software. A prospective study won't solve this issue, if it is challenging to achieve adequate angiographic images for the software to run.

Thank you for this insightful comment. In the revised manuscript, we have added text to the methods section (line 157-163) where we describe the exclusion criteria:

"*We excluded from the study: i) patients admitted with an acute coronary syndrome who had an ambiguous culprit lesion, or a borderline negative FFR on a possible culprit lesion; ii) patients with left main stem disease, ostial left anterior descending, circumflex or right coronary artery lesions, coronary bypass grafts lesions, or lesion located in a stented segment (in-stent restenosis) or within 5mm from the edge of a stent (stent-edge restenosis); iii) and patients with inadequate angiographic data for 3D-QCA analysis (poor angiographic image quality, insufficient DICOM information, or lack of two angiographic projections ≥30° apart).*"

and created a flowchart (Supplementary Material, page 11) which displays why some patients and lesions were excluded from our final analysis:

"Supplementary Figure 2. Flowchart of the patients and lesions included in the final analysis.

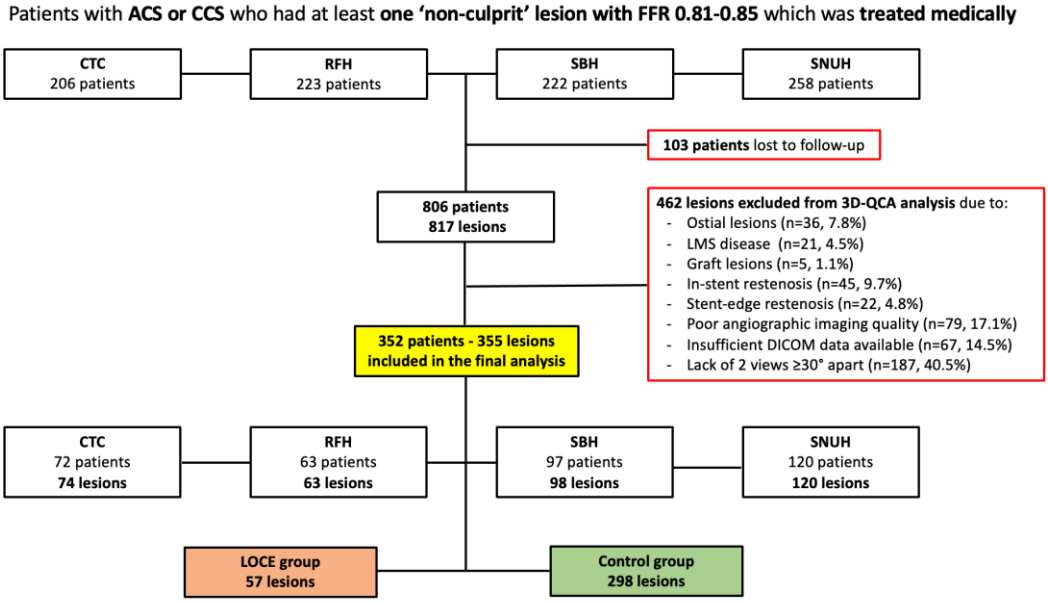


Figure footnote: ACS, acute coronary syndrome; CCS, chronic coronary syndrome; 3D-QCA, three-dimensional quantitative coronary angiography; CTC, Essex CardioThoracic Centre; DICOM, Digital Imaging and Communications in Medicine; FFR, fractional flow reserve; LMS, left main stem; LOCE, lesion-oriented clinical events; RFH, Royal Free Hospital; SBH, St Bartholomew's Hospital; SNUH, Seoul National University Hospital."

Indeed, as the reviewer stated >50% of the lesions (462/817, 56.5%) were excluded from the analysis. The main reason for excluding these lesions were insufficient DICOM data available (14.5%), poor angiographic image quality (17.1%) and lack of two angiographic views that were at least 30^{o} apart which are needed for vessel reconstruction (40.5%).

Conversely, the number of cases that were exclude because of lesion location – ostial lesions, LMS disease or graft cases – was relatively small accounting for <15% of the total number of lesions. Like

other software that have been designed to assess lesion severity from 3D-QCA data (e.g. vFFR, QFR), the CAAS WSS Workstation cannot operate in LMS and RCA disease. (Ramasamy et al., 2020) Reconstruction of ostial LCx or LAD lesions was technically feasible, but it was found to be challenging in our data because in these segments there is often overlapping, or we had to use the spider view where there is increased background noise that can affect accurate vessel modelling which is detrimental for the WSS computation. In addition, we have excluded cases of in-stent and stent-edge restenosis as it is likely that not only the WSS, but also other mechanisms have been involved in the formation of these lesions. Nevertheless, we and others believe that WSS play a detrimental role in vessel wall response following stent implantation and that future studies should look on the value of this software to predict stent failure. (Papafaklis et al., 2010)

It should be noticed that a similar exclusion rate was reported in our previous study (Tufaro et al., 2021), when we used a different 3D-QCA software (Medis QAngio®XA 3D), which requires 2 views at least 25° degrees apart, to reconstruct vessel architecture. This indicates that most of the cases were excluded from this study not because of deficiencies of the software, but because of the limitations of the available data. This is a well-recognized problem in the field and has been reported in numerous retrospective studies that used 3D-QCA-based software to assess lesion physiology. For the QFR software, 1/3 of the cases were excluded from the analysis in retrospective studies (median 28%, range 6% to 92%), whereas only 1 in 6 cases was excluded in prospective studies (median 17%, range 7% to 52%). (https://www.nice.org.uk/guidance/dg43/resources/qangio-xa-3d-qfr-and-caas-vffr-imaging-software-for-assessing-coronary-stenosis-during-invasive-coronary-angiography-pdf-1053809508805).

A prospective study is also likely to enhance the performance of the CASS Workstation WSS prototype in measuring the WSS distribution as the interventional cardiologist will try to acquire optimal angiographic data for 3D-QCA reconstruction. This point has already been discussed in the originally submitted manuscript (line 393-396) in the limitations section, thus no additional changes have been made in the revised draft.

3. The manuscript deals with quite technical elements and parameters of wall shear stress, of which many readers will be learning about for the first time. There should likely be an introduction to these variables, ideally accompanied by a figure describing them in significantly greater detail.

We thank the reviewer for the valuable comments that we have tried to address in the revised draft.

We have added to the methods a paragraph named “WSS computation”. Here we describe the concept of time-averaged WSS, and we also explain the different multidirectional WSS variables in detail (line 204-238). Considering that this paper refers mainly to clinicians, we have avoided description of the mathematical formulas that we used to estimate the multidirectional WSS and focused on the clinical meaning of these metrics.

"WSS computation

Following 3D-QCA reconstruction, fast CFD analysis was performed by the software to compute the WSS across the SOI (Online Supplementary Material). The software assumes a pulsatile flow profile that allows not only measurements of the WSS, but also evaluation of the changes in the flow direction during the cardiac cycle that can been quantified with the computation of multidirectional shear stress indices (Supplementary Figure 1).[14] In pulsatile flow, the WSS was averaged over the entire phase of the cardiac cycle to estimate the time-averaged WSS (TAWSS). The TAWSS values correlate well with the WSS estimated under steady flow conditions which have been found in numerous studies to be predictors of cardiovascular events.[4,5,9,15] The multidirectional WSS metrics that have been found to affect plaque biology and determine its evolution include:

- The oscillatory shear index (OSI), which is used to quantify oscillations in coronary flow and is computed by the ratio of forward WSS to the total WSS variation (value of 0.5 being always-forward oscillation, whilst 0.0 being back-and-forth oscillation).

- The relative residence time (RRT), a parameter that combines TAWSS and OSI and reflects the time that a blood particle spends at a certain location on the vessel wall.

- The transverse WSS (transWSS), which corresponds to the WSS component that is perpendicular to the TAWSS vector.

- Crossflow index (CFI), which is defined as the transWSS normalized by the TAWSS and provides an estimate of the changes in the direction of the WSS throughout the cardiac cycle.

Each SOI was split in 3mm segments and in each segment the mean TAWSS as well as the maximum predominant and minimum predominant TAWSS were computed. The maximum and minimum predominant TAWSS were defined as the maximum and minimum values of the TAWSS computed in each 3mm segment in all surface data located in a 90° arc window across the circumference of the vessel wall.[4,10,11] The highest maximum predominant and the lowest minimum predominant TAWSS values in the 3mm segments encompassing the lesion corresponded to the "maximum" and "minimum" TAWSS, respectively. In addition, the highest mean TAWSS in the 3mm segments of the lesion, the mean TAWSS at the MLA and the mean TAWSS proximally to the MLA were recorded. The maximum predominant multidirectional WSS parameters such as OSI, RRT, transWSS and CFI were also computed at every 3mm segment; for each of these variables, the highest value within the lesion was recorded.

In diffusely diseased segments and in lesions with borderline negative FFR along the vessel, 3D-QCA and CFD analysis was performed in the entire segment. In tandem lesions we extracted the WSS only in the lesion with the higher AS – as this was the most powerful predictor of future events in previous studies[11,16] – and this lesion was included in the analysis. An event was censored in this setting only if there was disease progression in the lesion with the largest area stenosis."

We have also created an additional Supplementary Figure which shows the three types of flow environment to which an endothelial cell can be exposed (Supplementary Material, page 10).

"*Supplementary Figure 1. Three types of flow environments an endothelial cell can be exposed to.*

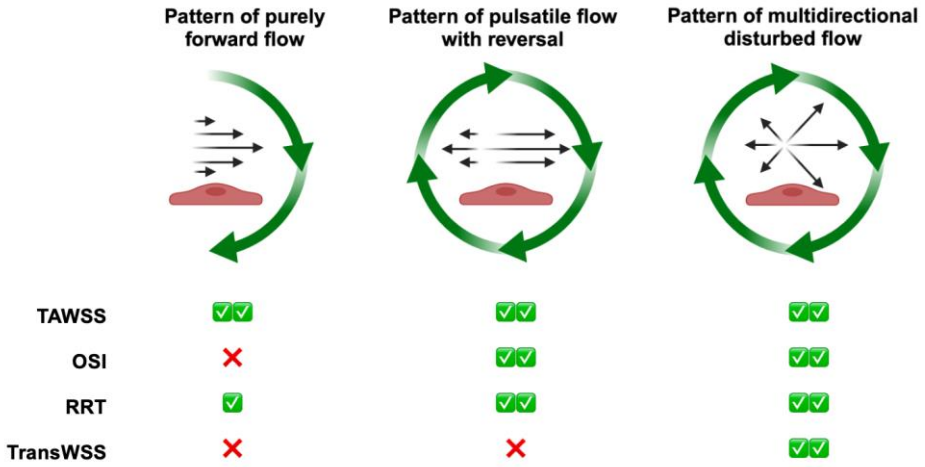


Figure footnote: The black arrows indicate wall shear stress vectors at different points during the cardiac cycle, while the green arrows depict their progression over time. The table displays the following shear stress variables: TAWSS (Pa), OSI, RRT (relative to purely forward flow) and transWSS (Pa) for the three environments. Figure adapted from Peiffer V et al.[6]
OSI, oscillatory shear index; RRT, relative residence time; TAWSS, time-averaged wall shear stress; transWSS, transverse WSS.

4. I would encourage the authors to temper their conclusions / discussion. Their results demonstrated at wall shear metrics are not an independent predictor of subsequent events. I find the data interesting that combined increase in area of stenosis + wall stress is associated with subsequent events, but it is hypothesis generating and may not be explained by wall stress itself.

We agree with the reviewer that the interpretation of the results is challenging as there are conflicting findings. We have found that the WSS variables were predictors of events in univariate Cox regression analysis (Table 1) and in the multivariable models built adding the WSS metrics to the baseline demographics (Figure 1). However, when the WSS metrics were added to the model including patient baseline demographics and area stenosis (AS), these were not predictors of LOCE. Conversely, the AS was a predictor of LOCE when this was added to the models that included baseline demographics and WSS variables (Figure 2).
The results, however, were different when we split the patients in groups based on the AS and WSS cutoffs that appear to predict LOCE. Kaplan-Meier curve (Figure 5) showed that the TAWSS provided incremental prognostic information to AS: patients who had lesions with maximum TAWSS and AS

above the pre-defined cut-offs were at higher risk of having an event than those with AS and max TAWSS below these cutoffs (cumulative event rat 26% vs. 7%, p <0.001) at 4-year follow-up.

During the preparation of the manuscript, we have requested the opinion of the statistician who performed the analysis - Prof Halil Tanboga - about the reported results. His view was that based on the performance metrics of the studied variables (Supplementary Figure 3) the WSS metrics did not provide incremental prognostic information to AS and baseline demographics and that this should be the main conclusion of the study. The findings of the Kaplan Meier analysis are interesting and require confirmation in larger studies. However, there is a concern that dichotomizing the continuous variables (AS and TAWSS) and dividing patients into groups may have led to a type I statistical error.

In the discussion, we have amended the original text to tone down the discussion and conclusions, highlighting the need to conduct further studies to fully investigate the prognostic value of the WSS computed in 3D-QCA-based models.

Discussion, line 326-329:

"3) *TAWSS and transWSS did not have an incremental value to AS in detecting lesions that will cause events in multivariable analysis; however, 4) in the Kaplan-Meier analysis, lesions with high maximum TAWSS and AS were more likely to cause an event than those with low maximum TAWSS and AS."*

Discussion, line 381-386:

"…, following the identification of optimal AS and WSS cut-offs for event prediction, Kaplan-Meier curves showed that patients with elevated AS and maximum TAWSS exhibit an increased risk of experiencing events. This finding suggests that local hemodynamic forces computed in 3D-QCA based models might offer supplementary prognostic insights beyond lesion geometrical characteristics (Graphical Abstract) and underscore the need to conduct large prospective studies to explore their incremental value in predicting events."

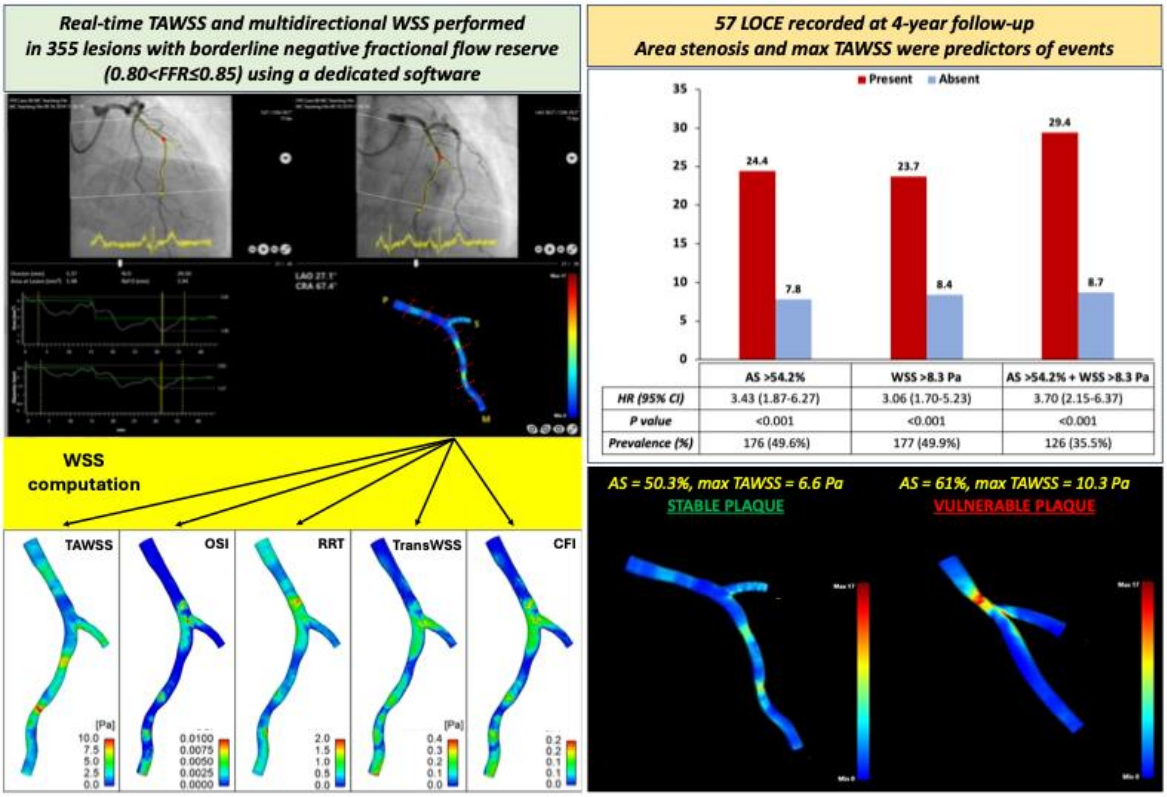
Conclusions, line 415-421:

"In patients with borderline negative FFR lesions, WSS metrics calculated using the CAAS Workstation WSS prototype did not provide additional prognostic value beyond patients' clinical characteristics and AS. However, Kaplan-Meier analysis revealed promising indications that the flow pattern distribution measured in 3D-QCA models could be useful in predicting adverse events. Further studies, involving a larger number of patients, is warranted to validate these observations and explore the full potential of this software in risk stratification."

5. The manuscript is challenging to read with the sheer amount of nontraditional abbreviations. I would encourage the authors to rethink how many of them are actually needed.

In this study, we have included 17 abbreviations in total and 5 of these (CFI, OSI, RRT, TAWSS and transWSS) refer to local hemodynamic forces. We hope that adding a detailed description of the multidirectional WSS indices to the methods section will help the reader to become familiar with the

abbreviations used in the results and discussion. Our graphical abstract should also help the readers to understand the process of splitting the lesion in 3mm segments and computing different WSS variables for each segment.



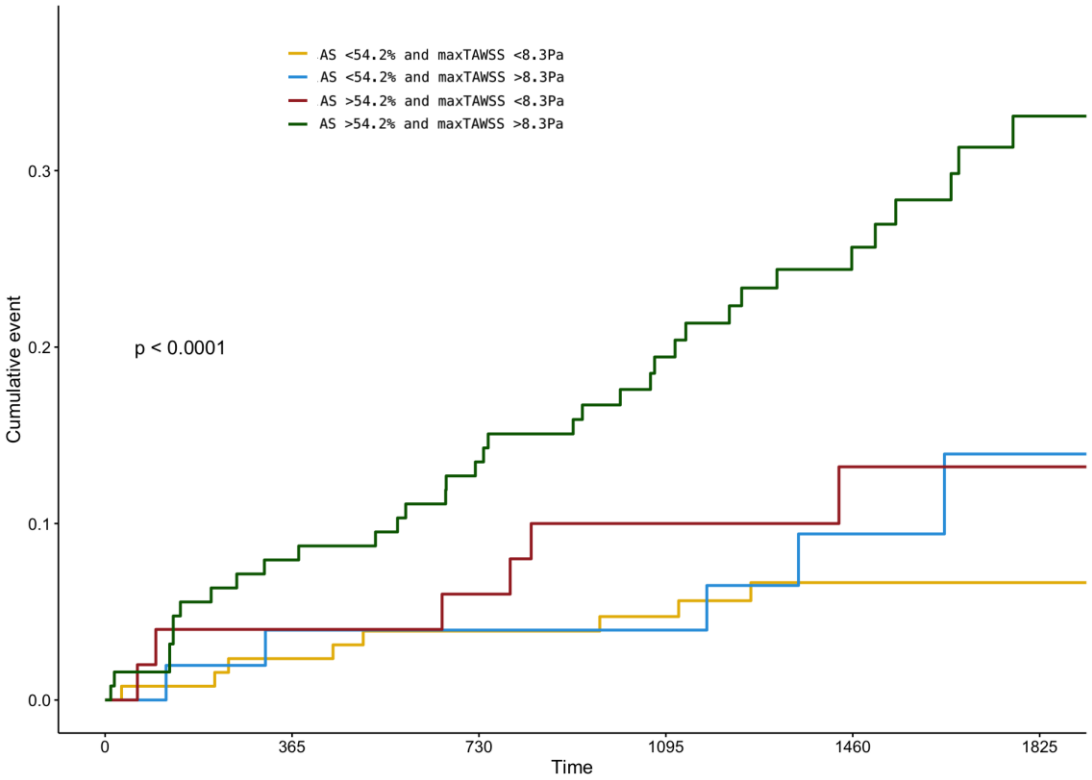
6. The discussion makes up 4.5/10 pages of the manuscript. I would encourage the authors to use more of the discussion room to better introduce the concepts of shear wall stress and its metrics.

In the revised draft we have shortened the discussion by 1 page and all the changes have been written in red as per Editorial office instructions. The concept of WSS and different multidirectional metrics have introduced in the methods.

7. Figure 5 labels needs to be relabelled from their current statistical software variable labels

Thank you for spotting this mistake.

Figure 5 has been re-labelled.


AS <54.2% and maxTAWSS <8.3Pa
AS <54.2% and maxTAWSS >8.3Pa
AS >54.2% and maxTAWSS <8.3Pa
AS >54.2% and maxTAWSS >8.3Pa
p < 0.0001
Cumulative event
0.0
0.1
0.2
0.3
0
365
730
1095
1460
1825
Time

References

Adjedj, J., De Bruyne, B., Flore, V., Di Gioia, G., Ferrara, A., Pellicano, M., . . . Barbato, E. (2016). Significance of Intermediate Values of Fractional Flow Reserve in Patients With Coronary Artery Disease. *Circulation, 133*(5), 502-508. doi:10.1161/CIRCULATIONAHA.115.018747

Ahn, J.-M., Park, D.-W., Shin, E.-S., Koo, B.-K., Nam, C.-W., Doh, J.-H., . . . Park, S.-J. (2017). Fractional Flow Reserve and Cardiac Events in Coronary Artery Disease. *Circulation, 135*(23), 2241-2251. doi:doi:10.1161/CIRCULATIONAHA.116.024433

Barbato, E., Toth, G. G., Johnson, N. P., Pijls, N. H., Fearon, W. F., Tonino, P. A., . . . De Bruyne, B. (2016). A Prospective Natural History Study of Coronary Atherosclerosis Using Fractional Flow Reserve. *J Am Coll Cardiol, 68*(21), 2247-2255. doi:10.1016/j.jacc.2016.08.055

Bourantas, C. V., Zanchin, T., Torii, R., Serruys, P. W., Karagiannis, A., Ramasamy, A., . . . Stone, G. W. (2020). Shear Stress Estimated by Quantitative Coronary Angiography Predicts Plaques Prone to Progress and Cause Events. *JACC Cardiovasc Imaging, 13*(10), 2206-2219. doi:10.1016/j.jcmg.2020.02.028

Candreva, A., Gallo, D., Munhoz, D., Rizzini, M. L., Mizukami, T., Seki, R., . . . Collet, C. (2024). Influence of intracoronary hemodynamic forces on atherosclerotic plaque phenotypes. *Int J Cardiol, 399*, 131668. doi:10.1016/j.ijcard.2023.131668

Candreva, A., Pagnoni, M., Rizzini, M. L., Mizukami, T., Gallinoro, E., Mazzi, V., . . . Collet, C. (2022). Risk of myocardial infarction based on endothelial shear stress analysis using coronary angiography. *Atherosclerosis, 342*, 28-35. doi:10.1016/j.atherosclerosis.2021.11.010

Chen, L., Chester, M. R., Crook, R., & Kaski, J. C. (1996). Differential progression of complex culprit stenoses in patients with stable and unstable angina pectoris. *J Am Coll Cardiol, 28*(3), 597-603. doi:10.1016/0735-1097(96)00203-3

Chen, S. L., Xu, B., Chen, J. B., Xu, T., Ye, F., Zhang, J. J., . . . Lin, S. (2013). Diagnostic accuracy of quantitative angiographic and intravascular ultrasound parameters predicting the functional significance of single de novo lesions. *Int J Cardiol, 168*(2), 1364-1369. doi:10.1016/j.ijcard.2012.12.010

Collet, C., Sonck, J., Vandeloo, B., Mizukami, T., Roosens, B., Lochy, S., . . . De Bruyne, B. (2019). Measurement of Hyperemic Pullback Pressure Gradients to Characterize Patterns of Coronary Atherosclerosis. *J Am Coll Cardiol, 74*(14), 1772-1784. doi:10.1016/j.jacc.2019.07.072

Engstrom, T., Kelbaek, H., Helqvist, S., Hofsten, D. E., Klovgaard, L., Holmvang, L., . . . Investigators, D.-P. (2015). Complete revascularisation versus treatment of the culprit lesion only in patients with ST-segment elevation myocardial infarction and multivessel disease (DANAMI-3-PRIMULTI): an open-label, randomised controlled trial. *Lancet, 386*(9994), 665-671. doi:10.1016/s0140-6736(15)60648-1

Erlinge, D., Maehara, A., Ben-Yehuda, O., Botker, H. E., Maeng, M., Kjoller-Hansen, L., . . . Investigators, P. I. (2021). Identification of vulnerable plaques and patients by intracoronary near-infrared spectroscopy and ultrasound (PROSPECT II): a prospective natural history study. *Lancet, 397*(10278), 985-995. doi:10.1016/S0140-6736(21)00249-X

Hakeem, A., Edupuganti, M. M., Almomani, A., Pothineni, N. V., Payne, J., Abualsuod, A. M., . . . Uretsky, B. F. (2016). Long-Term Prognosis of Deferred Acute Coronary Syndrome

Lesions Based on Nonischemic Fractional Flow Reserve. *J Am Coll Cardiol, 68*(11), 1181-1191. doi:10.1016/j.jacc.2016.06.035

Heitner, J. F., Senthilkumar, A., Harrison, J. K., Klem, I., Sketch, M. H., Jr., Ivanov, A., . . . Kim, R. J. (2019). Identifying the Infarct-Related Artery in Patients With Non-ST-Segment-Elevation Myocardial Infarction. *Circ Cardiovasc Interv, 12*(5), e007305. doi:10.1161/CIRCINTERVENTIONS.118.007305

Jeremias, A., Davies, J. E., Maehara, A., Matsumura, M., Schneider, J., Tang, K., . . . Stone, G. W. (2019). Blinded Physiological Assessment of Residual Ischemia After Successful Angiographic Percutaneous Coronary Intervention: The DEFINE PCI Study. *JACC Cardiovasc Interv, 12*(20), 1991-2001. doi:10.1016/j.jcin.2019.05.054

Johnson, N. P., Toth, G. G., Lai, D., Zhu, H., Acar, G., Agostoni, P., . . . Gould, K. L. (2014). Prognostic value of fractional flow reserve: linking physiologic severity to clinical outcomes. *J Am Coll Cardiol, 64*(16), 1641-1654. doi:10.1016/j.jacc.2014.07.973

Kato, K., Yonetsu, T., Kim, S. J., Xing, L., Lee, H., McNulty, I., . . . Jang, I. K. (2012). Nonculprit plaques in patients with acute coronary syndromes have more vulnerable features compared with those with non-acute coronary syndromes: a 3-vessel optical coherence tomography study. *Circ Cardiovasc Imaging, 5*(4), 433-440. doi:10.1161/CIRCIMAGING.112.973701

Kedhi, E., Berta, B., Roleder, T., Hermanides, R. S., Fabris, E., AJJ, I. J., . . . Wojakowski, W. (2021). Thin-cap fibroatheroma predicts clinical events in diabetic patients with normal fractional flow reserve: the COMBINE OCT-FFR trial. *Eur Heart J, 42*(45), 4671-4679. doi:10.1093/eurheartj/ehab433

Kerensky, R. A., Wade, M., Deedwania, P., Boden, W. E., Pepine, C. J., & Veterans Affairs Non, Q. W. I. S. i.-H. T. I. (2002). Revisiting the culprit lesion in non-Q-wave myocardial infarction. Results from the VANQWISH trial angiographic core laboratory. *J Am Coll Cardiol, 39*(9), 1456-1463. doi:10.1016/s0735-1097(02)01770-9

Kotoku, N., Ninomiya, K., Masuda, S., O'Leary, N., Garg, S., Naito, M., . . . Serruys, P. W. (2023). Preprocedural physiological assessment of coronary disease patterns to predict haemodynamic outcomes post-PCI. *EuroIntervention, 19*(11), e891-e902. doi:10.4244/EIJ-D-23-00516

Kumar, A., Thompson, E. W., Lefieux, A., Molony, D. S., Davis, E. L., Chand, N., . . . Samady, H. (2018). High Coronary Shear Stress in Patients With Coronary Artery Disease Predicts Myocardial Infarction. *J Am Coll Cardiol, 72*(16), 1926-1935. doi:10.1016/j.jacc.2018.07.075

Kuramitsu, S., Matsuo, H., Shinozaki, T., Horie, K., Takashima, H., Terai, H., . . . Investigators, J. C. (2022). Five-Year Outcomes After Fractional Flow Reserve-Based Deferral of Revascularization in Chronic Coronary Syndrome: Final Results From the J-CONFIRM Registry. *Circ Cardiovasc Interv, 15*(2), e011387. doi:10.1161/CIRCINTERVENTIONS.121.011387

Masrani Mehta, S., Depta, J. P., Novak, E., Patel, J. S., Patel, Y., Raymer, D., . . . Kurz, H. I. (2015). Association of Lower Fractional Flow Reserve Values With Higher Risk of Adverse Cardiac Events for Lesions Deferred Revascularization Among Patients With Acute Coronary Syndrome. *J Am Heart Assoc, 4*(8), e002172. doi:10.1161/JAHA.115.002172

Mol, J. Q., Volleberg, R., Belkacemi, A., Hermanides, R. S., Meuwissen, M., Protopopov, A. V., . . . van Royen, N. (2023). Fractional Flow Reserve-Negative High-Risk Plaques and Clinical Outcomes After Myocardial Infarction. *JAMA Cardiol, 8*(11), 1013-1021. doi:10.1001/jamacardio.2023.2910

Montone, R. A., Meucci, M. C., & Niccoli, G. (2020). The management of non-culprit coronary lesions in patients with acute coronary syndrome. *Eur Heart J Suppl, 22*(Suppl L), L170-L175. doi:10.1093/eurheartj/suaa175

Ninomiya, K., Serruys, P. W., Kotoku, N., Zhou, J., Kageyama, S., Masuda, S., . . . Onuma, Y. (2023). Anonymous Comparison of Various Angiography-Derived Fractional Flow Reserve Software With Pressure-Derived Physiological Assessment. *JACC Cardiovasc Interv, 16*(14), 1778-1790. doi:10.1016/j.jcin.2023.04.026

Opolski, M. P., Spiewak, M., Marczak, M., Debski, A., Knaapen, P., Schumacher, S. P., . . . Witkowski, A. (2019). Mechanisms of Myocardial Infarction in Patients With Nonobstructive Coronary Artery Disease: Results From the Optical Coherence Tomography Study. *JACC Cardiovasc Imaging, 12*(11 Pt 1), 2210-2221. doi:10.1016/j.jcmg.2018.08.022

Papafaklis, M. I., Bourantas, C. V., Theodorakis, P. E., Katsouras, C. S., Naka, K. K., Fotiadis, D. I., & Michalis, L. K. (2010). The effect of shear stress on neointimal response following sirolimus- and paclitaxel-eluting stent implantation compared with bare-metal stents in humans. *JACC Cardiovasc Interv, 3*(11), 1181-1189. doi:10.1016/j.jcin.2010.08.018

Pinilla-Echeverri, N., Mehta, S. R., Wang, J., Lavi, S., Schampaert, E., Cantor, W. J., . . . Sheth, T. (2020). Nonculprit Lesion Plaque Morphology in Patients With ST-Segment-Elevation Myocardial Infarction: Results From the COMPLETE Trial Optical Coherence Tomography Substudys. *Circ Cardiovasc Interv, 13*(7), e008768. doi:10.1161/circinterventions.119.008768

Ramasamy, A., Jin, C., Tufaro, V., Bajaj, R., Kilic, Y., Safi, H., . . . Baumbach, A. (2020). Computerised Methodologies for Non-Invasive Angiography-Derived Fractional Flow Reserve Assessment: A Critical Review. *J Interv Cardiol, 2020*, 6381637. doi:10.1155/2020/6381637

Reynolds, H. R., Srichai, M. B., Iqbal, S. N., Slater, J. N., Mancini, G. B., Feit, F., . . . Hochman, J. S. (2011). Mechanisms of myocardial infarction in women without angiographically obstructive coronary artery disease. *Circulation, 124*(13), 1414-1425. doi:10.1161/CIRCULATIONAHA.111.026542

Sakai, K., Mizukami, T., Leipsic, J., Belmonte, M., Sonck, J., Norgaard, B. L., . . . Collet, C. (2023). Coronary Atherosclerosis Phenotypes in Focal and Diffuse Disease. *JACC Cardiovasc Imaging, 16*(11), 1452-1464. doi:10.1016/j.jcmg.2023.05.018

Scarsini, R., Fezzi, S., Leone, A. M., De Maria, G. L., Pighi, M., Marcoli, M., . . . Ribichini, F. L. (2022). Functional Patterns of Coronary Disease: Diffuse, Focal, and Serial Lesions. *JACC Cardiovasc Interv, 15*(21), 2174-2191. doi:10.1016/j.jcin.2022.07.015

Sels, J. W., Tonino, P. A., Siebert, U., Fearon, W. F., Van't Veer, M., De Bruyne, B., & Pijls, N. H. (2011). Fractional flow reserve in unstable angina and non-ST-segment elevation myocardial infarction experience from the FAME (Fractional flow reserve versus Angiography for Multivessel Evaluation) study. *JACC Cardiovasc Interv, 4*(11), 1183-1189. doi:10.1016/j.jcin.2011.08.008

Smits, P. C., Abdel-Wahab, M., Neumann, F. J., Boxma-de Klerk, B. M., Lunde, K., Schotborgh, C. E., . . . Compare-Acute, I. (2017). Fractional Flow Reserve-Guided Multivessel Angioplasty in Myocardial Infarction. *N Engl J Med, 376*(13), 1234-1244. doi:10.1056/NEJMoa1701067

Stone, G. W., Maehara, A., Lansky, A. J., de Bruyne, B., Cristea, E., Mintz, G. S., . . . Investigators, P. (2011). A prospective natural-history study of coronary atherosclerosis. *N Engl J Med, 364*(3), 226-235. doi:10.1056/NEJMoa1002358

Stone, P. H., Maehara, A., Coskun, A. U., Maynard, C. C., Zaromytidou, M., Siasos, G., . . . Stone, G. W. (2018). Role of Low Endothelial Shear Stress and Plaque Characteristics in the Prediction of Nonculprit Major Adverse Cardiac Events: The PROSPECT Study. *JACC Cardiovasc Imaging, 11*(3), 462-471. doi:10.1016/j.jcmg.2017.01.031

Stone, P. H., Saito, S., Takahashi, S., Makita, Y., Nakamura, S., Kawasaki, T., . . . Investigators, P. (2012). Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation, 126*(2), 172-181. doi:10.1161/CIRCULATIONAHA.112.096438

Tian, J., Ren, X., Vergallo, R., Xing, L., Yu, H., Jia, H., . . . Jang, I. K. (2014). Distinct morphological features of ruptured culprit plaque for acute coronary events compared to those with silent rupture and thin-cap fibroatheroma: a combined optical coherence tomography and intravascular ultrasound study. *J Am Coll Cardiol, 63*(21), 2209-2216. doi:10.1016/j.jacc.2014.01.061

Tufaro, V., Safi, H., Torii, R., Koo, B. K., Kitslaar, P., Ramasamy, A., . . . Bourantas, C. V. (2021). Wall shear stress estimated by 3D-QCA can predict cardiovascular events in lesions with borderline negative fractional flow reserve. *Atherosclerosis, 322*, 24-30. doi:10.1016/j.atherosclerosis.2021.02.018

Vrints, C., Andreotti, F., Koskinas, K. C., Rossello, X., Adamo, M., Ainslie, J., . . . Group, E. S. C. S. D. (2024). 2024 ESC Guidelines for the management of chronic coronary syndromes. *Eur Heart J*. doi:10.1093/eurheartj/ehae177

Weerts, J., Pustjens, T., Amin, E., Ilhan, M., Veenstra, L. F., Theunissen, R., . . . Rasoul, S. (2021). Long-term outcome after deferred revascularization due to negative fractional flow reserve in intermediate coronary lesions. *Catheter Cardiovasc Interv, 97*(2), 247-256. doi:10.1002/ccd.28753

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.

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

Mr Jean-Paul Aben is an employee of Pie Medical Imaging.
The remaining authors have no conflicts of interest to disclose.

Figure 1

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3D-QCA variables	Hazard ratio(95% Conf. Int.)	p value
Area stenosis (%)	2.41(1.52 to 3.82)	<0.001
Mean Reference Area (mm2)	1.60(0.99 to 2.57)	0.110
Distal Reference Area(mm2)	1.50(0.93 to 2.43)	0.178
Proximal Reference Area (mm2)	1.45(0.92 to 2.30)	0.147
Lesion Length (mm)	0.97(0.65 to 1.45)	0.964
MLA (mm2)	0.80(0.55 to 1.17)	0.485
CFD-derived variables		
Maximum TAWSS (Pa)	2.79(1.50 to 5.19)	0.002
Highest mean TAWSS (Pa)	2.75(1.50 to 5.07)	0.001
Mean TAWSS at MLA (Pa)	2.64(1.43 to 4.87)	0.001
Maximum TransWSS (Pa)	2.34(1.29 to 4.25)	0.013
Mean TAWSS prox to MLA (Pa)	1.98(1.12 to 3.51)	0.012
Maximum OSI	1.40(0.86 to 2.29)	0.217
Maximum CFI	1.36(0.94 to 1.97)	0.236
Minimum TAWSS (Pa)	1.34(0.81 to 2.24)	0.497
Maximum RRT	1.13(0.69 to 1.85)	0.134

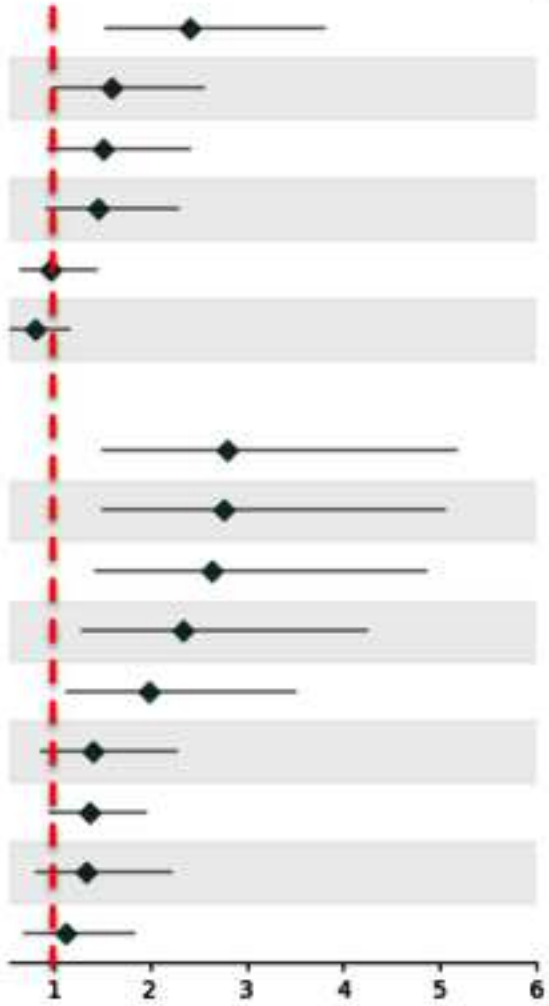


Figure 2

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Predictive value of 3D-QCA derived variables	Hazard ratio(95% Conf. Int.)	AUC before	AUC after	p value
AS (adjusted by Base, Highest mean TAWSS and Maximum TransWSS)	2.01(1.15 to 3.51)	0.690	0.717	0.042
AS (adjusted by Base and Highest mean TAWSS)	1.95(1.15 to 3.31)	0.682	0.705	0.043
AS (adjusted by Base, mean TAWSS at the MLA and Maximum TransWSS)	1.95(1.15 to 3.32)	0.693	0.720	0.043
AS (adjusted by Base and mean TAWSS at the MLA)	1.93(1.12 to 3.34)	0.683	0.707	0.052
Predictive value of the TAWSS metrics				
mean TAWSS at the MLA (adjusted by Base and AS)	1.68(0.82 to 3.42)	0.705	0.707	0.348
Highest mean TAWSS (adjusted by Base and AS)	1.66(0.85 to 3.27)	0.705	0.705	0.275
mean TAWSS at the MLA (adjusted by Base, AS and Maximum TransWSS)	1.40(0.67 to 2.91)	0.720	0.720	0.636
Highest mean TAWSS (adjusted by Base, AS and Maximum TransWSS)	1.29(0.54 to 3.05)	0.720	0.720	0.847
Predictive value of the multidirectional WSS metrics				
Maximum TransWSS (adjusted by Base and AS)	1.72(0.92 to 3.19)	0.705	0.720	0.23
Maximum TransWSS (adjusted by Base, AS and highest mean TAWSS)	1.52(0.72 to 3.22)	0.705	0.720	0.552
Maximum TransWSS (adjusted by Base, AS and mean TAWSS at the MLA)	1.49(0.74 to 2.97)	0.707	0.720	0.525

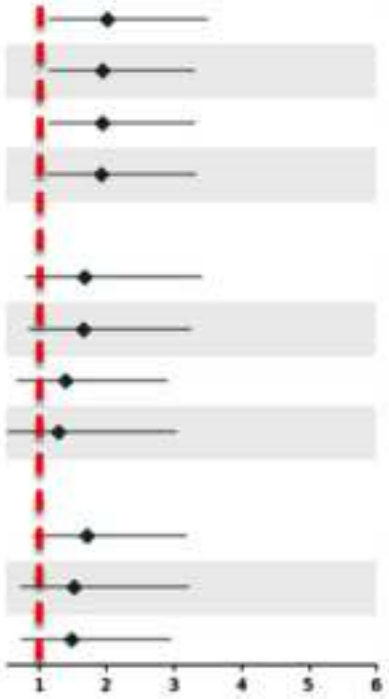


Figure 3

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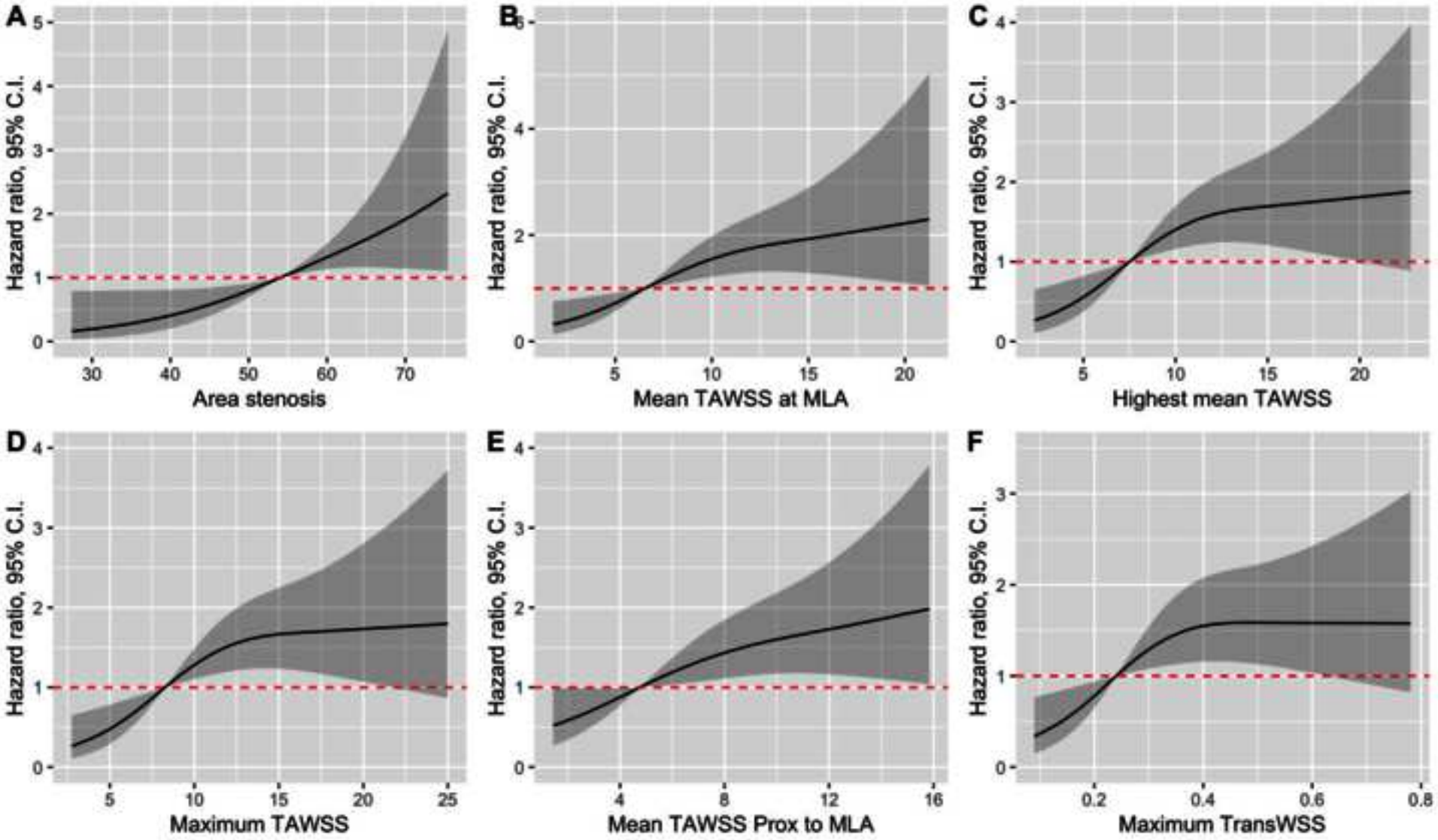


Figure 4

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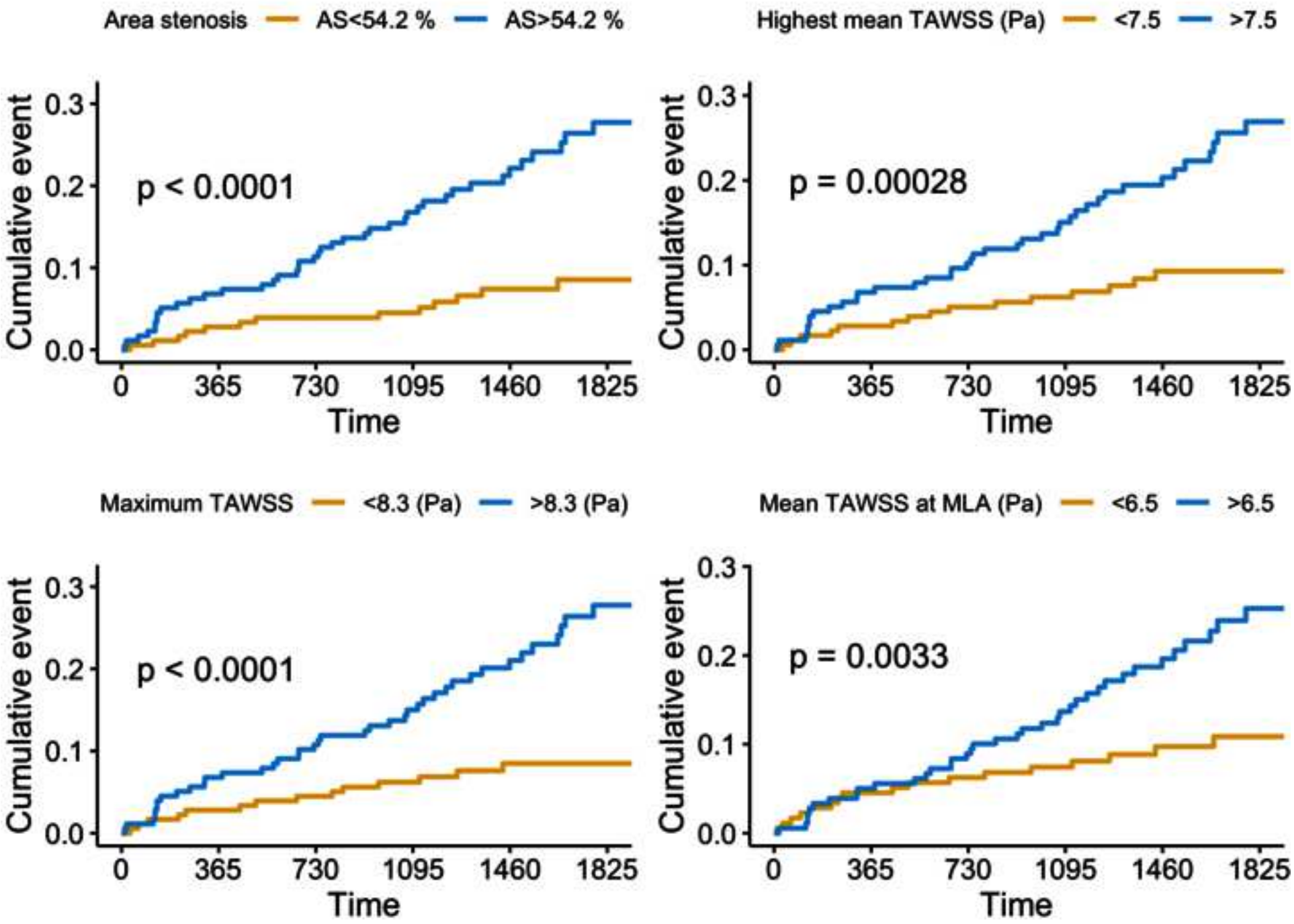


Figure 5

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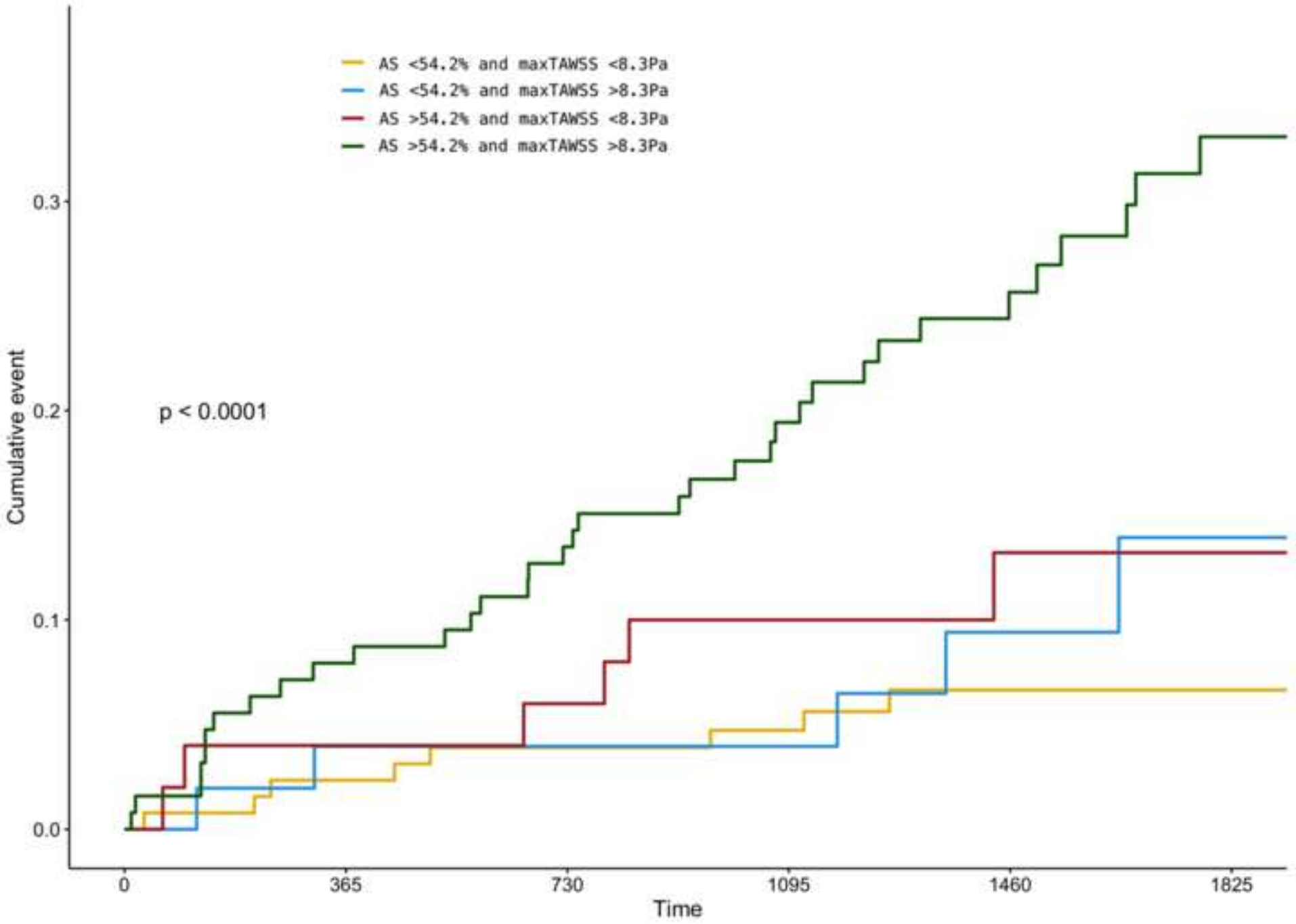
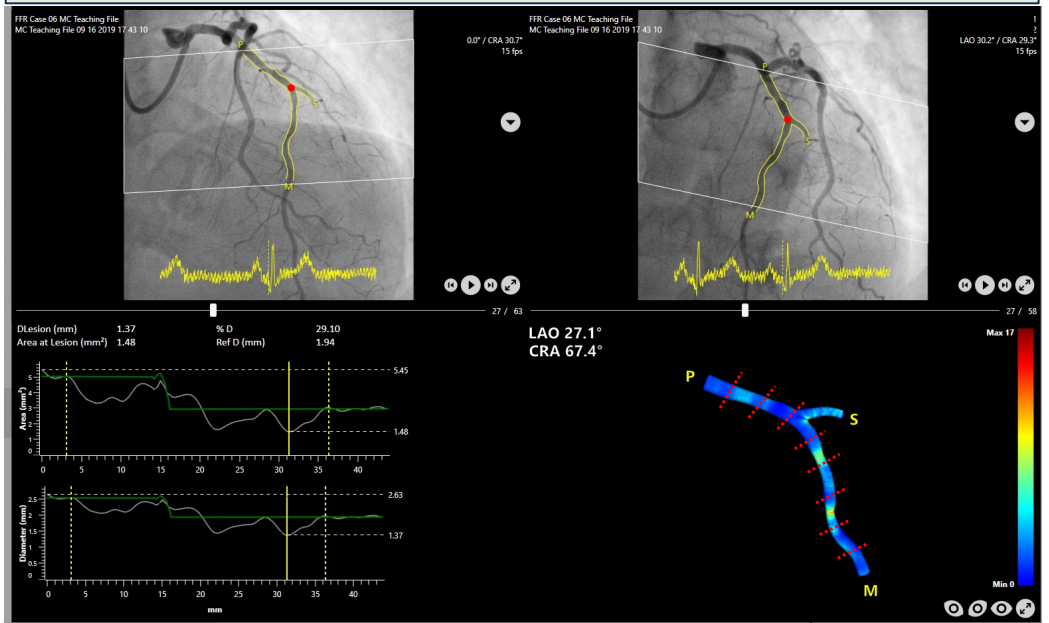


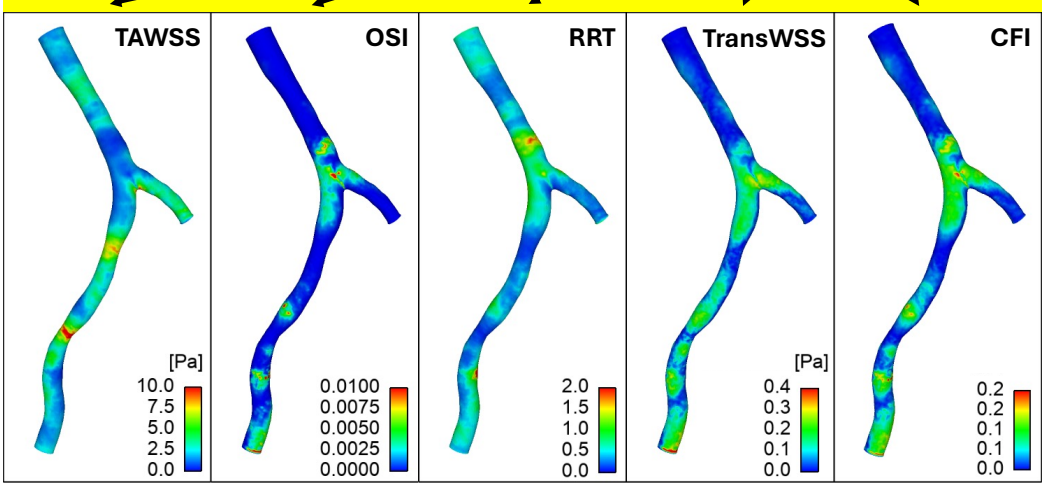
Figure 6

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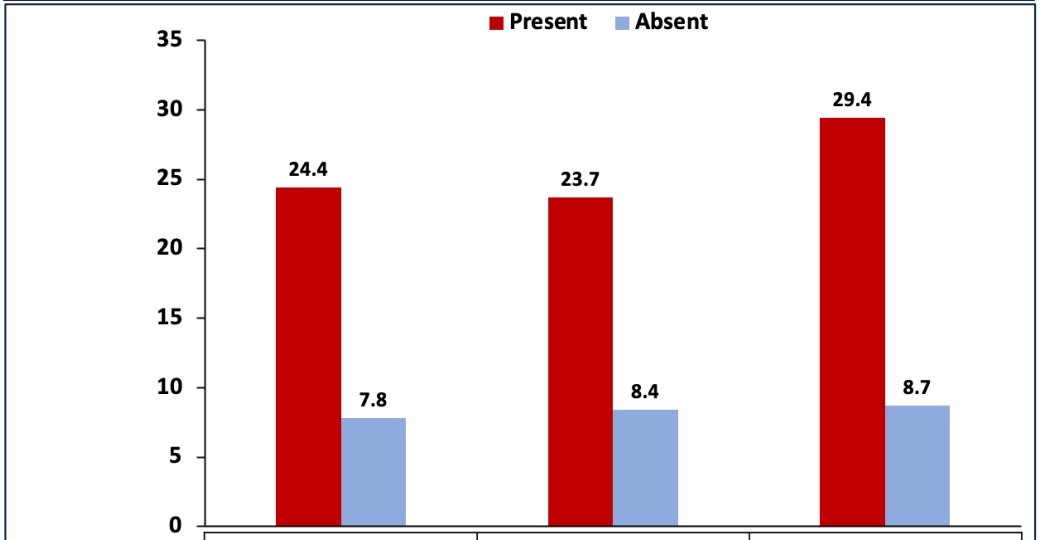
Real-time TAWSS and multidirectional WSS performed in 355 lesions with borderline negative fractional flow reserve (0.80<FFR≤0.85) using a dedicated software



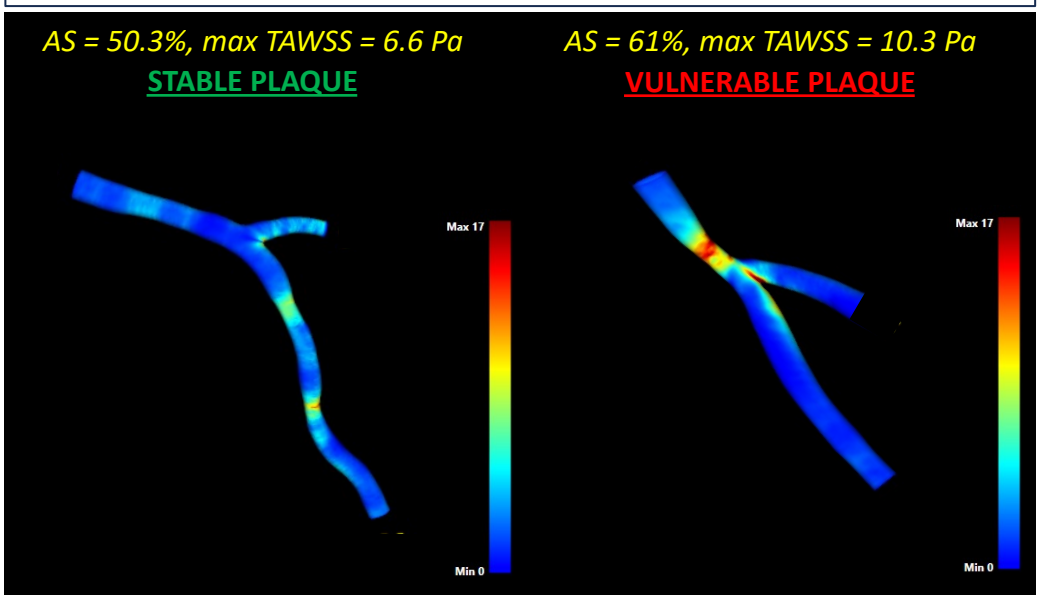
WSS computation



57 LOCE recorded at 4-year follow-up
Area stenosis and max TAWSS were predictors of events



	AS >54.2%	WSS >8.3 Pa	AS >54.2% + WSS >8.3 Pa
HR (95% CI)	3.43 (1.87-6.27)	3.06 (1.70-5.23)	3.70 (2.15-6.37)
P value	<0.001	<0.001	<0.001
Prevalence (%)	176 (49.6%)	177 (49.9%)	126 (35.5%)



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Are gene names italicized? **Yes** **No**

not applicable

Gene names should be italicized; protein products of the loci are not italicized.

For murine models, the gene and protein names are lowercase except for the first letter.
(e.g., gene: *Abcb4;* protein: Abcb4)

For humans, the whole gene name is capitalized.
(e.g., gene: *ABCB4;* protein ABCB4)

Mouse strains and cell lines

Are knock-out or transgenic mouse strains and cell lines italicized and the symbol superscripted? **Yes** **No**

not applicable

(e.g. *ob/ob* , $p53^{+/+}$, $p53^{-/-}$)

p values

Are *p* values consistently formatted according to the below style throughout the manuscript (including figures and tables)? **Yes** **No**

p <X
p >X
p=X

Language

Is your manuscript written in good English? **Yes** **No**

Please make sure that you consistently use either American or British English, but not a mixture of them.

Please make sure that words are written consistently in the same way throughout the manuscript.
e.g. non-significant or nonsignificant
e.g. down-regulation or downregulation

Artwork

Have you submitted high-resolution versions of your original artwork? **Yes** **No**

Please make sure to use uniform lettering and sizing in your original artwork, including letters to indicate panels, consistently throughout all figures.

Atherosclerosis policy on the use of proper terminology when referring to intima-media thickness (IMT)

Atherosclerosis has recently embraced a new editorial policy to clarify the use of proper terminology when referring to intima-media thickness (IMT):
IMT should be referred to as "arterial injury" or "arteriopathy", not atherosclerosis.
For more details, please see the following letter to the editor and reply published in *Atherosclerosis*

"IMT is not atherosclerosis", Spence 2020 (https://doi.org/10.1016/j.atherosclerosis.2020.09.016)

"Carotid intima-media thickness should not be referred to as subclinical atherosclerosis: A recommended update to the editorial policy at *Atherosclerosis*", Raggi and Stein 2020 (https://doi.org/10.1016/j.atherosclerosis.2020.09.015)

Use of the terms "sex" *vs.* "gender"

These terms should be used correctly, corresponding to the WHO definitions: http://www.who.int/gender/whatisgender/

Sex" refers to the biological and physiological characteristics that define men and women.
"Gender" refers to the socially constructed roles, behaviours, activities, and attributes that a given society considers appropriate for men and women.

Graphical abstract

The graphical abstract is mandatory at submission of the revised version of the paper.

Technical requirements

- **Size:** Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi
- **Font type and font size:** Calibri 18
- **Preferred file types:** TIFF, EPS, PDF or MS Office files
- **Content:** the abstract should consist of *one single panel*

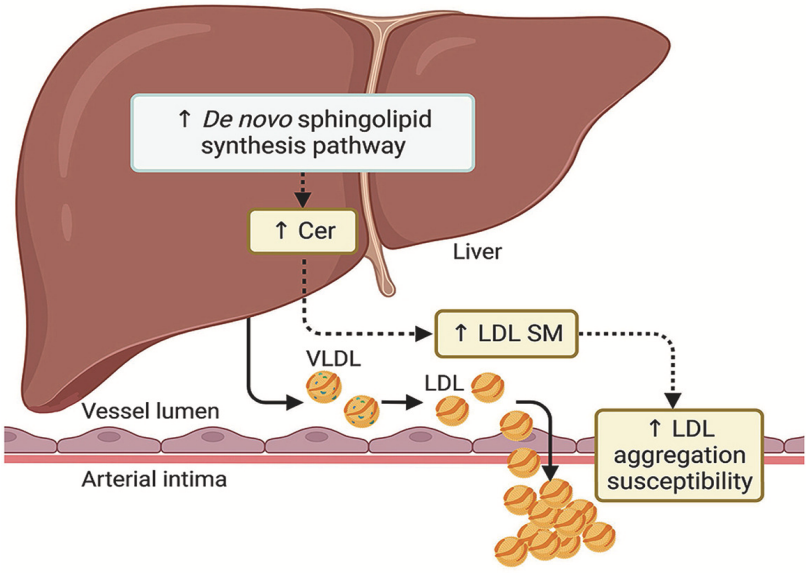
Content

Keep it simple

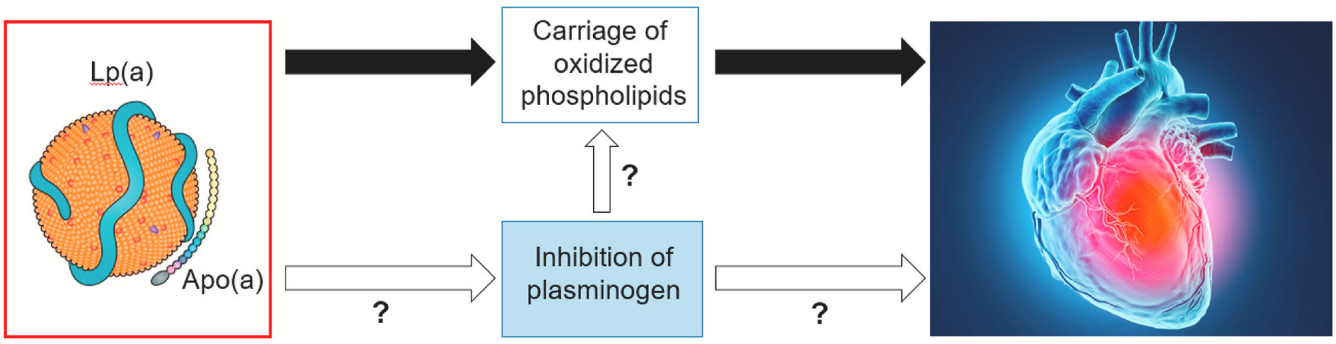
The graphical abstract should:

- Have a clear start and end, "reading" from top-to-bottom or left-to-right
- Emphasize the new findings from the current paper without including excess details from previous literature
- Avoid the inclusion of features that are more speculative (unless the speculative nature can be made apparent visually)
- Not be too text-heavy; most of the content should be in a graphical form
- Use simple labels

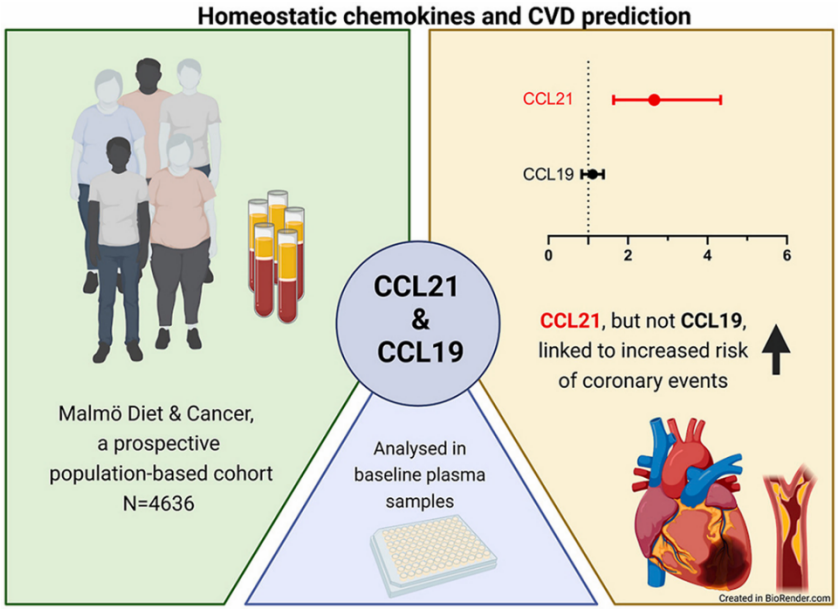
Some examples of graphical abstracts:



Lahelma et al. 2022, *Atherosclerosis*; Volume 363 Pages 22-29 (December 2022)



Clarke et al., *Atherosclerosis;* Volume 354 Pages 15-22 (August 2022)



Katra et al. *Atherosclerosis*; Volume 366 Pages 1-7 (February 2023)

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