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Multi-Center Multi-Device Hybrid Imaging Study of Coronary Artery Disease. Results from the EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease (EVINCI) Hybrid Imaging population

--Manuscript Draft--

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Abstract:	Aims Hybrid imaging provides a non-invasive assessment of coronary anatomy and myocardial perfusion. We sought to evaluate the added clinical value of hybrid imaging in a multi-centre multi-vendor setting. Methods and results Fourteen centres enrolled 252 patients with stable angina and intermediate (20-90%) pre-test likelihood of coronary artery disease (CAD) who underwent myocardial perfusion scintigraphy (MPS), CT coronary angiography (CTCA), and quantitative coronary angiography (QCA) with fractional flow reserve (FFR). Hybrid MPS/CTCA images were obtained by 3D image fusion. Blinded core-lab analyses were performed for CTCA, MPS, QCA and hybrid datasets. Hemodynamically

	significant CAD was ruled-in non-invasively in the presence of a matched finding (myocardial perfusion defect co-localized with stenosed coronary artery) and ruled-out with normal findings (both CTCA and MPS normal). Overall prevalence of significant CAD on QCA (>70% stenosis or 30-70% with FFR≤0.80) was 37%. Of 1004 pathological myocardial segments on MPS, 246 (25%) were reclassified from their standard coronary distribution to another territory by hybrid imaging. In this respect, in 45/252 (18%) patients, hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 42% of the cases. Hybrid imaging allowed non-invasive CAD rule-out in 41%, and rule-in in 24% of patients, with a negative and positive predictive value of 88% and 87%, respectively. Conclusions In patients at intermediate risk of CAD, hybrid imaging allows non-invasive co-localization of myocardial perfusion defects and subtending coronary arteries, impacting clinical decision-making in almost one every five subjects.
Response to Reviewers:	Please see separate extensive rebuttal letter appended to the submitted manuscript

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Ms. No. EHJCI-D-16-00104
Multi-Center Multi-Device Hybrid Imaging Study of Coronary Artery Disease. Results from the EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease (EVINCI) Hybrid Imaging population

Reply to reviewers' comments:

We are thankful to all of the reviewers for taking the time of a thoughtful and detailed review of our manucript. We are convinced that their comments have significantly contributed to improve the quality of the manuscript. Please find enclosed a point-by-point reply to the reviewers' comments. Changes within the manuscript are highlighted in red.

Reviewer #1:

1.
Reviewer: Indeed, hybrid- multi-modality imaging showed a great promise to show "more" than the individual modalities are able to do. I fully agree with the authors that a multi-center, multi-vendor setting is mandatory to evaluate these new technologies onto their possible clinical impact. Further evaluation using standardized operating procedures (SOP) is also very important to be able to compare the results at a high quality.
Having said this, and perhaps I overlooked it, if you compare the results of the core-lab to those generated by the individual centers themselves, is there a difference? In other words, I assume that every center will ultimately perform their own analysis? Can you elaborate on this? I assume that you do not use (need) the core-lab for daily clinical practice?
Authors: Many thanks for this thoughtful comment and the possibility to clarify this issue. Indeed, to improve the quality and generalizability of our findings, the trial was fully conducted with core-lab data (i.e. with individual and independent core labs for CT, SPECT, PET, and hybrid imaging). This was an attempt to avoid any center-bias. However, as highlighted by the recently published main EVINCI trial (Neglia D et al. Circ Cardiovasc Imaging. 2015 Mar;8(3). pii: e002179. doi: 10.1161/CIRCIMAGING.114.002179), there are significant differences between individual center- and core lab-analyses. Therefore, and following the suggestion of this reviewer, we have added a separate accuracy analysis with the individual center analysis in the supplementary material section (**Suppl figure 1**) of the revised manuscript, and the results are discussed on page 13 of the revised manuscript: Notably, on centre-based analysis the diagnostic accuracy of the different non-invasive imaging modalities was generally improved compared to the core-lab data. Nevertheless, even when only individual centre-data were considered, hybrid imaging maintained significantly elevated specificity and overall diagnostic accuracy, both at per-patient and vessel-based analysis.

2.
Reviewer: Speaking about the analysis at the core-lab, on page 5 you write that analysis was performed by an in-house developed software (PMOD 3.6, PMOD Technologies Ltd., Zurich, Switzerland). However, I am used to identification of "in-house" when the software (or product) is made in the institution that writes the publication. Now it is related to a commercial company, so I am a bit confused with the term of "in-house".
Authors: Many thanks for pointing this out. The PMod Software was developed in-house but is now commercially available. We have therefore changed the sentence acocording to this reviewers suggestion*: „In case of H215O-PET images, parametric myocardial blood flow datasets, showing flows on a segmental level, were generated based on quantitative analysis performed using a commercially available software, (PMOD 3.6 software package. PMOD Technologies Ltd., Zurich, Switzerland).“*

3.

Reviewer: The methodology to apply hybrid imaging is expensive and it also needs radiation. It is perhaps outside the scope of your paper, but do you think that your results could be used to justify the proposed method to evaluate the patients as in your cohort on a larger scale and make it standard clinical practice?
Authors: Many thanks for this important comment. Indeed the added costs and radiation exposure of hybrid imaging are a concern, and do not justify any unrestricted use of the techniques. The present manuscript is too limited to identify any subpopulation that may benefit from hybrid imaging, and we are unable to address issues of prognostic impact and cost-efficiency. However, in our personal opinion hybrid imaging may be judiciously used in selected patients (e.g. patients with prior CTCA documenting intermediate stenoses or multivessel disease as part of a sequential imaging approach). With regard to radiation exposure, previous studies suggest that the use of modern equipment and dose-optimization protocols (e.g. prospective ECG-triggering for CTCA, stress-only for SPECT) may consistently reduce the radiation burden of hybrid imaging, favouring its clinical application on a larger scale. We have added this information in the limitations section on page 14 of the revised manuscript.

4.
Reviewer: Over which time period were the patients included?
Authors: Patients were enrolled between March 2009 and June 2012. We have added this to the methods section on page 4-5 of the revised manuscript.

5.
Reviewer: Almost 2/3 of the patients were dropped somewhere during the protocol. I most likely overlooked it, but could you identify why so many patients were dropped?
Author: Many thanks for the opportunity to clarify this issue: Figure 1 shows in detail the drop-outs and specific reasons for it. The majority of drop-outs were not specifically related to the hybrid substudy. Per EVINCI protocol, all patients included in the EVINCI trial had to undergo CT coronary angiography plus at least one functional imaging test (either stress echo, stress wall motion MRI, SPECT of PET). For the hybrid imaging substudy, only patients could be included who had myocardial perfusions imaging by SPECT or PET performed, in addition to a CT scan. Accordingly, patients submitted to wall motion imaging modalities were not included in the analysis, because their format precludes formation of 3D hybrid datasets with CTCA, which accounts for the large number of excluded EVINCI patients (n=404). The remaining 41 drop-outs were „true drop-outs“ due to either lack of core lab analysis data or inability to generate a hybrid data sets due to software incompatibility or incomplete or corrupted datasets. We have now clarified this in the revised methods section on page 5.

Reviewer #2:

1.
Reviewer: The paper is well written, methods and results clearly explained. I would skip the examples since these are already known from literature.
Authors: Many thanks for these kind comments. According to this reviewer's suggestion we have skipped figure 6, but decided to keep figure 3 to have at least one practical example in the manuscript. We believe that having a practical example in the manuscript will make the topic easier to comprehend and demonstrate the clinical relevance of the findings better, particularly for readers who are not experienced with such complex imaging protocols.

2.
Reviewer: Methods: It is amazing that in a major multicenter trial dropout rate was as high as mentioned in this study. From the initial 697 patients finally only 252 could be included in this study (with different reasons, some also technical). This induces a first major weakness of the study: are the conclusions of the study still relevant for the group of patients with low to intermediate risk of CAD or only for a very selected subgroup?

Authors: Many thanks for this important comment. Figure 1 shows in detail the drop-outs and specific reasons for it. The majority of drop-outs were not specifically related to the hybrid substudy. Per EVINCI protocol, all patients included in the EVINCI trial had to undergo CT coronary angiography plus at least one functional imaging test (either stress echo, stress wall motion MRI, SPECT of PET). For the hybrid imaging substudy, only patients could be included who had myocardial perfusions imaging by SPECT or PET performed, in addition to a CT scan. Accordingly, patients submitted to wall motion imaging modalities were not included in the analysis, because their format precludes formation of 3D hybrid datasets with CTCA, which accounts for the large number of excluded EVINCI patients (n=404). The remaining 41 drop-outs were „true drop-outs" due to either lack of core lab analysis data or inability to generate a hybrid data set due to software incompatibility or incomplete or corrupted datasets. Additionally, table 1 shows that our study population did not differ appreciably from the entire EVINCI cohort with regard to the baseline characteristics (age, gender, risk factors, symptomatology) (e.g. our pretest probability was 59% compared to 65% in the entire EVINCI population). We have now clarified this issue in the Methods section on page 5 and added a paragraph in the Results section on page 8 and in the Limitations section on page 13 of the revised manuscript. Furthermore, we are giving a modified table 1 in the supplementary appendix (**Supplementary table A,** see below) showing no significant differences between our study population compared to the entire EVINCI population as published in the main manuscript (Neglia et al. Circ Cardiovasc Imaging. 2015 Mar;8(3). pii: e002179. doi: 10.1161/CIRCIMAGING.114.002179.)

Supplementary Table A. Patients' baseline characteristics

Parameter	EVINCI Hybrid Substudy Population (n=252)	Overall EVINCI Study Population (475)*
Demographics, n (%)		
Age, years (mean±SD)	61±9	60±9
Male gender	161 (64)	291 (61)
Clinical characteristics, n (%)		
Typical angina	62 (25)	121 (25)
Atypical angina	148 (59)	288 (61)
Non-anginal chest pain	42 (17)	66 (14)
Pre-test probability of CAD [median (IQR)]	69 (28)	65 (42)
Left ventricular ejection fraction >50%	238 (94)	451 (95)
Cardiovascular risk factors, n (%)		
Family history of CAD	75 (30)	160 (34)
Diabetes mellitus	68 (27)	115 (24)
Hypercholesterolemia	161 (64)	267 (56)
Hypertension	155 (62)	290 (61)
Smoking	60 (24)	120 (25)
Obesity	72 (29)	112 (24)
Invasive coronary angiography data, n (%)		
Normal coronaries or non-obstructive CAD	158 (63)	335 (70)
Single-vessel disease	60 (23)	99 (21)
Multi-vessel disease	34 (14)	41 (9)

Data is given in absolute numbers and percentages (%), unless otherwise stated; CAD denotes coronary artery disease

All comparisons were between both groups were not significant (by Mann *Whitney-U* test or χ^2 test where appropriate).

* as published in *Neglia D, Rovai D, Caselli C, Pietila M, Teresinska A, Aguadé-Bruix S, et al. Detection of significant coronary artery disease by noninvasive anatomical and functional imaging. Circ Cardiovasc Imaging 2015;8. pii:e002179. Doi:11.1161/CIRCIMAGING.114.002179*

3.
Reviewer: Methods: The penetration rate of FFR was extremely low in this study (23%) and on top of this 34% of the patients with intermediate lesions were not interrogated by FFR. The first article referring to the multimodality imaging technique used in this article dates from 2007. FAME I was published in January 2010. The results of the complete study EVINCI was only published very recently 2015. Data collection was concluded in june 2012: 2.5 years of inclusion into EVINCI occurred in an era were FAME proved already the value of FFR. This is a very important limitation of this study. The argument of the known gap between EBM and everyday clinical practice is an argument in everyday clinical practice but not in a research study as EVINCI.
Authors: We are thankful to this reviewer for bringing up this topic, and fully agree with him that the low FFR penetration is a limitation of the study. One reason for this low penetration, is that the EVINCI protocol considered a coronary stenosis >70% already hemodynamic significant (without evidence of ischemia by FFR). FFR, however, was mandated in stenoses of 30-70%. In june 2010, (i.e. 14 months after enrolment of the first patient in the EVINCI study), the FAME trialists published, that at least 20% of stenoses with 70-90% stenosis severity were not hemodynamically significant by FFR. These findings clearly conflicted with the definition of obstructive CAD by the EVINCI protocol, however, the protocol was not amended. Finally, the lack of FFR use in one third of intermediate lesions clearly represents a protocol violation. This may have financial reasons, as FFR is not reimbursed in all European countries. This is now discussed on page 13 in the limitations section of the revised manuscript highlighting that the low FFR penetration is a clear limitation of this study.

4.
Reviewer: Methods: These methodological shortcomings have an important impact on the interpretation of the results and on the conclusions.
Authors: We are not sure to which limitation the reviewer refers in this particular sentence. However, we believe that the two main limitations were the high „drop-out rate“ and the low penetration of FFR. We have addressed this two limitations in the comments above and have added some paragraphs in the limitations section to highlight the importance of these two limitations.

5.
Reviewer: Results: False negative hybrid studies are still an important problem: 41 were false negative and probably most of them were really missed ischaemia regions by MPI since from the 17 FFR measured lesions 13/17 were indeed positive: the mentioned 32% are somewhat misleading since this implicates that from the 41 false negatives 77% had indeed lesions creating ischaemia although missed by MPI. On the false positive cases, nothing can be concluded since FFR was not performed in these patients.
Authors: Many thanks for this important comment. Indeed, the high false negative rate observed in this trial was a concern. However, as shown in figure 4, the false negative rate varies considerably based on the definition of what is considered a pathological hybrid study, i.e. whether mismatched findings are considered as positives or not. This adds flexibility and at the same time also complexity to the definition of coronary artery disease which is no longer a binary „Yes/No“ disease but considers the entire anatomofunctional spectrum of disease. Indeed, it is possible that in some of the false negative findings, MPI may have failed to detect ischemia as evidenced by the positive FFR value. However, this finding is not very suprising given the number of reports documenting rather poor agreement between perfusion imaging techniques and FFR (e.g. Melikian N, et al. JACC Cardiovasc Interv 2010;3:307-14). Myocardial perfusion imaging integrates micro- and macrovascular effects on myocardial blood flow while FFR only interrogates a single coronary artery segment (i.e. a small snap-shot of the entire coronary vasculature). Furthermore, the cut-off of 0.80 may overestimate the functional significance of coronary lesions based on the first comparative studies with non-invasive

imaging which determined an ideal cut-off of 0.72-0.75 (Pijls NH, et al. N Engl J Med. 1996 Jun 27;334(26):1703-8.) This may explain partly some of the false negative findings, since a significant number (37%) of FFR-positive lesions had „borderline“ significance. We have added this data on page 9 of the results section and the supplementary table A and have expanded the discussion on this issue on page 12 of the revised discussion section.

6.
Reviewer: Discussion: The authors claim a high feasibility of the technique. Given the extreme high dropout authors cannot claim this.
Authors: Drop-outs for technical reasons affecting the feasibility of imaging occurred only in 7% (see table 1). We would argue that this documents reasonable feasiblility and have changed accordingly the term to: "... highlighting the robustness of the technique" on page 11 of the revised discussion section.

7.
Reviewer: Discussion: In a matched positive hybrid finding 70% were revascularized and the authors claim that this is extremely high. I am puzzled with this conclusion: all of these patients had an invasive intervention, angio, and had a matched lesion, why was reperfusion not 100%? In a matched negative hybrid finding authors claim that invasive evaluation can be safely spared. Strange again if 10% of these patients were revascularized!
Authors: Since the decision for revascularization was left entirely to the judgement of the treating physician, i.e. in this case the interventional cardiologist performing the invasive study we can only speculate on the answer for for this question: Possible reasons for deferring any revascularization procedure may be: small ischemia, technical difficulty (calcified tortuous verssels, chronic occlusions), poor target vessel quality, high surgical risk, patient refusal, severe comorbidities and others. Potential reasons for performing a revascularization procedure in the group of negative hybrid patients could be visual overestimation of stenosis severity, and medicolegal considerations. Nevertheless, the reported frequencies for revascularization procedures are well in line with those of previously published reports (Pazhenkottil et al. Eur Heart J. 2011 Nov;32(22):2824-9; Schaap J et al. Heart. 2013 Feb;99(3):188-94).

8.
Reviewer: Discussion: In the absence of CAD on CTCA, MPI was + in 39/252 patients and 26% of these patients were revascularized! In an era where CTCA is considered as a technique with an extremely high negative predictive value this is a rather poor result.
Authors: Thank you for this important comment. Indeed, CTCA has demonstrated a very high negative predictive value in several trials where the gold standard was invasive coronary angiography only (without FFR). The fact that we used a more comprehensive anatomofunctional gold standard (ICA+FFR) may explain to some extent the lower sensitivity. Moreover, the sensitivity of CTCA by core lab analysis in the main EVINCI trial was lower than by individual-center analysis (Neglia D, et al. Circ Cardiovasc Imaging 2015;8. pii:e002179). As a result, some lesions may have been underestimated accounting for the small number of revascularizations in this group. We have added this information to the discussions section on page 12 of the revised manuscript and have added a supplementary figure (**Suppl figure 1**) with the accuracy analysis performed with individual centre-based data (as opposed to core lab data).

10.
Reviewer: Discussion: As always in diagnostic imaging, adding another technique on top of another one results in a dilemma: if you define a positive hybrid exam as a positive CTCA or positive MPI, you increase sensitivity at the expense of a reduction in specificity. On the contrary if you consider only real positive hybrid imaging as a positive CTCA and a positive MPI, you increase specificity but with a dramatic decline in sensitivity. The overall results are in my opinion not that spectacular and nothing (or almost nothing) is said about cost-efficiency neither on radiation hazard. The only reason why the community would accept a supplementary load on cost and radiation would be the fact that a new diagnostic strategy resulted in better patient

outcome and by this often also a better cost-effective result. These elements are lacking in this study.
Authors: We are very thankful for this important comment and fully agree with this reviewer: The assessment of accuracy when two imaging modalities are added becomes immediately more complex based on how mismatched findings are considered in the analysis. The main objective of the present study was to highlight the complementary role of anatomofunctional modalities and their synergistic value over standalone techniques for identifying functionally significant coronary lesions (and thereby guide revascularization decisions). The accuracy analysis is only a secondary objective and the authors are very aware of the limitations pointed out by this reviewer. Unfortunately, the design of the study and the lack of follow-up data precludes to assess impact on patient management and cost-efectiveness. Accordingly, we have added the limitations with regards to radiation exposure and the need for further studies assessing cost-effectiveness in the limitations section on page 13 and 14 of the revised manuscript

Reviewer #3:

1.
Reviewer: This is a substudy of the EVINCI trial reporting on 252 patients with stable angina and an intermediate pre-test probability for the existence of significant epicardial coronary stenoses. Hybrid imaging consisting of either SPECT or PET perfusion imaging plus CT coronary angiography was performed in all patients. The result of this extensive and radiation intensive diagnostic work up was that 1. 25% of segments were reclassified from their standard coronary distribution to another territory and 2. hybrid imaging was able to exclude relevant CAD in 41% of patients and confirm relevant CAD in 24% of patients. With respect to the first result one wonders about the practical consequences of this finding because patients with regional ischaemia based on the findings of SPECT or PET would undergo invasive coronary angiography according to current guidelines and also have their coronary perfusion beds reassigned. The second result is rather disappointing because it leaves 35% of patients out a definite diagnosis with respect to the presence of haemodynamically relevant epicardial stenoses.
Authors: We are very thankful to this reviewer for his/her important comments. The reviewer has accurately summarized the main findings of the study and rightfully points out concerns about the clinical impact of the findings. We are grateful for the opportunity to reply on his comments: 1. We respectfully disagree with this reviewer that every patient with myocardial ischemia will undergo immediately invasive coronary angiography. In fact, the recently published SPARC trial (J Am Coll Cardiol. 2012 Jan 31;59(5):462-74) showed that more than 80% with mildly anormal and more than 50% of patients with moderately to severely abnormal SPECT and PET results are not referred for cardiac catheterization (i.e. conflicting with recommendations from current guidelines). This underutilisation of coronary angiography and revascularization may be improved through the use of hybrid imaging (Pazhenkottil et al. Eur Heart J. 2011 Nov;32(22):2824-9; Schaap J et al. Heart. 2013 Feb;99(3):188-94). Correct allocation of myocardial perfusion defects to their tributaries may for example downstage suspected 3-VD to 2-VD (as shown in figure 3) for which medical treatment may be justified rather than CABG or the opposite, and thereby improve downstream ressource utilisation. 2. The fact that 35% of patients in our study had mismatched findings on hybrid imaging and therefore, as this reviewer points out, „no clear diagnosis“, may be disturbing but to some degree represents the reality of CAD diagnosis. With our manuscript we emphasize that we should move away from a binary „yes/no“ paradigm of coronary artery disease, to embrace the entire spectrum of coronary disease which includes not only obstructive lesions of the epicardial coronary arteries but also non-obstructive disease, diffuse disease, and microvascular dysfunction. Hybrid imaging allows to interrogate this entire spectrum yielding a number of mismatched findings for which further management decisions are more complex and will need further integration of invasive findings and more clinical information. Finally, the added radiation dose from hybrid imaging procedures is an important concern and presently a deterrant for the unrestricted use of hybrid imaging. However, the use of modern equipment

and dose-optimization protocols (e.g. prospective ECG-triggering for CTCA, stress-only for SPECT) may consistently reduce the radiation burden of hybrid imaging, favouring its clinical application on a larger scale. We have accordingly highlighted the high radiation burden as an important limitation of the study in the limitations section on page 14 of the revised manuscript.

2.
Rewiewer: A certain weakness of the paper is that not all patients had as prespecified FFR performed in vessels with stenoses with an angiographic degree of obstruction between 30 and 70%. In addition, as correctly pointed out by the authors in the discussion, FFR should also be performed according to the FAME study in stenoses of 70 to 90% angiographic severity.
Authors: We are thankful to this reviewer for bringing up this topic, and fully agree with him that the low FFR penetration is a limitation of the study. As pointed out by this reviewer, the EVINCI protocol considered a coronary stenosis >70% already hemodynamically significant (without evidence of ischemia by FFR). FFR, however, was mandated in stenoses of 30-70%. In june 2010, (i.e. 14 months after enrolment of the first patient in the EVINCI study), the FAME trialists published, that at least 20% of stenoses with 70-90% stenosis severity were not hemodynamically significant by FFR. This findings clearly conflicted with the definition of obstructive CAD by the EVINCI protocol, however, the protocol was not amended. This is now discussed on page 13 in the limitations section of the revised manuscript, highlighting that the low FFR penetration is a clear limitation of this study.

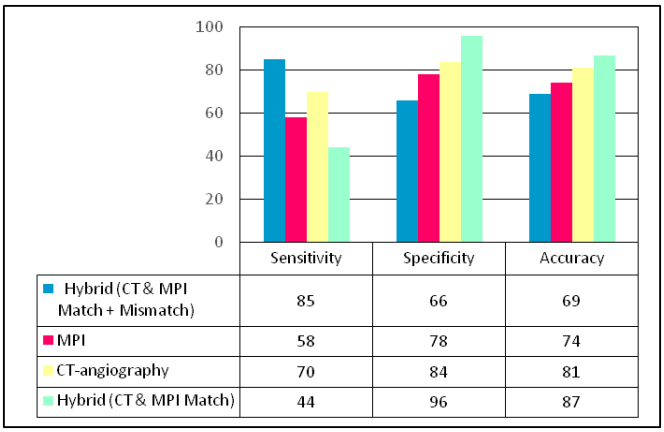
3.
Reviewer: Abstract: In the conclusions the authors talk about "patients at low-intermediate risk of CAD". However, according to the US guidelines (Fihn et al. JACC 2012) intermediate pre-test probability is defined as a probability between 20 and 70% whereas the European guidelines define the intermediate range as existing between 15 and 85%. The NICE guidelines extend the intermediate range to 10 to 90%. Thus, the choice of 20 to 90% as in this paper is unusual. Definitely, these patients were not in the low-intermediate risk group but in the intermediate risk group.
Authors: We thank the reviewer for having pointed out this aspect of the previous version of the manuscript. As correctly remarked, the definition of intermediate probability of CAD varies quite significnatly accrording to the specific guidelines or scientific statements that are considered. In the EVINCI study the specific range of probabilities was defined according to the best scientific evidence available at the time of the conception of the study. Specifically, the 2006 ESC guidelines for the management of CAD were chosen (Fox K, Garcia MA, Ardissino D, Buszman P, Camici PG, Crea F, et al. Eur Heart J doi:10.1093/eurheartj/ehl001). In that document a pre-test probability of CAD >90% was considered "elevated", while a probability <10% was defined "very low". In line with that evidence, a range of probabilities of 20-to-90% ollowed to reasonably exclude patients at low risk of CAD, selecting only the intermediate risk group. We have changed accordingly the sentence in the abstract from „low-to-intermediate“ to „intermediate“ risk and added a new reference (ref. 11) in the reference list.

4.
Reviewer: Page 6, myocardial perfusion scintigraphy: How was the presence of myocardial scar interpreted for diagnostic purposes in this context?
Authors: In our primary analysis, a scar was considered as a pathological MPS finding (i.e. the presence of a scar defined as a summed rest score ≥2 was considered as positivity for MPS). We are now stating this clearly on page 6 of the revised manuscript. The rationale for using myocardial scar as a criterion for positivity was that in the majority of cases the presence of a scar in a CAD-naive patient with signs and symptoms of CAD would probably prompt further diagnostic testing. Nonetheless, to address this reviewer's comment, we performed a separate analysis where only ischemia was accepted as a criterion for MPS positivity. We did not find any major differences in accuracy comparing ischemia only to ischemia+scar as the positivity criterion for SPECT or PET (see below). We are now stating this on page 13 of the revised manuscript, however, we chose not to inlude an additional figure in the manuscript in order not

to overload the manuscript with accuracy analyses (see also comment #8 by the second reviewer).

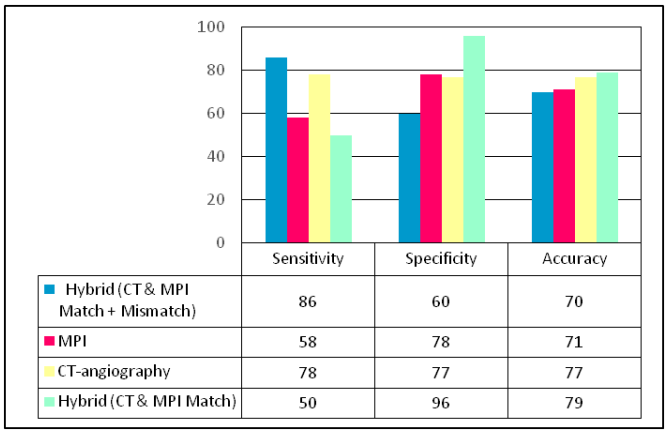
Accuracy in detecting significant CAD using the presence of reversible ischemia as the only positivity criteria for MPI and Hybrid imaging.

Per vessel analysis



	Sensitivity	Specificity	Accuracy
Hybrid (CT & MPI Match + Mismatch)	85	66	69
MPI	58	78	74
CT-angiography	70	84	81
Hybrid (CT & MPI Match)	44	96	87

Per patient analysis



	Sensitivity	Specificity	Accuracy
Hybrid (CT & MPI Match + Mismatch)	86	60	70
MPI	58	78	71
CT-angiography	78	77	77
Hybrid (CT & MPI Match)	50	96	79

5.
Reviewer: Page 12, end of paragraph 1: the authors point out that of 19 coronary lesions with a pathological FFR only 4 had a matched perfusion defect. This clearly suggests that the currently used cut-off for a pathologic FFR measurement of 0.80 is too liberal resulting in a substantial overestimation of clinically relevant ischaemia caused by the stenosis in question. Indeed, the original cut-off proposed by the inventors of FFR was 0.75. Yet, the authors suggest that it would be clinically justified based on their results to have symptomatic patients with pathologic CT coronary angiography yet normal myocardial perfusion scans undergo invasive FFR measurements.
Authors: We totally agree with this reviewer, that the FFR cut-off at 0.80 may be too liberal and overestimate the severity of lesions in some instances. Indeed, 37% of lesions without myocardial ischemia on MPS but positive FFR had values in the range of 0.75 to 0.80, which is a grey zone for which clinical recommendations are not yet totally clear. We have added this information on page 12 of the discussions section in the revised manuscript. The DEFER study (now with 15 year follow-up: Zimmermann et al. Eur Heart J. 2015 Dec 1;36(45):3182-8), for example, suggested that it is safe to defer revascularization in patients with single vessel disease if FFR values were higher than 0.75. On the other hand, the FAME II trial (De Bruyne et al. N Engl J Med. 2014 Sep 25;371(13):1208-17) showed that in multivessel disease patients revascularization of all lesions with an FFR value lower than 0.80 improved outcomes. As a result, some interventional cardiologists consider the 0.80 cut-off valid in main branches (e.g. left main, proximal LAD), while they are more liberal in side branches. Nevertheless, in some of these patients, it may be wise to take the final decision about revascularization after all information from noninvasive and invasive imaging has been integrated with regard to extent and severity of disease, justifying a complete noninvasive/invasive assessment.

6.
Reviewer: Page 13: After reading this study one wonders whether hybrid imaging is really worth the added expenses and radiation.
Authors: We agree that concerns of added costs and radiation exposure exist. However, at least with regard to radiation exposure many new developments in the field of CT and SPECT exist, which have already substantially lowered radiation exposure compared to the techniques used

in the original EVINCI trial. We have therefore added a paragraph dealing with the issue of radiation reduction on page 14 of the revised manuscript.

Reviewer #4:

1.
Reviewer: The EVINCI study, first presented in June 2012 and with primary results published in Circ Imaging (2015;8: pii: e002179), aimed to study combined anatomic and functional imaging for the assessment of coronary artery disease in patients with intermediate likelihood. The primary publication found that coronary CT angiography (CCTA) had the highest diagnostic accuracy of the modalities considered, in comparison to a reference standard of quantitative coronary angiography and fractional flow reserve (in the small minority of patients in which it was performed). The present manuscript focuses on hybrid imaging with 3D image fusion of myocardial perfusion scintigraphy (MPS) and CCTA images, in the 252 EVINCI patients who underwent CCTA, MPS, and invasive angiography. The authors' major findings here were: 1) hybrid (fused) imaging reassigned 25% of visually-classified abnormal myocardial segments, in 18% of patients, from one coronary vascular distribution to another; 2) fusion imaging could be performed in 93% of patients, with high inter-rater agreement. Reclassification occurred in 49% of myocardial segments visually classified as belonging to the left circumflex (36% to LAD and 13% to RCA), in 32% classified to the right coronary (19% to LAD and 13% to LCx), and in only 2% of segments classified to the left anterior descending. Correlating these changes with CCTA findings, the reclassification moved the perfusion defect to the distribution of a coronary artery with a stenosis on CTA in 16 patients ("matched finding"), and away from a stenosis in 3 of the 252 patients.
Fused imaging generates pretty pictures but requires two costly tests, which for most patients is not justified (excepting those with equivocal first tests). It requires specialized software which is not necessary since each study can be interpreted independently and the results combined without the images being combined. For example if MPS shows an inferior perfusion defect and CCTA shows a stenosis only of the circumflex, it is clear that that defect reflects ischemia from the circumflex, not from the RCA, even without fancy fused pictures. And in any event, if the patient is symptomatic despite optimal medical therapy and has either one of these tests performed demonstrating significant coronary disease, then invasive angiography is probably this right next test to perform. If on invasive angiography the inferior defect seen on MPS is found to be associated with only a circumflex stenosis then it is clear that the circumflex should be intervened upon, not the right coronary. The additional performance of pre-cath CCTA would not significantly impact clinical decision-making in this patient - they would wind up with a circumflex stent in any event. An analogous argument can be made were CCTA the initial test - then MPS would not impact clinical decision-making. Thus I take issue with the authors' conclusion that hybrid imaging impacts clinical decision-making in 18% of patients. Pre-catheterization segmental reclassification is not equivalent to an impact on clinical decision-making, and the authors do not provide any strong data regarding the latter.
Authors: Many thanks to this reviewer for his/her insightful comments. We fully agree with this reviewer that limitations for hybrid imaging exist, and that its indiscriminate should not be recommended. The incremental costs and radiation exposure of the technique are limitations. We have thus added an additional paragraph on page 14 of the revised manuscrip highlighting this limitation. Furthermore, the study was not designed to obtain follow-up information, therefore it is beyond the scope and the design of the manscript to assess the impact of hybrid imaging on downstream ressource ustilisatio, patient management and outcomes. We have further added this as a limitation on page 13 of the revised manuscript.
We agree that in the majority of patients, the fact that hybrid imaging may reassign coronary perfusion territories will have limited clinical impact. We rather see the added clinical value in patients with multivessel disease where revascularization decisions are more complex. This is best demonstrated by the example given in figure 3, where reassigning ischemic segments from the LCX territory to the RCA territory was able to downstage suspected 3-vessel disease (where

CABG is the preferred revasc technique) to 2-vessel disease (where PCI or even medical treatment may be considered). Another potential scenario may be reassigning of a perfusion defect from a territory subtended by a difficult chronic total occlusion (where PCI would most likely fail and CABG would be required) to a territory subtended by a easy type A stenosis. However, we fully agree with this reviewer that such complex coronary cases are rare, and that the majority of patients are appropriately managed without hybrid imaging.

2.
Reviewer: The authors do not seem to use the term "intention-to-diagnose" correctly. For example they state "According to an intention-to-diagnose strategy, any non-diagnostic segment was considered abnormal." My understanding of "intention-to-diagnose" is that it is analogous to "intention-to-treat", whereby patients are classified as to the group corresponding to the diagnostic test which was intended to be performed for the patient, irrespective of what test(s) were or weren't actually performed. As such "intention-to-diagnose" has nothing to do with classification of non-diagnostic segments. You can simply state that a prior a decision was made to classify non-diagnostic segments as abnormal.
Authors: Many thanks for this comment. We have changed this accordingly on page 6 of the revised manuscript.

3.
Reviewer: The authors acknowledge that incomplete use of FFR may account for some false-negative and false-positive hybrid findings. Why was a uniform reference standard not applied?
Authors: We are thankful to this reviewer for bringing up this topic, and fully agree with him that the low FFR penetration is a limitation of the study. Nonetheless, it was a declared aim of the EVINCI trialists to avoid a purely anatomical gold standard of invasive coronary angiography which would intrinsically favour angiographical tests like CT coronary angiography, and thereby (by using a comprehensive anatomofunctional gold standard consisting of ICA plus FFR) overcome the limitations of innumerable previous diagnostic studies. However, one reason for the low FFR penetration is that the EVINCI protocol considered a coronary stenosis >70% already hemodynamic significant (without evidence of ischemia by FFR). FFR, however, was mandated in stenoses of 30-70%. In june 2010, (i.e. 14 months after enrolment of the first patient in the EVINCI study), the FAME trialists published, that at least 20% of stenoses with 70-90% stenosis severity were not hemodynamically significant by FFR. This findings clearly conflicted with the definition of obstructive CAD by the EVINCI protocol, however, the protocol was not amended. Finally, the lack of FFR use in one third of intermediate lesions clearly represents a protocol violation. This may have financial reasons, as FFR is not reimbursed in all European countries. We have added a senstence in the limitations section on page 13 highlighting that the low FFR penetration is a clear limitation of this study.

Reviewer #5:

1.
Reviewer: Out of 697 patients, only 252 = 36% are included in this hybrid imaging substudy due to various reasons. How were these patients different from the 445 patients that were not included? How did this bias affect the results of the study?
Authors: We are very thankful to this reviewer for his insighful comments. The high „drop-out“ rate is indeed a concern and a limitation of the study. Table 1 shows that our study population did not differ appreciably from the entire EVINCI cohort with regard to the baseline characteristics (age, gender, risk factors, symptomatology) (e.g. our pretest probability was 59% compared to 65% in the entire EVINCI population). We have now clarified this in the Methods section on page 5 and added a paragraph on this in the Results section on page 8 and in the Limitations section on page 13 of the revised manuscript. Furthermore, we are giving a modified table 1 in the supplementary appendix (**Supplementary table A,** see below) showing no significant differences between our study population compared to the entire EVINCI population

as published in the main manuscript (Neglia et al. Circ Cardiovasc Imaging. 2015 Mar;8(3). pii: e002179. doi: 10.1161/CIRCIMAGING.114.002179.)

Supplementary Table A. Patients' baseline characteristics

Parameter	EVINCI Hybrid Substudy Population (n=252)	Overall EVINCI Study Population (475)*
Demographics, n (%)		
Age, years (mean±SD)	61±9	60±9
Male gender	161 (64)	291 (61)
Clinical characteristics, n (%)		
Typical angina	62 (25)	121 (25)
Atypical angina	148 (59)	288 (61)
Non-anginal chest pain	42 (17)	66 (14)
Pre-test probability of CAD [median (IQR)]	69 (28)	65 (42)
Left ventricular ejection fraction >50%	238 (94)	451 (95)
Cardiovascular risk factors, n (%)		
Family history of CAD	75 (30)	160 (34)
Diabetes mellitus	68 (27)	115 (24)
Hypercholesterolemia	161 (64)	267 (56)
Hypertension	155 (62)	290 (61)
Smoking	60 (24)	120 (25)
Obesity	72 (29)	112 (24)
Invasive coronary angiography data, n (%)		
Normal coronaries or non-obstructive CAD	158 (63)	335 (70)
Single-vessel disease	60 (23)	99 (21)
Multi-vessel disease	34 (14)	41 (9)

Data is given in absolute numbers and percentages (%), unless otherwise stated; CAD denotes coronary artery disease

All comparisons were between both groups were not significant (by Mann *Whitney-U* test or χ^2 test where appropriate).

* as published in *Neglia D, Rovai D, Caselli C, Pietila M, Teresinska A, Aguadé-Bruix S, et al. Detection of significant coronary artery disease by noninvasive anatomical and functional imaging. Circ Cardiovasc Imaging 2015;8. pii:e002179. Doi:11.1161/CIRCIMAGING.114.002179*

2.

Reviewer: Similarly, only 23% underwent FFR and a distal 34% of the intermediate lesions had FFR. While this was a pragmatic, comparative effectiveness study, this is a very low percent of FFR use for a study where QCA and FFR form the reference standard and in the background of FAME and FAME-2, not reflective of current use of FFR either. The rate may reflect the rate of clinical use of FFR in the participating countries at the time of the study, but this makes for a poor reference standard from a study perspective.

Authors: We fully agree with this reviewer that the low FFR penetration is a limitation of the study. One reason for the low FFR penetration, is that the EVINCI protocol considered a coronary stenosis >70% already hemodynamic significant (without evidence of ischemia by FFR). FFR, however, was mandated in stenoses of 30-70%. In june 2010, (i.e. 14 months after enrolment of the first patient in the EVINCI study), the FAME trialists published, that at least 20% of stenoses with 70-90% stenosis severity were not hemodynamically significant by FFR. This findings clearly conflicted with the definition of obstructive CAD by the EVINCI protocol, however, the protocol was not amended. Finally, the lack of FFR use in one third of intermediate lesions clearly represents a protocol violation. This may have financial reasons, as FFR is not

reimbursed in all European countries. We have added a senstence in the limitations section on page 13 highlighting that the low FFR penetration is a clear limitation of this study.

3.

Reviewer: The average dose of radiation for SPECT/CTCA, which was done in 71% of the included patients was quite high at 18.5 mSv, which is very concerning given the risk of secondary cancer. In the era of cardiac MR perfusion imaging, which involves no radiation, and CT-FFR, which involves no extra radiation over CTCA, this is a major issue and not very well discussed in the manuscript. According to the AHA Scientific Statement on "Approaches to Enhancing Radiation Safety in Cardiovascular Imaging" (Fazel et al. Circulation 2014;130), "when a cardiac imaging study is appropriate, if a comparable test that does not use ionizing radiation (e.g., echocardiography or cardiac magnetic resonance imaging) is able to provide the clinical information needed with comparable accuracy, cost, and convenience but lower overall risk (taking into consideration other potential risks, such as those related to use of gadolinium contrast agents or anesthesia), then it may be the preferred approach". I would argue that hybrid imaging may improve over CTCA or SPECT individually, but the increased radiation presents a significant risk, which could be avoided with use of a comparable test that does not use ionizing radiation (e.g., echocardiography or cardiac magnetic resonance imaging).

Authors: Many thanks for this important comment. Indeed the radiation exposure for hybrid studies is a clear safety concern and limits the indiscriminate use of the technique. However, many now developments in the field of CT and SPECT exist, which have already substantially lowered radiation exposure compared to the techniques used in the original EVINCI trial. We have therefore added a paragraph dealing with the issue of radiation reduction on page 14 of the revised manuscript. With regard to alternative techniques (CMR perfusion or stress echo), technical issues prevent their use for 3D hybrid imaging. Both, CMR perfusion and stress echocardiography obtain two-dimensional datasets of myocardial perfusion which are very challenging to warp onto three-dimensional CT coronary angiograms. And even if this was possible the low interplane resolution of CMR (only three short axis slices of the entire left ventricle are obtained) is insufficient for hybrid imaging. Novel faster CMR perfusion sequences will soon become available which will allow to obtain a full 3D dataset of LV myocardial perfusion more suitable for fusion with CT coronary angiography (Manka R. Eur Heart J. 2011 Nov;32(21):2625), but until these sequences are widely implemented, nuclear/CT fusion remains the most practical and robust hybrid technique and the only one that has been tested in diagnostic studies.

4.

Reviewer: Overall, this study is a good proof-of-concept study that hybrid imaging may be better than CTCA or nuclear imaging alone, but the difficulty in conducting per-protocol imaging studies even in this multi-center research study, and the high radiation used for hybrid imaging suggest that hybrid imaging is unlikely to have a significant clinical impact in the current era.

Authors: Many thanks for this comment. We fully agree with this reviewer, that limitations exist however, we believe that with further technological developments (including the increased availability of hybrid devices, the efforts in lowering radiation from CT and radionclide imaging) hybrid imaging may play an ever increasing role in the future. Furthermore, studies like this one may set an example for applying hybrid imaging to other areas on cardiology (e.g. detection of infective endocarditis with PET/CT, use of hybrid imaging to determine responders of CRT, assessment of plaque structure and biology with hybid imaging, real-life hybrid imaging in structural heart disease interventions, etc.).

Abstract

Aims Hybrid imaging provides a non-invasive assessment of coronary anatomy and myocardial perfusion. We sought to evaluate the added clinical value of hybrid imaging in a multi-centre multi-vendor setting.

Methods and results Fourteen centres enrolled 252 patients with stable angina and intermediate (20-90%) pre-test likelihood of coronary artery disease (CAD) who underwent myocardial perfusion scintigraphy (MPS), CT coronary angiography (CTCA), and quantitative coronary angiography (QCA) with fractional flow reserve (FFR). Hybrid MPS/CTCA images were obtained by 3D image fusion. Blinded core-lab analyses were performed for CTCA, MPS, QCA and hybrid datasets. Hemodynamically significant CAD was ruled-in non-invasively in the presence of a matched finding (myocardial perfusion defect co-localized with stenosed coronary artery) and ruled-out with normal findings (both CTCA and MPS normal).

Overall prevalence of significant CAD on QCA (>70% stenosis or 30-70% with FFR≤0.80) was 37%. Of 1004 pathological myocardial segments on MPS, 246 (25%) were reclassified from their standard coronary distribution to another territory by hybrid imaging. In this respect, in 45/252 (18%) patients, hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 42% of the cases. Hybrid imaging allowed non-invasive CAD rule-out in 41%, and rule-in in 24% of patients, with a negative and positive predictive value of 88% and 87%, respectively.

Conclusions In patients at intermediate risk of CAD, hybrid imaging allows non-invasive co-localization of myocardial perfusion defects and subtending coronary arteries, impacting clinical decision-making in almost one every five subjects.

Multi-Center Multi-Device Hybrid Imaging Study of Coronary Artery Disease.

Results from the EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease (EVINCI) Hybrid Imaging population

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Abstract

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Overall prevalence of significant CAD on QCA (>70% stenosis or 30-70% with FFR≤0.80) was 37%. Of 1004 pathological myocardial segments on MPS, 246 (25%) were reclassified from their standard coronary distribution to another territory by hybrid imaging. In this respect, in 45/252 (18%) patients, hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 42% of the cases. Hybrid imaging allowed non-invasive CAD rule-out in 41%, and rule-in in 24% of patients, with a negative and positive predictive value of 88% and 87%, respectively.

Conclusions In patients at intermediate risk of CAD, hybrid imaging allows non-invasive co-localization of myocardial perfusion defects and subtending coronary arteries, impacting clinical decision-making in almost one every five subjects.

***Keywords*:** hybrid imaging**;** myocardial perfusion scintigraphy; CT coronary angiography; coronary artery disease

Introduction

The risk of patients with stable coronary artery disease (CAD) varies considerably based on the extent of anatomical involvement and of myocardial ischemia.[1] Unfortunately, there is disagreement between the angiographic severity of CAD and myocardial perfusion abnormalities.[2,3] Thus, current guidelines recommend a comprehensive anatomo-functional assessment to decide on the most appropriate treatment, with patients at low-risk treated conservatively, while high-risk patients are generally referred for more aggressive therapies.[1] Specifically, revascularization strategies should be guided by the presence of hemodynamically significant coronary stenosis, while non-significant coronary stenoses may be treated conservatively.[4,5]

Recently, hybrid cardiac imaging has emerged as a non-invasive way of assessing CAD by integration of myocardial perfusion images with individual coronary anatomy.[6] Small studies have suggested superior diagnostic accuracy compared with the separate imaging modalities,[7] whereas others have reported incremental prognostic value.[8] While the technique is finding increasing acceptance in clinical practice, questions remain over the clinical role of hybrid imaging. Furthermore, the impact of the technique has never been tested in a multi-centre, multi-device, real-world setting.

This study sought to assess the clinical role of hybrid cardiac imaging in a multi-centre study using different equipment and practice, and to explore its value for the diagnosis of hemodynamically significant CAD.

Methods

Study Design.

The EVINCI (EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease) study is a "European Commission 7th Framework Program for Research and Innovation" sponsored multi-modality imaging project in 14 centres from 9 European countries.[9] The characteristics of the study population have been already described in detail,[9] and are summarized in **Table**

1. Briefly, between March 2009 and June 2012, patients with symptoms suggestive of CAD and intermediate pre-test probability (20-90%)[10, 11] underwent a study of coronary anatomy by CT coronary angiography (CTCA) and at least one coronary functional imaging test by myocardial perfusion scintigraphy (MPS) (single-photon emission computed-tomography (SPECT) or positron emission tomography (PET) and/or wall motion imaging (stress echocardiography or cardiac magnetic resonance), with the recommendation to perform invasive coronary angiography (ICA) with fractional flow reserve (FFR) in intermediate lesions. Each patient was followed-up for 30 days and the referral for coronary revascularization recorded. Ethical approval was provided by each centre and all subjects gave written informed consent.

Image acquisition

Acquisition protocols were agreed on for each technique based on best available clinical practice. Individual core labs were responsible for harmonization and quality control of imaging protocols. Details on imaging procedures and protocols can be found in the EVINCI publication.[9] All EVINCI subjects in whom core-lab analyses of CTCA, MPS and ICA were available were selected for the present hybrid sub-study (**Figure 1**). Accordingly, patients submitted to wall motion imaging modalities were not included in the analysis, because their format precludes formation of 3D hybrid datasets with CTCA. No further exclusion criterion was considered.

Image Fusion

MPS and CTCA datasets were transferred to a dedicated hybrid core-lab blinded to clinical history and imaging findings (Cardiac Imaging, University Hospital Zurich, Switzerland). Image fusion of MPS and CTCA datasets was performed on a dedicated workstation (Advantage Workstation 4.4, GE Healthcare) using the CardIQ Fusion software package (GE Healthcare) as previously described.[12] In case of $H_2{}^{15}O$-PET images, parametric myocardial blood flow datasets, showing flows on a segmental level, were generated based on quantitative analysis performed using a commercially available software, (PMOD 3.6 software package. PMOD Technologies Ltd., Zurich, Switzerland).

Hybrid analysis was performed using an optimized alignment tool, allowing projection of the MPS image on the left ventricular epicardial surface obtained from the CTCA, allowing a panoramic view of the coronary artery tree projected onto the left ventricular myocardial perfusion territories. In all patients, the image fusion procedure (including image generation and reading) was performed by two independent and blinded operators. Disagreement with regard to allocation of myocardial perfusion defects was resolved by consensus reading.

Image interpretation and definitions

Image interpretation was performed in dedicated core-labs as follows:

CT coronary angiography

CTCA was assessed using a modified 16-segment system[13] and considered abnormal if at least one coronary segment had a diameter stenosis >50%. Significant left main stem stenosis were assigned to both left anterior descending (LAD) and left circumflex (LCX) coronary arteries. To limit any selection bias, any non-diagnostic segment was considered abnormal.

Myocardial perfusion scintigraphy

Perfusion in each of 17 segments[14] was visually classified as 0=normal, 1=mild reduction, 2=moderate reduction, 3=severe reduction or 4=absent perfusion, and the segmental scores were summed for the stress (SSS) and rest (SRS) images. ^{15}O-H_2O PET data were processed and parametric perfusion images were scored similarly. The difference between SSS and SRS was calculated as the summed difference score (SDS). On per patient analysis, a reversible perfusion defect (ischemia) was defined as a SDS ≥2, either from a score ≥1 in at least two contiguous segments or ≥2 in at least one segment. Myocardial scar was defined similarly as a SRS ≥2. Accordingly, MPS studies were considered pathological in the presence of significant myocardial ischemia and/or scar.

For per-vessel analysis, a reversible perfusion defect (ischemia) was defined as a territorial difference score ≥1, and a scar as a rest score ≥1. Each perfusion defect was assigned to one or more coronary territories

according to the standardized myocardial segmentation model[14]. Similarly to CTCA analysis, any non-diagnostic segment was considered abnormal.

Invasive coronary angiography

Coronary angiograms were subdivided using the previously mentioned segmentation model[13] and analysed using quantitative coronary angiography (QCA). A stenosis was considered hemodynamically significant if causing a >50% diameter reduction in the left main stem or >70% elsewhere, or between 30% and 70% with a FFR ≤0.80.

Hybrid images

All hybrid MPS/CTCA images were analysed by consensus of two independent readers with regard to the presence of matched, mismatched or normal findings. A matched finding was defined as a perfusion defect in a territory subtended by a stenotic coronary. All other combinations of pathological findings were classified as mismatched. In the absence of pathological findings on both CTCA and MPS hybrid images were considered normal. Finally, all pathological MPS segments were assigned to the pertinent vascular territory by spatial co-registration according to individual coronary anatomy by both operators to determine inter-observer agreement and repeatability of hybrid-based co-registration.

Statistical analysis

Statistical analysis was performed using the SPSS software. Continuous variables were expressed as mean±SD, and categorical variables as percentages. Numerical values were compared using the Mann-Whitney U test or Student's *t* test, and categorical values using the χ^2 test. Inter-observer agreement was assessed using Cohen's kappa statistic. Sensitivity, specificity, and accuracy were calculated for each imaging method (MPS, CTCA, and hybrid imaging) on a per-vessel and per-patient basis. The McNemar test was performed to compare the accuracy of the different imaging methods against QCA±FFR. A value of $P<0.05$ was considered significant.

Results

Patient population

A total of 252 patients underwent CTCA, MPS and ICA, and were included in the analysis (**Figure 1**). The characteristics of the study populations are shown in **Table 1**. Compared with the overall EVINCI population,[9] there were no significant differences in baseline characteristics except for a slightly higher CAD prevalence in our patient population (37% vs 30%, P=0.05) **(Supplementary table A).**

Interestingly, as in the case of the main EVINCI population, also in the present study traditional criteria for calculating pre-test probability[11] overestimated the prevalence of hemodynamically significant CAD, which was 37% at QCA±FFR. FFR was performed in 58/252 patients (23% of all patients and 66% of patients with intermediate coronary stenoses), and was abnormal (≤0.80) in 19 patients.

Imaging results: MPS and CTCA

One-hundred and eighty (71%) patients were submitted to SPECT while 72 (29%) underwent PET (**Table 2**). Overall, 104 (41%) patients presented myocardial perfusion abnormalities in one (8%), two (41%) or three (51%) vascular territories. At core-lab analysis, MPS images were judged of non-diagnostic quality (having at least 1 non-diagnostic segment) in 11 patients.

On CTCA, 111 (44%) patients presented significant CAD in one (48/111, 43%), two (41/111, 37%) or three (22/111, 20%) vessels (**Table 2**) with no significant difference between patients submitted to SPECT or PET. At core-lab analysis, CT images were judged of non-diagnostic quality (having at least 1 non-diagnostic segment) in 8 patients.

Hybrid imaging: feasibility and repeatability

In 18/270 (7%) patients originally submitted to CTCA and MPS, hybrid imaging could not be accomplished due to corruption of original data-sets (8 patients) or software incompatibility (10 patients).

Inter-rater agreement of hybrid-based co-registration was good (k=0.75 95% CI 0.70-0.80) with both observers agreeing in the classification of 92% of all pathological myocardial segments.

Hybrid Imaging: segment reclassification

A total of 4284 myocardial segments were analyzed, of which 1004 (23%) were pathological. According to the standard myocardial segmentation model, 397 (39%), 269 (27%), and 338 (34%) abnormal segments were allocated to the LAD, LCX and right coronary artery (RCA) vascular territory, respectively. After image fusion, 246 (25%) of the 1004 abnormal myocardial segments were reclassified from their standard coronary distribution to another territory **(Table 3**). Segment reclassification was highest for the standard LCX (49%) and RCA (32%) segments, while it was very low for standard LAD segments (2%; P <0.001 vs both LCX and RCA). **Figure 2** shows the proportion of pathological segments reassigned by hybrid imaging.

In 45/252 (18%) patients hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 19 cases (from a mismatched to a matched finding in 16 patients, and the opposite in 3). Interestingly, in 16 (84%) of those patients the myocardial perfusion abnormality was correctly assigned to a territory subtended by a hemodynamically significant stenosis at QCA±FFR. The role of hybrid analysis in the anatomo-functional characterization of patients and in identifying significant CAD is exemplified in **Figure 3**.

"Rule-in/rule-out" clinical algorithm

The diagnostic accuracy of hybrid imaging and of stand-alone imaging modalities in detecting significant CAD (QCA±FFR) is reported in **Figure 4**

Specifically, a matched finding at hybrid imaging was found in 61 patients (24%) while103 patients (41%) had normal hybrid findings. Of the remaining 88 patients with mismatched abnormal findings (35%), 45 presented a positive CTCA in the absence of perfusion abnormalities at MPS, while 39 showed a pathological MPS despite the absence of obstructive CAD at CTCA. Revascularization rates were 70% for matched hybrid images, 36% for mismatched findings, and 10% for normal findings (P<0.001). **(Figure 5)**.

Interestingly, among the 41 "false negative" hybrid studies (either normal or mismatched findings in the presence of significant CAD at QCA), the majority (80%) showed negative MPSs, despite a stenotic vessels on CTCA in 64% of the cases. FFR was performed in 17/41 patients and was positive in 13 (76%). (**Suppl. Table B**). On the other hand, the "false-positive" hybrid studies were almost exclusively associated

with the presence of intermediate coronary lesions (>30% and ≤70%) on QCA mainly in the absence of an invasive assessment of the hemodynamic relevance of stenoses by FFR (**Suppl. Table C**).

Radiation burden of the non-invasive imaging protocol

Average radiation doses in the study population were 7.9 mSv (range 0.6-24 mSv) for CTCA, 10.4 mSv (range 3.2-17.5 mSv) for SPECT, and 1.8 mSv (range 1.7-3.5 mSv) for PET. The average radiation dose of hybrid imaging was 9.4 mSv (range 5.2-21 mSv) for PET/CTCA and 18.5 mSv (range 6-31 mSv) for SPECT/CTCA (P<0.001).

Discussion

The EVINCI hybrid sub-study is one of the largest studies to assess the clinical value of non-invasive hybrid imaging in stable CAD. Several methodological advantages, including the use of dedicated blinded core-lab image analysis, the multicenter and multivendor design, and the use of an accepted invasive gold standard (QCA±FFR), distinguish it from previously published reports and provide greater uniformity and generalizability of its results. The main findings of the study are: (i) large variability of coronary anatomy leading to systematic errors of standardized myocardial segmentation in predicting culprit coronary vessels. (ii) Hybrid imaging (by 3D co-registration of CTCA and MPS) is feasible and reproducible. (iii) A hybrid anatomo-functional protocol allows non-invasive "rule-in and rule-out" of hemodynamically significant CAD.

Standardized myocardial segmentation models are widely used to assign myocardial territories to subtending coronary arteries.[14] However, coronary anatomy is highly variable, which may frequently lead to mistaken identification of culprit vessels by standard models. In this respect, it has been previously suggested that hybrid imaging may help in the individual co-localization of myocardial perfusion abnormalities and subtending coronary arteries.[15–18]

We identified systematic deviation from the standardized assignment of myocardial segments in 25% of pathological segments, localized almost exclusively in the standard LCX and RCA territories (i.e. the lateral and inferior myocardial wall). This turned out to be clinically significant in almost every fifth

patient, in whom the entire perfusion defect was reassigned to another coronary artery, changing the final diagnosis in almost half of them. This result might be of particular relevance in patients considered for revascularization, where only hemodynamically significant lesion deserve treatment. [5,19]

Previous reports have shown the feasibility and reproducibility of 3D fusion of anatomical (CTCA) and functional (MPS) imaging.[12] In this study, hybrid analysis was successfully performed in 93% of the EVINCI patients originally submitted to MPS and CTCA with good inter-observer repeatability, highlighting the robustness of the technique. In fact, technical image fusion failure occurred in only 7% of patients mainly in the case of early generation SPECT devices with incomplete or corrupted datasets or software incompatibility.

Given the heterogeneity of hybrid results (combining various anatomo-functional patterns) we considered that a binary diagnostic approach disregards the complexity of CAD. Conversely, a "rule-in/rule-out" hybrid-based approach appears more clinically meaningful, since matched positive findings allow rule-in of CAD and matched normal findings CAD rule-out (**Figure 5**). Accordingly, although in the EVINCI study the clinical management of patients, including the decision for coronary revascularization, was entirely left to the judgment of the local clinician, possibly introducing a bias in the analysis of the data, a matched positive hybrid finding was still associated with a high early revascularization rate (70%). On the other hand, in patients with a completely negative hybrid report the revascularization rate was extremely low (≈10%), making ICA theoretically superfluous. It should be emphasized that the majority of false negatives hybrid studies was due to negative MPS downstream a stenotic coronary vessel at CTCA which was confirmed by a >70% lumen diameter reduction at QCA (considered as hemodynamically significant). After the FAME study,[2] published almost at the end of the EVINCI study, coronary stenoses between 70% and 90% should be also submitted to FFR since a considerable proportion of these lesions have a normal FFR. On the other hand, the false positive hybrid imaging studies were essentially associated with the presence of intermediate coronary lesions (>30% and ≤70%) that did not undergo an invasive evaluation of their hemodynamic relevance through FFR and, thus, considered as not significant. It is conceivable that, if FFR would have been more extensively performed, the number of "false negative" and "false positives" results could have been considerably reduced. Interestingly, a consistent proportion of those patients were still submitted to coronary revascularization despite the absence of an objective proof of

myocardial ischemia (either by MPS or through FFR) (**Suppl. Table B and Suppl. Table C**), further highlighting the existing gap between evidence-based patient management[1, 3, 5, 19] and everyday clinical conduct.[20]

Patients with mismatched findings (positive MPS/negative CTCA or negative MPS/positive CTCA) represent a heterogeneous group. In the absence of coronary stenoses on CTCA, myocardial perfusion defects may represent either artefacts or microvascular/endothelial dysfunction. Accordingly, in this group CAD prevalence and revascularization rates were low (**Figure 5**). CTCA has a very high negative predictive value as demonstrated by a vast number of studies comparing it to the angiographical gold standard of ICA.[21] The fact that we used a more comprehensive anatomofunctional gold standard (ICA+FFR) may explain to some extent the low sensitivity. Moreover, the sensitivity of CTCA by core lab analysis in the main EVINCI trial was lower than by individual-center analysis.[9] As a result, some lesions may have been underestimated accounting for the small number of revascularizations in this group.

Conversely, patients with significant coronary stenoses on CTCA but absence of perfusion defects had a substantial CAD prevalence and revascularization rate (40 and 42%, respectively). This finding has several explanations. On one hand, the gold standard used in the present study was mainly anatomical (QCA), favouring agreement with CTCA rather than MPS. On the other hand, as already shown[22] the cut-off chosen for FFR ($\leq$0.80)[5,19] may overestimate the hemodynamic significance of CAD compared with non-invasive ischemia testing. In line with this evidence, among the 19 patients with a pathological FFR evidenced in this study, only 21% had a matched finding on hybrid imaging. Interestingly, only 12/19 (63%) of those lesions presented a FFR $\leq$0.75, as a more stringent cut-off for positivity[3]. However, the incomplete FFR penetration observed in the present study, mainly due to protocol violations, does not allow defining whether the use of a lower cut-off value of FFR would have better correlated with hybrid findings.

Such a "rule-in/rule-out" protocol is supported by follow-up data, indicating low event rates in patients with normal hybrid findings, high event rates for pathological matched findings, and intermediate event rates with mismatched findings.[8] Moreover, in selected cases, our integrated protocol may overcome the limitations of the more simplistic binary (i.e. either functional or anatomic) approach usually applied to CAD diagnostics, as recently reported.[23]

Limitations

Like the overall EVINCI population, our study had a significant drop-out rate, as not every patient underwent all protocol-specified imaging studies. Additionally, data corruption and incomplete datasets accounted for further drop-outs. Accordingly, 252 of the 697 patients originally enrolled in the EVINCI study were included in the present sub-study. However, those represented all the EVINCI patients that underwent MPS, CTCA and ICA and in whom, thus, hybrid analysis could be practically performed. In fact, only a marginal portion of those patients (7%) was excluded because of technical reasons, confirming the overall robustness of 3D image fusion. Moreover, since the demographical, clinical and angiographic characteristics of the present patients were almost superimposable to those of the main EVINCI population,[9] the presence of a significant selection bias can be excluded (**Supplementary table A**). Second, no long-term follow-up data was obtained precluding any analysis on the impact of hybrid imaging on downstream patient management and outcomes. Third, FFR rate was only 23%, and 34% of patients with intermediate lesions were not interrogated with FFR. Incomplete FFR penetration due to frequent protocol violations highlights the sub-optimal FFR use across Europe and may have been responsible for some of the "false negative" hybrid findings and prevents any conclusive analysis on the "false positive" studies (**Suppl. Table B and C)**. In our study, the respective sensitivities of CTCA and MPS were lower than anticipated from small single-centre studies (particularly for CTCA: 78%). This may be explained by selecting higher risk patients who had additional MPS performed, as well as by the inclusion of patients with intermediate stenosis (30-70%) without invasive functional evaluation, and by the exclusive use of independent core-lab data for the present analysis. In fact, the accuracies of standalone imaging modalities reported were almost superimposable to those of the overall EVINCI study when only core-lab data were considered.[9] Notably, on centre-based analysis the diagnostic accuracy of the different non-invasive imaging modalities was generally improved compared to the core-lab data. Nevertheless, even when only individual centre-data were considered, hybrid imaging maintained significantly elevated specificity and overall diagnostic accuracy, both at per-patient and vessel-based analysis (**Suppl. Figure 1**).

Moreover, in the accuracy analyses, MPS was considered pathological in the presence of ischemia and/or scar. Interestingly, the presence of a matched hybrid finding showed comparable sensitivity,

specificity and accuracy if myocardial ischemia (and not scar) was considered as the only positivity criteria (50%, 96%, and 79%, respectively).

Finally, the added radiation exposure from hybrid protocols must be also considered. In the present study, average radiation doses varied considerably, depending on the imaging technique (PET vs SPECT) and on the acquisition protocol employed. Specifically, the theoretical risk related to the radiation exposure of a SPECT/CTCA hybrid protocol may appear rather high, particularly if compared to PET/CTCA imaging or other non-invasive imaging modalities.[24] However, previous results suggest that the use of modern equipment and dose-optimization protocols (e.g. prospective ECG-triggering for CTCA, stress-only for SPECT) may consistently reduce the radiation burden of hybrid imaging,[25] favouring its clinical application on a larger scale. Nevertheless, further long-term comparative studies are probably needed to conclusively define the cost-efficiency and quantitate the added radiation hazard that may be related to hybrid imaging, and to definitively assess its possible prognostic impact.

Conclusions

Hybrid imaging allows more reliable co-localization of myocardial perfusion defects with subtending coronary arteries than standardized myocardial segmentation models accounting for variations in individual coronary anatomy. In two-thirds of patients at intermediate pre-test probability of CAD, hybrid imaging may offer a non-invasive "rule-in or rule-out" of patients with hemodynamically significant CAD.

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Conflict of Interest

None declared'

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Figure Legends

Figure 1 Patient flow chart. Coronary CT angiography (CTCA); invasive coronary angiography (ICA); fractional flow reserve (FFR); myocardial perfusion imaging (MPS)

Figure 2 (A) Standardized myocardial segmentation model used in this study with number codes for each segment (see Table 3) (13). (B) Reassignment rates by hybrid imaging for the 1004 pathological segments (the intensity of colours in each segment indicates the frequency of reassignment of that segment when pathological). (C) Pie chart indicating proportion of reassignment and reassignment fate for pathological segments in each standard coronary territory. Shades of red indicate standard LAD, of green standard LCX, of blue standard RCA territories. Standard LCX segments were most often reassigned to LAD (36%), while standard RCA segments where equally distributed between LAD and LCX.

Figure 3 A 55-year old gentleman with atypical chest pain. (A) SPECT shows a reversible perfusion defect inferiorly with lateral extension, and in addition, there is a separate reversible perfusion defect involving the the apical region and the midventricular anteroseptal wall. (B) The perfusion polar maps show the SPECT core lab interpretation (white=normal, yellow=mildly reduced, orange=moderately reduced, and red=severely reduced radiotracer uptake) with pathological segments assigned to all three coronary territories. (C) CTCA reveals two 70-90% mid LAD stenoses, a 50% proximal LCX stenosis, and a probable occlusion of the mid RCA (arrows). (D) On hybrid imaging, the entire inferolateral perfusion defect is reassigned to the RCA, effectively changing the diagnosis from 3-vessel to 2-vessel disease. (E) Imaging findings were confirmed on QCA showing two high-grade lesions in the mid LAD, diffuse non-significant disease in the LCX, and a chronic total occlusion of the mid RCA.

Figure 4 Accuracy analysis of stand-alone and hybrid protocols for the diagnosis of significant CAD (by QCA±FFR) on per-vessel (A) and per-patient (B) analysis. On a per-vessel basis, when positivity was defined by the presence of at least one positive test (either matched or mismatched findings), hybrid imaging had higher sensitivity than single modalities ($P<0.001$ vs MPS and CTCA), at the price of lower

specificity (P<0.001 vs both MPS and CTCA) and accuracy (P<0.001 vs both MPS and CTCA). When only matched findings were considered positive, hybrid imaging increased accuracy (P<0.001 vs both MPS and CTCA) driven by higher specificity (P<0.001 vs both MPS and CTCA) but with lower sensitivity (P<0.001 vs MPS and CTCA).

Figure 5 Hybrid-based "rule in/rule out" clinical protocol.

Supplementary Figure 1 Accuracy analysis of stand-alone and hybrid protocols for the diagnosis of significant CAD (by QCA±FFR) on per-vessel (A) and per-patient (B) analysis using individual centres data. On a per-vessel basis, when positivity was defined by the presence of at least one positive test (either matched or mismatched findings), hybrid imaging had higher sensitivity than single modalities (P<0.001 vs MPS and CTCA), at the price of lower specificity and accuracy. When only matched findings were considered positive, hybrid imaging increased accuracy driven by higher specificity (P<0.001 vs both MPS and CTCA) but with lower sensitivity.

Table 1

Table 1. Patients' baseline characteristics

Parameter	Overall Population (n=252)
Demographics, n (%)	
Age, years (mean±SD)	61±9
Male gender	161 (64)
Clinical characteristics, n (%)	
Typical angina	62 (25)
Atypical angina	148 (59)
Non-anginal chest pain	42 (17)
Pre-test probability of CAD *	59±23
Left ventricular ejection fraction	59±9
Cardiovascular risk factors, n (%)	
Family history of CAD	75 (30)
Diabetes mellitus	68 (27)
Hypercholesterolemia	161 (64)
Hypertension	155 (62)
Smoking	60 (24)
Obesity	72 (29)
Invasive coronary angiography data, n (%)	
Normal coronaries or non-obstructive CAD	158 (63)
Single-vessel disease	60 (23)
Multi-vessel disease	34 (14)
Myocardial perfusion imaging, n (%)	
Single-photon emission computed tomography	180 (71)
• ^{99m}Tc-Sestamibi	103 (57)
• ^{99m}Tc-Tetrofosmin	77 (43)
Positron emission tomography	72 (29)
• ^{15}O-Water	63 (88)
• ^{13}N-Ammonia	8 (11)
• ^{82}Rubidium	1 (1)

Data is given in absolute numbers and percentages (%), unless otherwise stated; CAD denotes coronary artery disease

* pre-test probability was calculated based on Ref. 10

Table 2. Noninvasive imaging data

Parameter	Overall Population (n=252)	SPECT (n=180)	PET (n=72)	P value
Myocardial perfusion imaging data				
Normal perfusion	148 (59)	111 (62)	37 (58)	0.175
Scar	41 (16)	35 (19)	6 (8)	0.037
Inducible ischemia	88 (35)	54 (30)	34 (47)	0.013
Computed tomography data				0.599
One-vessel disease	48 (19)	38 (21)	10 (14)	
Two-vessel disease	41 (16)	29 (16)	12 (17)	
Three-vessel disease	22 (9)	15 (8)	7 (10)	
Hybrid imaging				0.054
Hybrid Match	61 (24)	39 (22)	22 (31)	
Hybrid Mismatch	88 (35)	68 (38)	20 (28)	
MPS positive and CT negative	39 (15)	26 (14)	13 (18)	
MPS negative and CT positive	49 (19)	42 (23)	7 (10)	
Normal Hybrid	103 (41)	73 (41)	30 (42)	

Data is given as numbers and percentages, n (%); MPS denotes myocardial perfusion imaging

Table 3. Hybrid-based reclassification of myocardial perfusion abnormalities

Standard coronary distribution	Myocardial Segments (17 segments LV model)*	Perfusion abnormality, n	Abnormal segment reclassified, n (%)	To LAD n (%)	To LCX n (%)	To RCA n (%)
LAD	Segment 1	50	0 (0)	-	0 (0)	0 (0)
	Segment 2	51	1 (2)	-	0 (0)	1 (100)
	Segment 7	56	0 (0)	-	0 (0)	0 (0)
	Segment 8	48	0 (0)	-	0 (0)	0 (0)
	Segment 13	62	0 (0)	-	0 (0)	0 (0)
	Segment 14	51	1 (2)	-	0 (0)	1 (100)
	Segment 17	79	6 (8)	-	2 (33)	4 (67)
LCX	Segment 5	72	25 (35)	0 (0)	-	25 (100)
	Segment 6	43	20 (47)	19 (95)	-	1 (5)
	Segment 11	58	20 (34)	17 (85)	-	3 (15)
	Segment 12	44	31 (70)	30 (97)	-	1 (3)
	Segment 16	52	35 (67)	30 (86)	-	5 (14)
RCA	Segment 3	55	13 (24)	10 (77)	3 (23)	-
	Segment 4	82	15 (18)	0 (0)	15 (100)	-
	Segment 9	52	15 (29)	12 (80)	3 (20)	-
	Segment 10	76	15 (20)	0 (0)	15 (100)	-
	Segment 15	73	49 (67)	42 (86)	7 (14)	-

LV denotes left ventricle; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery

* For exact location of perfusion segment within the LV see **figure 2A**.

Figure 1

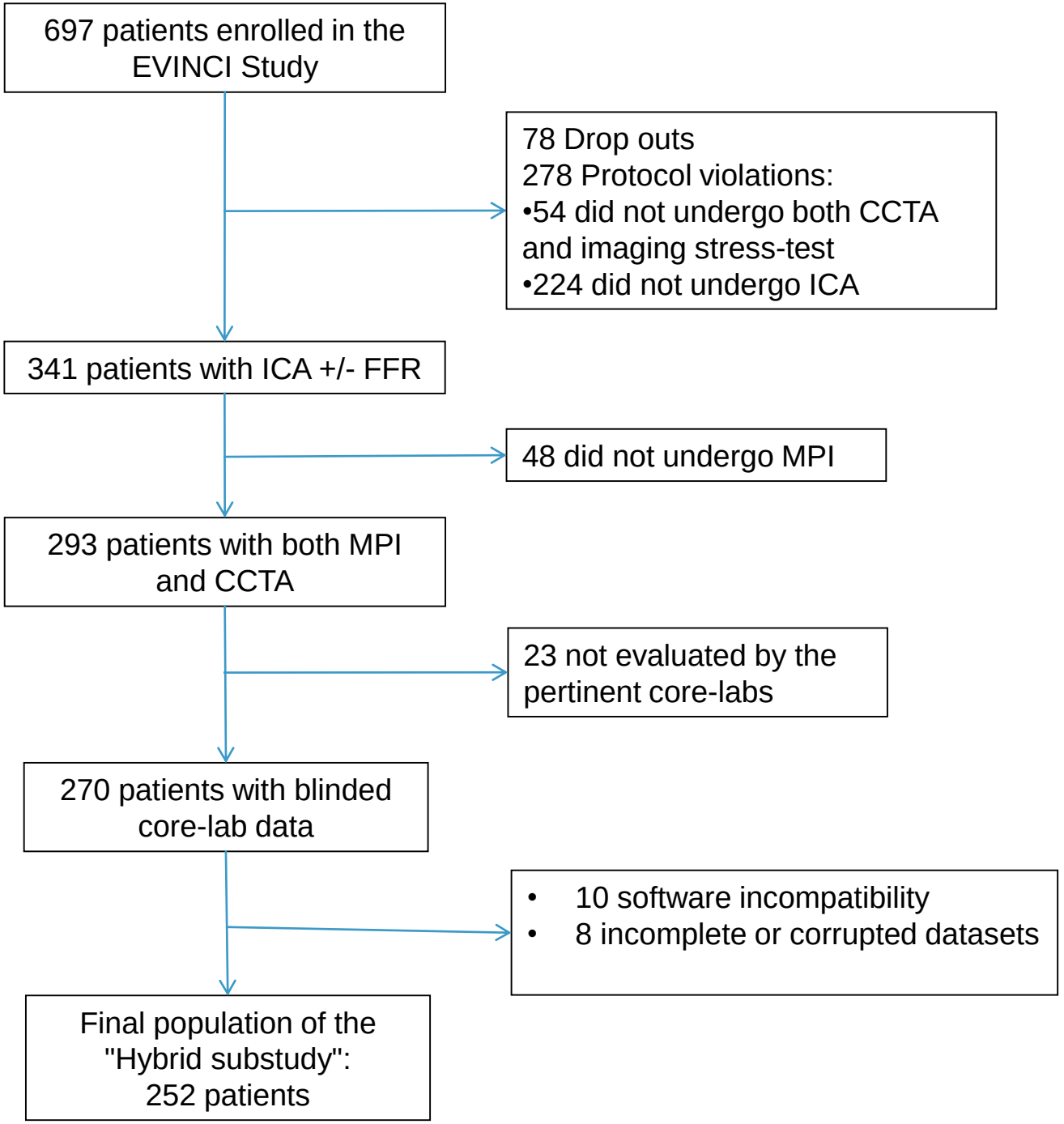
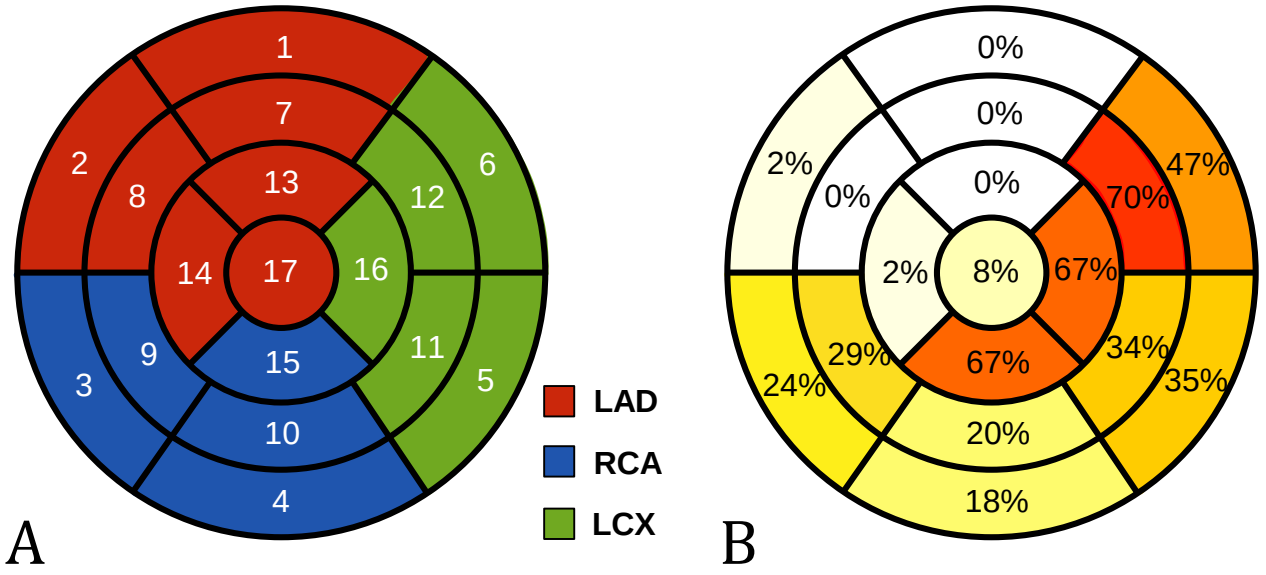


Figure 1

Figure 2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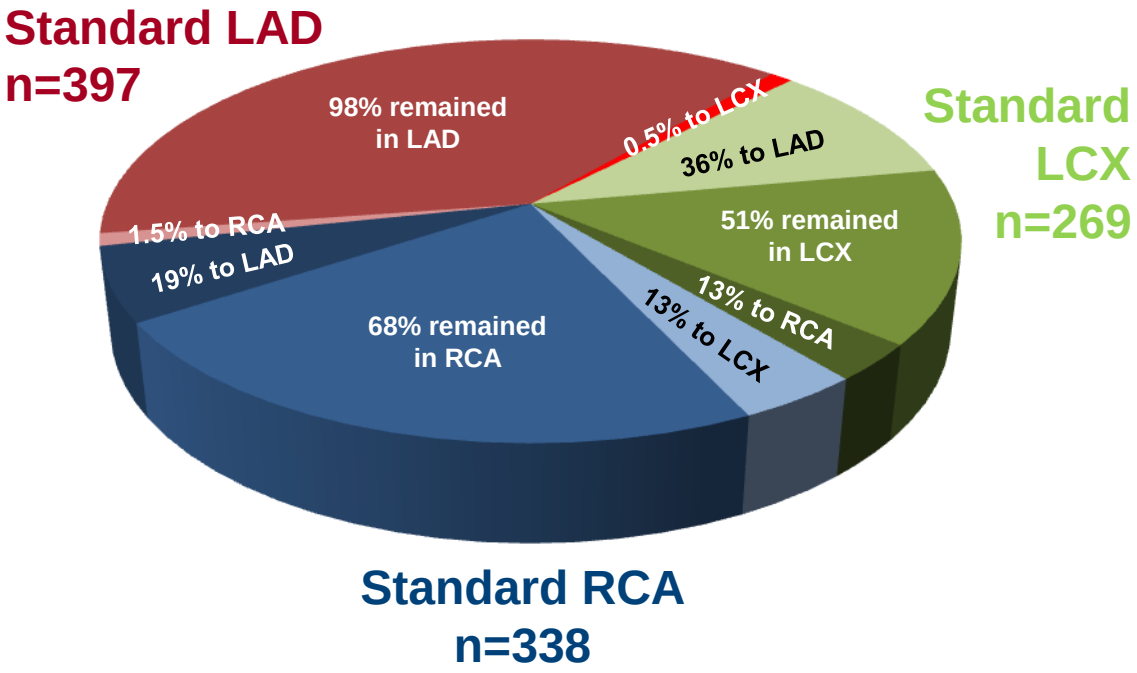

Standard LAD
n=397
98% remained
in LAD
0.5% to LCX
36% to LAD
1.5% to RCA
19% to LAD
68% remained
in RCA
Standard
LCX
n=269
51% remained
in LCX
13% to RCA
13% to LCX
C
Standard RCA
n=338

Figure 3

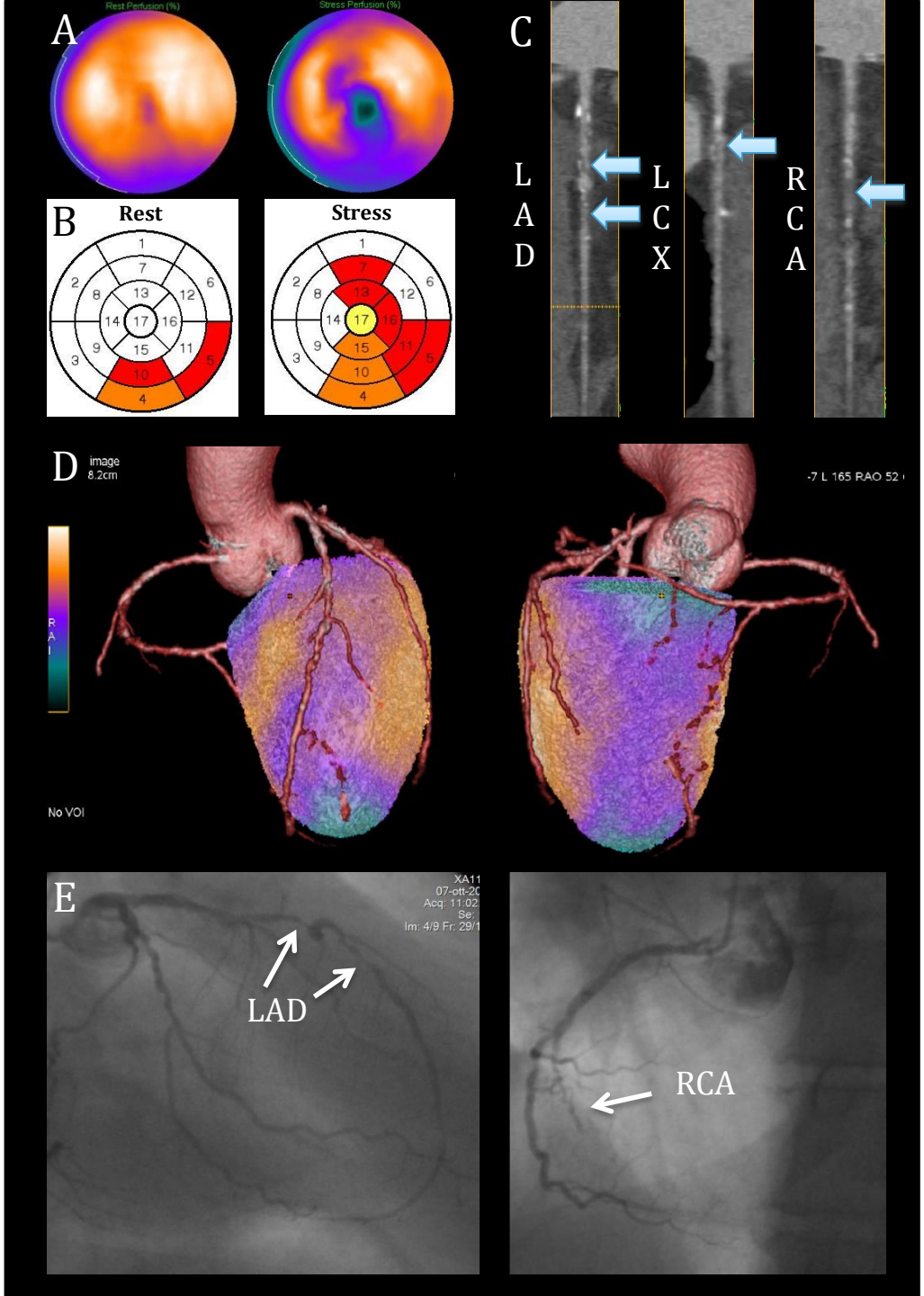
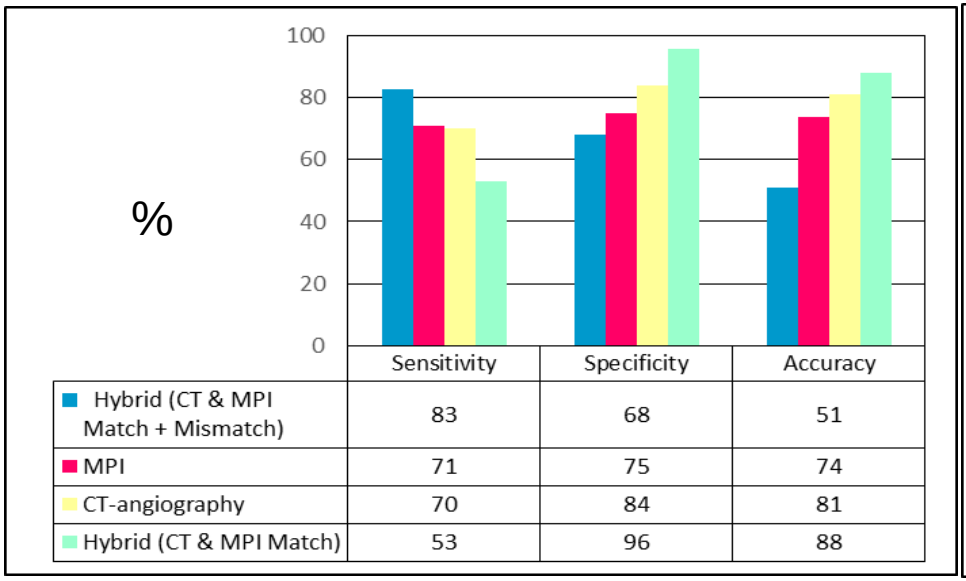


Figure 3.

Figure 4

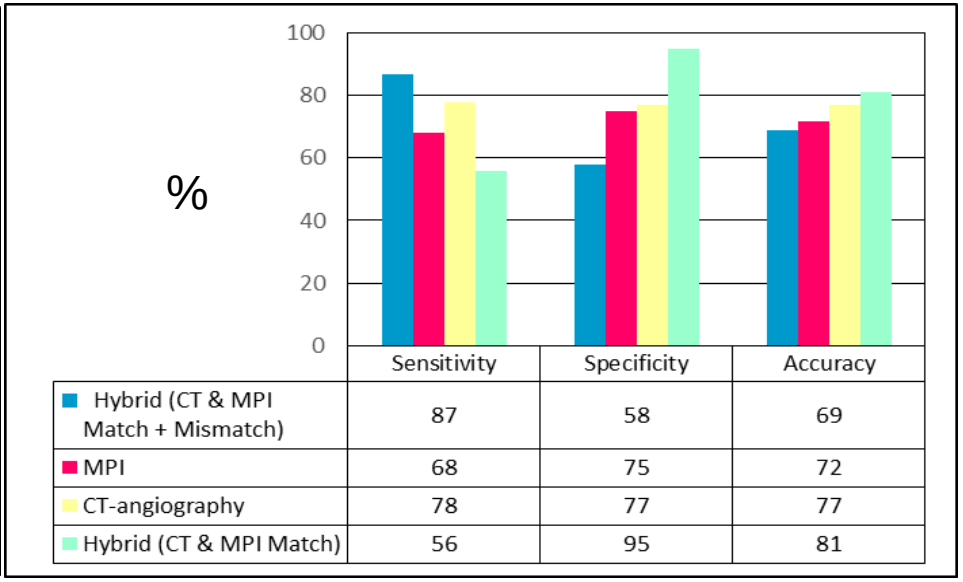
Accuracy in detecting significant CAD (QCA±FFR)

A. Per-vessel analysis



	Sensitivity	Specificity	Accuracy
Hybrid (CT & MPI Match + Mismatch)	83	68	51
MPI	71	75	74
CT-angiography	70	84	81
Hybrid (CT & MPI Match)	53	96	88

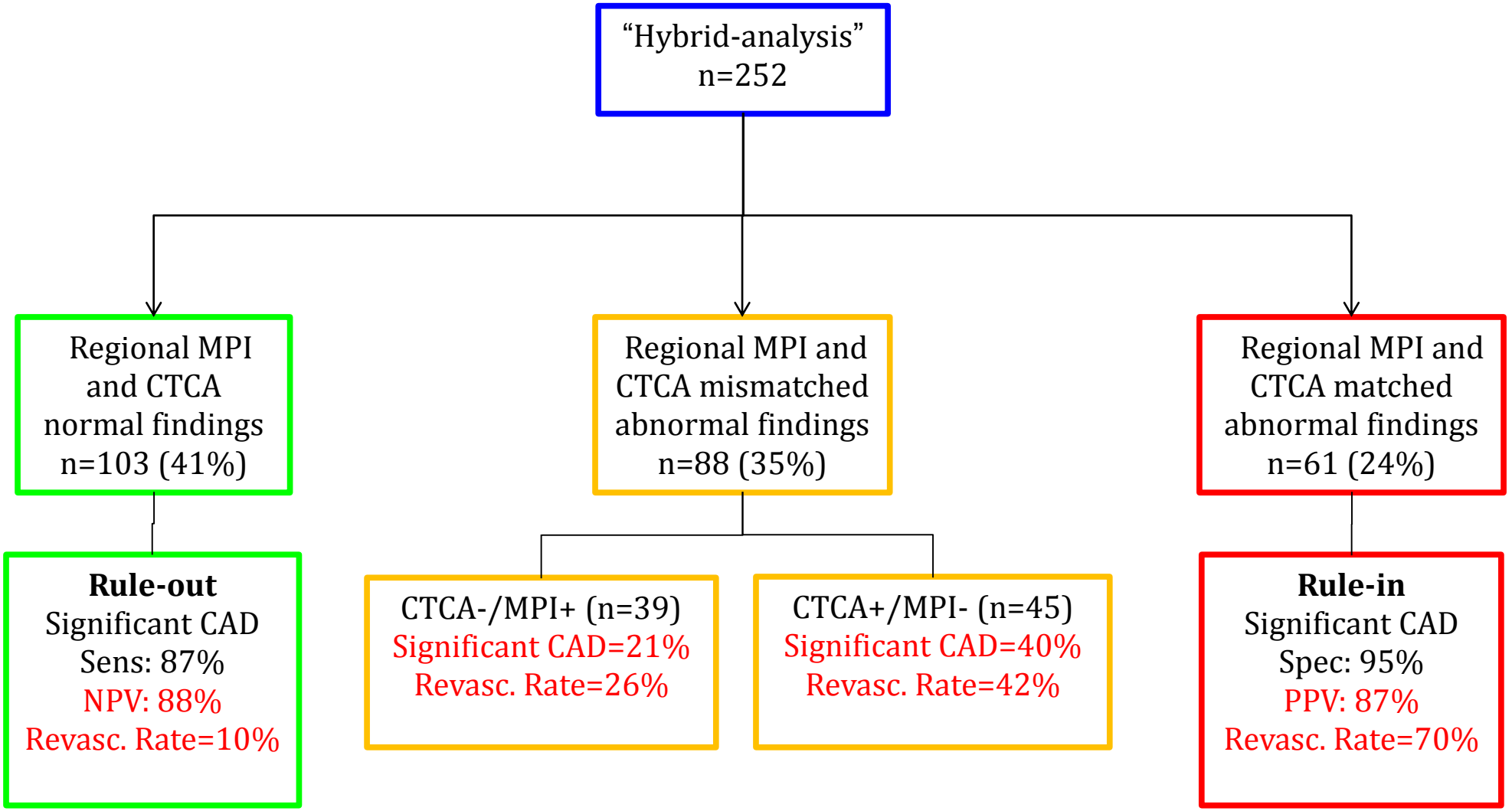
B. Per-patient analysis



	Sensitivity	Specificity	Accuracy
Hybrid (CT & MPI Match + Mismatch)	87	58	69
MPI	68	75	72
CT-angiography	78	77	77
Hybrid (CT & MPI Match)	56	95	81

Figure 4

Figure 5


"Hybrid-analysis"
n=252
Regional MPI and CTCA normal findings
n=103 (41%)
Regional MPI and CTCA mismatched abnormal findings
n=88 (35%)
Regional MPI and CTCA matched abnormal findings
n=61 (24%)
Rule-out
Significant CAD
Sens: 87%
NPV: 88%
Revasc. Rate=10%
CTCA-/MPI+ (n=39)
Significant CAD=21%
Revasc. Rate=26%
CTCA+/MPI- (n=45)
Significant CAD=40%
Revasc. Rate=42%
Rule-in
Significant CAD
Spec: 95%
PPV: 87%
Revasc. Rate=70%

Supplemental material tables

Supplemental material figure