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Derivation of a novel angiography-based method to assess coronary microvascular dysfunction in patients with acute myocardial infarction

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Coordinator: Prof. Giuseppe Faggian

Tutor: Prof. Flavio Luciano Ribichini

Doctoral Student: Dr. Roberto Scarsini

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Dott. Roberto Scarsini

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ABBREVIATIONS

AAR: area at risk
AUC: area under the curve
CAD: coronary artery disease
CFR: coronary flow reserve
CMR: cardiovascular magnetic resonance imaging
cTFC: corrected TIMI frame count
DES: drug eluting stent
DS%: percentage diameter stenosis
EDV: end diastolic volume
EF: ejection fraction
eGFR: estimated glomerular filtration rate
ESV: end systolic volume
FFR: fractional flow reserve
GPIIbIIIa: glycoprotein IIbIIIa
IMR: index of microcirculatory resistance
IQR: interquartile range
IRA: infarct related artery
IS: infarct size
IS%: percentage of infarct size
LAD: left anterior descending
LCx: left circumflex
LV: left ventricle
MLD: minimal lumen diameter
MVO: microvascular obstruction
MVO%: percentage of microvascular obstruction
OXAMI: Oxford Acute Myocardial Infarction
Pa: aortic pressure
PCI: percutaneous coronary intervention
Pd: distal pressure
PICSO: pressure-controlled intermittent coronary sinus occlusion
PPCI: primary percutaneous coronary intervention
QCA: quantitative coronary angiography
RCA: right coronary artery
STEMI: ST elevation myocardial infarction
TIMI: thrombolysis in myocardial infarction
T2W: T2 weighted

Summary

ST-segment elevation myocardial infarction (STEMI) is still associated with a 10% one-year mortality and up to 25% risk of heart failure. The pressure-wire index of microcirculatory resistance (IMR) may have an important role in the assessment of the downstream microcirculatory function of the IRA, providing prognostically relevant information and identifying patients at risk of suboptimal reperfusion who are eligible for additional novel therapies. However, the penetration of IMR in the clinical practice is still limited mainly because of the technical complexity of the procedure and increased costs and procedural time. Nevertheless, the implementation of a risk stratification using coronary physiology in patients with STEMI would be highly desirable to further improve the clinical outcomes.

In this PhD thesis we aimed to assess the long-term prognostic implications of CMD investigated using IMR. Furthermore, we aim to develop alternative methods to simplify the assessment of CMD in the catheterization laboratory and increase the penetration of physiology in the clinical practice. The current thesis consists of five main chapters. In Chapter one we explored the long-term clinical outcome of patients with STEMI stratified according to IMR and cardiovascular magnetic resonance imaging (CMR) in the cohort of the OxAMI Study. Importantly, CMD defined by IMR>40 U or by MVO demonstrated a more than 4-fold increase in mortality, heart failure or cardiac arrest at a median follow-up of 40 months.

In Chapter two, pressure-bounded coronary flow reserve (pb-CFR), an index derived using standard pressure-wire technology was compared with IMR and CFR in predicting microvascular obstruction and the extent of the infarct size at CMR imaging. Pb-CFR provided a fair prognostic stratification identifying a subgroup of patients with satisfactory myocardial reperfusion after PPCI. Nonetheless, the prognostic value of pb-CFR was inferior compared with IMR. Chapter three reports the derivation of an angiography-derived pressure-wire free index of microcirculatory resistance (IMR_{angio}). IMR_{angio} has been developed to overcome some of the limitations of IMR, using the Quantitative Flow Ratio (QFR) algorithm to obtain Pd and contrast frame count to estimate coronary flow. IMR_{angio} demonstrated to be significantly correlated with invasive IMR in a prospective

cohort of patients with STEMI. Importantly, IMR_{angio} was also correlated with the presence of MVO at CMR.

In Chapter four, IMR_{angio} was assessed in a prospective cohort of patients across the spectrum of acute and chronic coronary syndromes. Interestingly, IMR_{angio} was well-correlated with IMR not only in STEMI but also in patients with NSTEMI e stable coronary syndromes. Moreover, we observed that IMR_{angio} measured in non-hyperemic conditions (NH-IMR_{angio}) provided good diagnostic performance in the subgroup of patients with STEMI.

Chapter five reports on the long-term prognostic implications of patients with STEMI stratified according to NH-IMR_{angio} in a retrospective analysis of the OxAMI Study. Notably, NH IMR_{angio} demonstrated a prognostic value equivalent to invasively measured IMR.

In conclusion, CMD has important prognostic implications at long-term after STEMI. IMR_{angio} has the potential to guide additional novel additional therapies in patients undergoing PPCI. Abolishing the need for pressure-wire, IMR_{angio} may increase the penetration of CMD assessment in the catheterization laboratory and physiology-guided additional therapies. Further additional data are needed to explore the role of IMR_{angio} as a routine addition to diagnostic and interventional procedures in STEMI patients.

INTRODUCTION

Coronary microvascular dysfunction in patients with STEMI

Prompt patency restoration of the occluded coronary epicardial vessel is a cornerstone of the modern management of patients presenting with ST-segment elevation myocardial infarction (STEMI)[1]. Thrombolysis first, and then primary percutaneous coronary intervention (PPCI) reduced dramatically the early mortality after STEMI. However, despite a satisfactory angiographic result achieved in more than 90% of the cases, heart failure (HF) and cardiac death still limit the long-term survival after STEMI. Importantly, in the last 15 years, mortality after STEMI reached a plateau with no further improvement despite an impressive reduction of the door-to-balloon time[2].

The discrepancy between successful PPCI and poor clinical outcome may be explained by suboptimal myocardial reperfusion that occurs in up to 40% of the patients and may be easily misrecognized using standard clinical and angiographic assessment[3].

The extent of infarct size is ultimately responsible for the risk of mortality and HF after STEMI. Notably, every 5% increase in infarct size contribute to a 20% increase in the relative hazard for mortality or hospitalization for HF within 1 year after STEMI[4]. Time delay and microvascular obstruction (MVO) at cardiovascular magnetic resonance imaging (CMR) are the major determinants of infarct size. Every 1% increase in MVO extent is associated with 14% relatively increase in mortality and 8% increase in HF within 1 year after STEMI[5].

The main determinant of poor myocardial perfusion despite patent epicardial arteries in STEMI is the occurrence of coronary microvascular dysfunction (CMD). The pathogenesis of CMD in STEMI is a multifactorial and dynamic process involving: distal embolization and mechanical small vessel occlusion; endothelial and smooth muscle cells dysfunction; ischemia-reperfusion injury[6].

Pathophysiology of CMD in STEMI

Within less than an hour of ischemia in territory of the IRA, oedema develops from structural alterations of cardiomyocytes, resulting in cardiomyocyte death after the

first 3 hours. PCI is able to restore coronary blood flow in the IRA but may have also detrimental effects on the microcirculation causing dislodgement of atherothrombotic debris and distal embolization[3]. Despite endothelial cells are more resilient to ischemia compared to cardiomyocytes, eventually prolonged ischemia results in endothelial dysfunction. As a consequence, capillary permeability is initially increased with oedema formation. Furthermore, endothelial dysfunction leads to impaired vasomotion, stasis and release of deleterious substances such as vasoconstrictors, inflammatory cytokines and reactive oxygen species. These substances co-exist with atherosclerotic debris that lead to MVO and combined, cause destruction of the capillaries and haemorrhage[7, 8].

It is well established that intramyocardial haemorrhage (IMH) and MVO are closely associated. However, IMH probably reflects a more irreversible myocardial damage compared with MVO that has been observed to shrink and disappear at follow up. MVO assessed by CMR, is an independent predictor of worse outcome irrespective of infarct size, while patients with larger MVO more commonly develop heart failure post-ACS leading to an increase in mortality**.**

Ischemia and reperfusion injury contribute to CMD by formation of neutrophil-platelet aggregates that obliterate the vessel lumen and enhance the production of vasoconstrictors and inflammatory mediators. Immediately after reperfusion, swelling and disruption of capillaries endothelium integrity cause blood extravasation in the interstitial space and intramyocardial haemorrhage[6]. This phenomenon causes external compression of the microvasculature and further oedema formation.

The functional or structural obliteration of coronary microcirculation in the watershed zones adjacent to the infarcted area limits the healing process and promote myocardial necrosis. As a result, in patients with CMD, infarct size is larger, myocardial salvage is reduced and left ventricle ejection fraction is impaired[7].

Temporal changes of coronary physiology in the infarct-related artery

Cuculi et al. assessed the changes in coronary physiology change over time after STEMI[9]. In that study, 43 STEMI patients underwent repeated IRA physiological

assessment at the time of the PPCI, at day 1 and at 6 months of follow-up. Notably, the resting coronary flow, estimated via thermodilution, did not change over time after STEMI. Conversely, the hyperaemic coronary flow increased significantly at follow-up (CFR 1.8±0.9 vs 3.1±1.1, p<0.001). Consistently, the index of microcirculatory resistance (IMR) decreased progressively after STEMI, being 37.0±22.3 after PPCI, 30.6±21.4 at day 1 and 24.0±22.0 at 6 months (p=0.002).

Interestingly, the epicardial coronary physiology in the IRA showed significant variations over time as well. In particular, FFR decreased from 0.93±0.06 after PPCI to 0.92±0.06 at day 1 and 0.89±0.06 at 6 months (p<0.001). On the contrary, resting coronary physiology estimated by baseline Pd/Pa did not change significantly over time (after PPCI: 0.96±0.04; day 1: 0.95±0.05; 6 months: 0.96±0.04. p=0.22).

Notably, FFR variations over time were significant in patient with evidence of MVO at CMR (0.94±0.04 vs 0.88±0.06, p=0.006) but not in patients without MVO (0.94±0.05 vs 0.93±0.04, p=0.21).

These interesting findings suggest that coronary microcirculation generally recovers after STEMI in the IRA and tend to normalize at 6 months after STEMI.

The hyperemic response to adenosine is blunted in the IRA especially in patients with evidence of MVO. Therefore, the reliability of FFR in the acute phase of STEMI is questionable in the IRA territory.

How to assess CMD in the catheterization laboratory

Role of conventional angiographic scores

Angiographic-based techniques provide an immediate overview of coronary microvascular status expressed as no-reflow and defined as zero, partial or delayed anterograde coronary flow. These indices are limited by their low sensitivity for CMD and by their low inter and intra-observed reproducibility. Nevertheless, most of the clinical and procedural decisions in the catheterization laboratory are still based on angiographic assessment alone and, moreover, it represents a main inclusion criterion for clinical trials aiming to reduce CMD and infarct size in STEMI (e.g. PICSO-AMI I trial [NCT03625869])

The *Thrombolysis in Myocardial Infarction* (TIMI) flow is the most commonly used angiographic scoring system to grade antegrade coronary flow. TIMI ≤2 flow is associated with worse clinical outcome in patients with STEMI compared with TIMI 3 flow[10]. However, TIMI flow is a suboptimal measure of microvascular function. In fact, nearly 60% of patients with TIMI 3 flow at completion of PPCI demonstrated MVO at CMR imaging[11].

The *corrected TIMI frame count* (CTFC) has been introduced to increase the reproducibility of TIMI flow assessment providing a quantitative estimation of coronary flow. CTCF is obtained counting the number of angiographic frames required for contrast to reach a distal landmark. CTCF can be used to assess CMD but its association with prognosis is still unclear[11].

The *myocardial blush grade (MBG)* is a more specific measure of CMD compared with TIMI flow and it is associated with survival[12]. MBG is estimated as the myocardial contrast blush after injection, grading from no myocardial contrast density (MBG=0) to normal myocardial blush, comparable to the contralateral non-infarct related artery (MBG=3). However, similar to what observed for TIMI flow, a normal MBG do not exclude coronary microvascular and MVO at CMR imaging is often observed in patients with MBG ≥2[11]. Other angiographic indices of coronary microvascular dysfunction are reported in Table 1.

Role of invasive coronary physiology

A number of coronary physiology measures has been proposed to assess coronary microvascular function in patients with STEMI (Table 1), including CFR, IMR, hyperaemic microvascular resistance (HMR), resistive reserve ratio (RRR), pressure-bounded CFR (pb-CFR), and zero-flow pressure (Pzf). Table 1 summarises the characteristics of physiology indices potentially usable to assess CMD in STEMI and guide novel therapies.

IMR is the preferred method for the assessment of CMD in the catheterization laboratory.

It is derived using pressure-and-thermodilution wire and it is calculated as distal coronary pressure (Pd) multiplied with the hyperaemic transit time (Tmn). IMR is specific for the coronary microcirculation and less influenced by presence of

epicardial disease and by the baseline hemodynamic conditions compared with CFR[13]. Moreover, being based on thermodilution transit time and pressure, obtaining reliable IMR measurements is feasible during primary PCI and less challenging compared with Doppler velocity-derived hyperaemic myocardia resistance (HMR) index.

IMR has been validated against CMR imaging and hard clinical outcome. In particular, IMR demonstrated superior and independent prognostic value compared with CFR in detecting CMD[14, 15]. In the setting of STEMI, the threshold of IMR >40 U is associated with severe CMD and adverse clinical outcome. Patients with IMR >40 U at completion of primary PCI have more frequently evidence of MVO and larger infarct size at 6 months[16]. Moreover, De Maria GL and colleagues demonstrated that measuring IMR immediately after flow restoration and before stent implantation is feasible in the acute setting of STEMI. Pre-stenting IMR predicts post procedural microvascular injury and larger infarct size with high accuracy[3]. Possible limitations to the penetration of IMR in the clinical practice include the pressure-wire manipulation in the setting of a thrombus-containing infarct related artery, extra procedural time and perceived technical complexity for operators who do not perform physiology studies routinely.

Prognostic implications of CMD

An increasing body of evidence provides insights on the prognostic value of invasive physiology assessed at the time of PPCI in regards of acute and final infarct size, MVO and residual systolic function and clinical outcome after STEMI. IMR at completion of PPCI has been associated with the extent of MVO (rho=0.29, p=0.002) and infarct size in the subacute phase post-STEMI (rho=0.21, p=0.03) and at 6 months follow-up (rho=0.43, p=0.001)[7].

Moreover, patients with IMR >40 U have a >2-fold higher risk of mortality and heart failure at 12 months after STEMI (HR=2.1, 95%CI 1.1-4.1, p=0.03)[16]. Moreover, IMR ≥40 U has demonstrated an excellent performance to predict major in-hospital cardiac complications post-PPCI (AUC=0.90, 95% CI: 0.85-0.93)[17].

A preserved vasodilatory capacity, reflecting an intact and functional coronary microvasculature, is an important predictor of myocardial functional recovery at 6

months after STEMI. The resistive reserve ratio (RRR) has been proposed to assess the vasodilatory capacity of the coronary circulation and it is calculated as the ratio between the baseline microcirculatory resistance (BMR) and the hyperemic microcirculatory resistance expressed as IMR[18]. Recently, RRR demonstrated incremental prognostic value in a small cohort of STEMI patients undergoing PPCI. In particular, patients with impaired RRR (<1.98) at completion of PPCI showed larger MVO (3.5 [0.0-5.9], p=0.026), larger infarct size at 6 months (22.7 [10.2-35.0] vs 8.8 [6.9-12.3], p=0.006) and lower myocardial salvage index (34.0 [22.0-59.2] vs 53.2 [37.7-71.0], p=0.032) compared with patients with preserved RRR[19].

Table 1. Angiography and Physiology based methods for the assessment of coronary microvascular dysfunction in STEMI							
	Method	**Definition/ Calculation**	**Cut-off for CMD in STEMI**	**Pros**	**Cons**	**Main evidences**	**Reference**
Angiography							
TIMI	Angiography	Semiquantitative index grading from 0 (no flow) to 3 (normal flow)	< 3	Ready available in the cath-lab	Semiquantitative. Low sensitivity. Inter and intra-observer variability. Influenced by force of contrast injection, coronary anatomy, catheter engagement.	Post-PCI TIMI 3 is associated with better MACE free survival. >50% of patients with TIMI 3 show CMR-defined MVO.	Mehta RH et al J Am Coll Cardiol 2003 Caixeta A et al Eurointervention 2013 Konijnenberg LSF et al Cardiovasc Res 2020
CTFC	Angiography	Quantitative index (number of frames required for contrast to reach a standardized landmark of the vessel)	na	Superior inter and intra-observer reproducibility compared with TIMI flow	Influenced by force of contrast injection, coronary anatomy, catheter engagement	Good correlation with CFR. Association with mortality after STEMI.	Gibson CM et al Circulation 1996 Gibson CM et al J Am Coll Cardiol 1999
MBG	Angiography	Semiquantitative assessment of contrast blush intensity.	< 3	Higher specificity for CMD than TIMI flow	Semiquantitative. Inter and intra-observer variability. Influenced by	Association with infarct size, MVO and mortality	van 't Hof AW et al Circulation 1998

		Grading from 0 to 3.			force of contrast injection, coronary anatomy, catheter engagement		Costantini CO et al J Am Coll Cardiol 2004 Henriques JP et al Circulation 2003
TIMI myocardial perfusion grade	Angiography	Semiquantitative assessment of contrast blush wash out. Grading from 0 to 3.	< 3	Higher specificity for CMD than TIMI flow	Semiquantitative. Inter and intra-observer variability. Influenced by force of contrast injection, coronary anatomy, catheter engagement	Association with infarct size, MVO and mortality	van 't Hof AW et al Circulation 1998 Costantini CO et al J Am Coll Cardiol 2004 Henriques JP et al Circulation 2003
QuBE	Angiography	Automated computer-assisted myocardial blush quantification	< 9.3 (associated with CMR-based MVO)	Quantitative index. Automatic software based analysis	Lack of clinical validation	QuBE is associated with the presence of MVO and intramyocardial hemorrhage at CMR. Independent predictor of mortality	Porto I et al Am J Cardiol 2011 Gu YL et al Catheter Cardiov Interv 2011 Vogelzang M et al Eur Heart J 2009
FLASH	Angiography	Fluoroscopy assisted scoring of myocardial hypoperfusion. Calculated by	na	Higher sensitivity than TIMI flow or MBG	Lack of clinical validation	Independent predictor of cardiac mortality	Biesbroek PS et al Int J Cardiol 2016 Konijnenberg LSF et al

		contrast passage velocity multiplied with the mean cross-sectional area					Cardiovasc Res 2020
Physiology							
CFR	Thermodilutio-wire	Ratio of resting Tmn divided by Tmn during hyperaemia.	< 2	Association with outcomes	Combined measure of epicardial and microvascular resistance (less specific for CMD). Affected by haemodynamic conditions including pressure and heart rate	Association with MVO and myocardial functional recovery after STEMI	Cuculi F et al J Am Coll Cardiol 2014 Cuculi F et al Eur Heart J 2014
CFVR	Doppler-wire	Ratio of hyperaemic average peak velocity (APV) divided by resting APV	< 2	Association with outcomes	Combined measure of epicardial and microvascular resistance (less specific for CMD) Affected by haemodynamic conditions including pressure and heart rate	Association with long-term clinical outcome	van de Hoef et al. *Circ Cardiovasc Interv*. 2013;6:207-15

Pb-CFR	Pressure-wire	Calculation of pressure bounds	< 2	Easy to obtain using standard pressure-wire	Inferior prognostic value comped with IMR. Undetermined result in a significant proportion of cases	Associated with IMR, MVO and infarct size after STEMI	Scarsini R et al *Eurointerventio n* 2019
IMR	Pressure-Thermodilution-wire	Distal coronary pressure (Pd) multiplied with the hyperaemic Tmn	>40 U	Available as intraprocedura l tool (potential for guiding additional therapies). Independent from baseline haemodynami c conditions	In presence of a severe epicardial stenosis a correction using the wedge pressure or applying the Yong formula is required	Associated with larger infarct size and MVO. IMR >40 U post-PPCI is associated with mortality and hospitalization for heart failure. Superior prognostic value compared with CFR	Yong AS et al JACC Cardiovasc Interv 2013 Fearon WF et al Circulation 2013 De Maria GL et al Eur Heart J 2015 Carrick at al Circulation 2016 Maznyczka AM et al Circ Cardiovasc Med 2020
HMR	Pressure-Doppler wire	Pd divided by the hyperaemic APV	>2.5 mmHg/cm/se c	Available as intraprocedura l tool (potential for guiding additional	Adequate doppler signal measurements technically challenging in	Associated with the extent infarct size and LV remodeling.	Kitabata H et al JACC Cardiovasc Interv 2013

				therapies) Independent from baseline haemodynamic conditions	~30% of the cases	Superior prognostic value compared with CVFR	Teunissen PF et al Circ Cardiov Interv 2015 de Waard GA et al Heart 2017
RRR	Pressure-Thermodilution wire	Dynamic index of microcirculatory function. Ratio of basal to hyperemic resistance.	na	RRR provides information on the vasodilatory microvascular capacity.	Dependent on baseline resting Tmn measurements. Clinical cut-off not well defined.	Associated with infarct size, MVO and clinical outcomes	Scarsini R et al Cardiovasc Revasc Med 2018 Maznyczka AM et al Circ Cardiovasc Med 2020
Pzf	Doppler-wire	Pzf represents the value of pressure at which flow would cease in the coronary vessel	42 mmHg	High predictive value in prediction myocardial injury after STEMI	Need for dedicated software. Online measurement not available	Pzf is increased in STEMI and it is associated with TIMI flow, MVO, infarct size and higher risk profile	Patel N et al *JACC Cardiovasc Interv*. 2015;8:1410-21 Teunissen PFA et al Circ Cardiovasc Interv. 2015;8:1-11
Absolute Flow and Resistance	Thermodilution, dedicated catheter	Continuous infusion of saline and calculation of coronary flow and resistance	na	Reduced variability related to saline manual injection	Lack of validation with clinical outcome; need for dedicated equipment	Assessment of absolute flow is feasible in STEMI. Technique validated with PET	Xaplanteris P et al Circ Cardiovasc Intervent 2018;11:1-8 Wijnbergen I et al. Cardiovasc Revasc Med 2016;17:81-87

CFR, coronary flow reserve; CVFR, coronary velocity flow reserve; CTFC, corrected TIMI frame count; FLASH, fluoroscopy assisted scoring of myocardial hypoperfusion; IMR, index of microcirculatory resistance; HMR, hyperaemic microvascular resistance; MBG, myocardial blush grade; MVO, microvascular obstruction; QuBE, quantitative blush evaluator; Pb-CFR, pressure-bounded CFR; Pzf, pressure at zero flow; RRR, resistive reserve ratio; TIMI thrombolysis in myocardial infarction.

Therapeutic options to treat CMD and improve Outcomes

In recent years, several device-based interventional techniques have been developed in the attempt to improve the outcome of patients undergoing PPCI. These novel therapies target one or more than one of the pathophysiological pathways responsible for CMD and for the extent of the infarct size by: preventing distal embolization; 2) reducing cardiac metabolism; 3) enabling cardiac and/or microvascular function to mitigate ischemia-reperfusion injury.

Figure 1 shows novel device-based therapies available in the setting of STEMI.

Being a readily available, intraprocedural tool, IMR is able to identify patients at high risk of poor reperfusion after stenting and may guide the application of novel additional therapies on top of conventional PPCI.

The Oxford Acute Myocardial Infarction-Pressure Intermittent Coronary Sinus Occlusion (OxAMI-PICSO) study was an IMR-guided application of the PICSO treatment in high-risk anterior STEMI[20]. The study demonstrated that the experimental treatment was effective in reducing the infarct size and improving microvascular function in the infarct-related artery. Notably, the final infarct size of high-risk patients treated with PICSO was similar to the one of the low-risk group of patients with pre-stenting IMR ≤40 Units[20] .

These results suggest that: 1) IMR is an effective early triage tool; 2) in high-risk patients, additional therapies on top of conventional PCI may have beneficial effects in terms of microvascular function and infarct size; 3) patients with IMR ≤40 Units respond well to stenting and are unlike to benefit from novel additional therapies.

Recently, we reported that PICSO appears to improve coronary microvascular function immediately after PPCI in patients with STEMI selected according to a threshold IMR criterion. The effect of PICSO on the microvascular function also advantageously influences measures of coronary microcirculatory vasodilatation, as shown by a greater increase in RRR after PICSO treatment compared to controls. Importantly, PICSO-assisted PPCI was associated with smaller infarct size at 6 months (26.0 [17.0-30.0 vs 30.0 [21.3-37.0, p=0.045) observed by CMR compared with standard treatment[21].

RATIONALE

Patency restoration of the occluded coronary artery is the cornerstone of the current treatment of patients presenting with STEMI. However, up to 40% of the patients undergoing PPCI presented suboptimal myocardial reperfusion and they are at high risk of adverse clinical outcome, including heart failure and cardiac death.

Coronary microvascular dysfunction (CMD) is the leading cause of incomplete myocardial reperfusion after PPCI and it may be related to a number of causes including distal embolization and mechanical small vessel occlusion; endothelial dysfunction; and ischemia-reperfusion injury.

IMR, a pressure-wire thermodilution-based technique, is the most commonly used index to assess CMD in the catheterization laboratory. IMR demonstrated high diagnostic accuracy in detecting CMD and provided prognostic stratification in the clinical setting of STEMI. Moreover, IMR was used in clinical trials to guide the application of novel additional therapies of patients with STEMI at high risk of suboptimal reperfusion. Notably, IMR-guided novel therapies showed favourable results in reducing microvascular obstruction and infarct size.

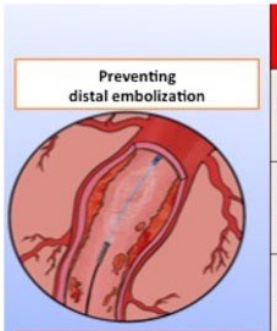
However, IMR is rarely used in the everyday clinical practice and it is mostly seen as a research tool only. The low penetration of IMR in the standard practice has several reasons including additional procedural time and costs, technical complexity of the procedure, concerns related to the instrumentation of the infarct-related artery and the use of vasodilators. Moreover, data on the long-term outcome of patients stratified according to IMR is scarce.

AIMS

In order to support the application of coronary physiology indices of CMD in the clinical practice, we aim to investigate the long-term clinical outcome of patients admitted with STEMI included in the Oxford Acute Myocardial Infarction (OxAMI) Study. This study cohort represents a unique opportunity to assess the value of invasive IMR evaluation performed systematically at completion of PPCI. Moreover, cardiovascular magnetic resonance imaging (CMR) was systematically performed within 48 hours from PPCI and at 6 months of follow up and provides an excellent comparator to IMR.

Once assessed the long-term clinical value of invasive IMR, we aim to develop alternative indices to overcome some of the limitations of IMR, in order to increase the penetration of CMD assessment in the clinical practice. In first instance, we aim to test the diagnostic performance of pressure-bounded coronary flow reserve (pb-CFR) in patients enrolled in the OxAMI Study. Pb-CFR is derived using standard pressure-wire technology without thermodilution or doppler measurements.

In order to further simplify and standardize the assessment of CMD in the catheterization laboratory we then aim to develop a novel angiography-based pressure-wire free index called angiography-derived IMR (IMR_{angio}). In order to do so, we designed a dedicated prospective study IMR_{angio} will be developed based on computational fluid dynamics using the QAngio® XA 3D software (Medis, Leiden, the Netherlands). IMR_{angio} will be then compared with invasive IMR and CMR. Furthermore, the long-term prognostic value of IMR_{angio} will be assessed retrospectively in the OxAMI cohort.



Device	Clinical Evidence	Time of application	Treatment Time	Extra vascular access	Ongoing Studies	Regulatory Status	*Suggested* Clinical Indication*
Manual thrombectomy	RCT	During PPCI	From Seconds to Minutes	No	No	FDA approved in STEMI CE marked in STEMI	Non routine and in highly thrombotic lesion
Mechanical thrombectomy	RCT	During PPCI	From Seconds to Minutes	No	CHEETAH (registry)	FDA approved in STEMI CE marked in STEMI	Bail-out when manual thrombectomy fails
Sonothrombolysis	RCT	Before & After PPCI	Average 15 minutes	No	SONOSTEMI-LYSIS (RCT)	No-FDA approved No-CE marked	In-ambulance / emergency room & post-PPCI
Myocardial Cooling	Non-RCT studies	Before PPCI	20 minutes	No	EURO-ICE (RCT)	FDA approved and CE marked in cardiac arrest	In STEMI presenting as out-of-hospital cardiac arrest
Left Ventricular Unloading	STEMI-DTU Pilot RCT	Before PPCI	30 minutes	Yes (Large Arterial (13-14 Fr)	STEMI-DTU Pivotal (RCT)	FDA approved and CE marked for high-risk PCI and cardiogenic shock	Anterior STEMI in the context of cardiogenic shock
PICSO	Non RCT studies	During PPCI	45 minutes	Yes (Venous 12 Fr)	PICSO AMI I (RCT)	FDA breakthrough designation (investigational) CE marked in STEMI	Anterior STEMI with TIMI Flow 0-1 and symptoms onset < 12 h
SSO_2	RCTs	After PPCI	60 minutes	No	No	FDA approved in STEMI CE marked in STEMI	Anterior STEMI with symptoms onset < 6 h
CoFI	Animal Model only	After PPCI	Cycles of 5 minutes	No	MOCA I (feasibility study)	FDA breakthrough designation (investigational)	Early Experimental phase

Figure 1. Summary of main novel device-based therapies for STEMI patients. Adapted from De Maria GL, Garcia-Garcia H, Scarsini R et al. Novel device-based therapies to improve outcome in St-segment elevation myocardial infarction. Eur Heart J Acute Care 2020.

CHAPTER 1.

Long-term prognostic implications of coronary microvascular dysfunction assessed using IMR and CMR after STEMI

ABSTRACT

Objectives. We sought to evaluate the long-term prognostic implications of coronary microvascular dysfunction (CMD) when assessed with both cardiovascular magnetic resonance (CMR) and index of microcirculatory resistance (IMR) in patients with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI).

Background. Post-ischaemic CMD can be assessed using the pressure-wire based IMR and/or by the presence of microvascular obstruction (MVO) on CMR.

Methods.198 patients with STEMI underwent IMR and MVO assessment. Patients were classified as follows: Group1, no significant CMD (low IMR (≤40U) and no MVO); Group 2, CMD with either high IMR (>40U) or MVO; Group3, CMD with both IMR>40U and MVO. The primary endpoint was the composite of all-cause mortality, diagnosis of new heart failure, cardiac arrest, sustained ventricular tachycardia/fibrillation and cardioverter defibrillator implantation.

Results.CMD with both high IMR and MVO was present in 23.7% of the cases (Group 3) and CMD with either high IMR or MVO was observed in 40.9% of cases (Group 2). At a median follow-up of 40.1(12.8-73.8) months, the primary endpoint occurred in 34 (17%) cases. At 1-year of follow-up, Group 3 (HR=12.6, 95%CI 1.6-100.6, p=0.017) but not Group 2 (HR=7.2, 95%CI 0.9-57.9, p=0.062) had worse clinical outcomes compared with those with no significant CMD in Group 1. However, in the long-term, patients in Group 2 (HR=4.2, 95%CI 1.4-12.5, p=0.009) and those in Group 3 (HR=5.2, 95%CI 1.7-16.2, p=0.004) showed similar adverse outcomes, mainly driven by the occurrence of heart failure.

Conclusion. Multimodality assessment CMD provides additional stratification of the risk of adverse events after STEMI. Post-ischaemic CMD defined by both high IMR and MVO is associated with high risk of events at 1 year after STEMI.

However, in the long-term, both this group and the group with IMR>40 or MVO have a significantly higher risk of poor clinical outcome when compared with having no significant post-STEMI CMD.

INTRODUCTION

Prompt coronary revascularization has drastically reduced the in-hospital mortality of patients with ST-segment elevation myocardial infarction (STEMI). However, the occurrence of heart failure (HF) after STEMI has not diminished and may be increasing[4, 22].

Unfortunately, suboptimal myocardial reperfusion is still observed in up to 40% of the cases despite rapid percutaneous revascularization. Post-ischaemic coronary microvascular dysfunction (CMD) plays an important role in the development of no-reflow and is a major determinant of suboptimal reperfusion[11]. Moreover, CMD is associated with larger infarct size and with a 2-fold higher risk of mortality and hospitalization for HF[14, 16].

Post-ischaemic CMD is considered a heterogenous entity and can be assessed by multiple invasive and non-invasive modalities[11] . Index of microcirculatory resistance (IMR), a pressure-wire-based and thermodilution-derived index, and microvascular obstruction (MVO) as detected on cardiovascular magnetic resonance (CMR) are the most commonly used indices for assessment of CMD after STEMI. Index of microcirculatory resistance (IMR) performed at the time of primary percutaneous coronary intervention (PPCI) has shown good accuracy in detecting MVO and in predicting large infarct size[3]. Both high IMR (>40 Units) and MVO after STEMI are associated with adverse clinical outcome including higher risk of mortality and HF[5, 14, 16].

It is unclear whether IMR and MVO describe the same pathophysiology, or whether IMR and MVO reflect distinct features of post-ischaemic CMD. Indeed, we previously reported that ~30% of patients with STEMI exhibited discordance in CMD with these two indices; they had either IMR >40 U or MVO on CMR [7] and that these patients had smaller infarct size at 6 months when compared to patients with both high IMR >40U and MVO. Therefore, we hypothesized that patients with both elevated IMR and MVO have a more severe form of post-ischaemic CMD

when compared to those with either of the indices. We aimed to study if the long-term clinical outcomes of these two groups of patients would be different from each other when compared with patients with preserved microvascular function.

METHODS

Patients with STEMI admitted to the Oxford Heart Centre for PPCI were prospectively considered for enrolment in the Oxford Acute Myocardial Infarction (OxAMI) Study from 2011 to 2019. Patients who underwent post-procedural IMR assessment and CMR prior to discharge from hospital were included in this analysis. Details of the study protocols have been previously reported[3].

STEMI was diagnosed in the presence of chest pain lasting at least 30 minutes, within 12 hours from onset of symptoms, and ST-segment elevation of >2 mm (0.2 mV) in at least 2 contiguous leads on ECG. Patients were excluded in case of symptom duration longer than 12 hours, presence of severe hemodynamic instability, severe left main disease, contraindications to adenosine infusion and general contraindications to CMR.

PPCI was performed in a standard fashion and decisions about direct stenting technique, thrombectomy and glycoprotein IIb/IIIa adoption were all left to operator's discretion. All patients were loaded with dual antiplatelet therapy. Weight-adjusted unfractionated heparin or bivalirudin was adopted as antithrombotic regimen.

The study protocol was approved by the local ethics committee (REC number 10/H0408/24) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Angiographic analysis

Coronary flow was graded using the standard TIMI criteria[23]. Angiographic thrombus score was graded from 0 to 5 after the passage of the guidewire, as previously described[24]. Myocardial blush grade at the end of the procedure was evaluated according to van't Hof[25].

Index of microcirculatory resistance

A standard pressure and temperature-monitoring guidewire (Abbott, Santa Clara, CA) was advanced in the distal segment of the culprit vessel at completion of PPCI.

Coronary flow was estimated using thermodilution to derive mean transit time. Maximal hyperaemia was induced with Adenosine i.v. infusion (140 mcg/kg/min). IMR was defined as the mean distal pressure multiplied by the mean transit time at hyperaemia as previously described[3]. IMR >40 Units was considered indicative of clinically significant CMD in patients with STEMI as previously reported[3].

CMR analysis

CMR was performed using a 3.0 Tesla magnetic resonance scanner (either MAGNETOM TIM Trio or MAGNETOM Verio; Siemens Healthcare, Erlangen, Germany) within 48 hours after PPCI. The CMR protocol is described in detail in Supplementary Material. In 144 (72.7%) cases CMR was also performed at 6-month follow-up to assess final infarct size. Cvi42 image analysis software (Circle Cardiovascular Imaging Inc, Calgary, Canada) was used for image analysis. Left ventricular (LV) volumes and ejection fraction (EF%) were assessed from steady-state free precession images. To quantify the percentage of LV mass infarct size (IS%), as depicted by late gadolinium enhancement (LGE), the signal intensity threshold was set at 5 standard deviations above the mean SI of the remote reference myocardium[26]. The MVO was defined as the hypointense area within the LGE region and its size was quantified by manual delineation of the area.

Groups definition

CMD was defined as the presence of MVO and/or high IMR (>40 U). Patients were categorized as follows: no significant CMD with low IMR (≤ 40U) and without MVO (Group 1), CMD with either high IMR (>40U) or MVO (Group 2) and CMD with both high IMR (>40U) and MVO (Group 3).

Endpoints and definitions

The primary endpoint of the study was the composite of all-cause mortality, HF, resuscitated cardiac arrest, malignant ventricular arrhythmias (sustained ventricular tachycardia/ventricular fibrillation) and the need for a primary prevention implantable cardioverter defibrillator (ICD). Secondary endpoints were all-cause mortality, HF and recurrent myocardial infarction (MI). Diagnosis of HF was obtained from electronic patient records (both hospital and primary care facilities) and from OxAMI study follow up visits. HF was defined by the development of new symptoms of HF and/or prescription of diuretics with documented evidence of

LV systolic dysfunction (LVEF <50%) on CMR or echocardiogram and/or raised levels of natriuretic peptide.

Statistical analysis

The normal distribution of the variables was tested using Shapiro-Wilk test and histograms. Continuous variables were reported as mean ± standard deviation or as median and interquartile range as appropriate. Categorical variables were reported as numbers and percentages. Continuous variables were compared with Student's t-test or analysis of variance with Scheffe' post hoc comparison. Mann-Whitney U test or Kruskal-Wallis test were used for non-normal distributed variables. Frequencies were compared with chi-square test or Fisher exact test, as appropriate. Survival analysis and endpoint comparison between groups were performed with the Cox regression analysis for the calculation of hazard ratio with 95% confidence interval and the log-rank test. Kaplan Meier curves were constructed. Survival analysis of the primary endpoint was adjusted for variables with p-value <0.1 at the univariate analysis. The test for proportional-hazards assumption was applied to confirm the validity of the model. Logistic regression analysis was performed to assess variables associated with CMD after STEMI. Multicollinearity of variables included in the final model used was assessed using variance inflation factor analysis. The validity of the model was tested using the Hosmer-Lemeshow goodness-of-fit test. Statistical analysis was performed with Stata version 15.1 (StataCorp LLC, College Station TX). A p-value <0.05 was considered significant.

RESULTS

Study population

Between September 2011 and September 2019, 222 patients with STEMI underwent both IMR assessment at completion of PPCI and CMR at a median time of 40.8 hours (IQR 24.7-47.8 hrs) after STEMI. Sixteen patients were excluded because of insufficient quality of CMR imaging (n=7), gadolinium-based contrast agent not administered (n=4) or inadequate pressure-wire traces (n=5). Eight patients were lost to follow-up after hospital discharge (Supplementary Table 1). Therefore, a total of 198 patients with STEMI were included in this study. The overall characteristics of the study cohort are presented in Table1. The mean age

was 60.2±10.7, 170 (85.9%) were male and 39 (19.8%) had diabetes. The median duration of follow up was 40.1(12.8-73.8) months.

IMR and MVO

High IMR (>40 U) was observed in 72 (36.4%) patients at completion of PPCI. Conversely MVO was observed in 100 (50.5%) patients.

70 (35.4%) patients had no significant CMD after STEMI (Group 1). CMD defined as presence of either high IMR or MVO (Group 2) was present in 81 (40.9%) patients (low IMR with MVO = 56 [28.3%]; high IMR without MVO = 25 [12.6%]). CMD defined as presence of both high IMR and MVO was observed in 47 (23.7%) patients (Group 3).

Significant differences in the infarcted myocardial territory and the severity of myocardial injury were observed across the groups (**Table 1-3**). In particular, patients with CMD with both high IMR and MVO (Group 3) had higher troponin release, lower coronary flow reserve, lower post-PPCI LVEF and larger IS%, both at 48 hours and at 6 months, compared with the other groups (**Table 1-3**).

Table 1. Overall clinical characteristics

Variable	Overall	Group 1 (No significant CMD)	Group 2 (CMD with high IMR or MVO)	Group 3 (CMD with high IMR and MVO)	p-value
No. patients	198(100)	70(35.4)	81(40.9)	47(23.7)	
Age, years	60.2±10.7	59.2±11.6	61.5±10.2	59.3±10.4	0.352
Male sex, %	170(85.9)	59(84.3)	68(83.9)	43(91.5)	0.446
Hypertension, %	88(44.9)	31(44.9)	40(50.0)	17(36.2)	0.318
Hypercholesterolemia, %	65(38.5)	18(25.7)	30(37.0)	17(36.2)	0.485
Diabetes, %	39(19.8)	9(12.9)	21(26.5)	9(19.1)	0.120
Smoking, %	72(36.7)	35(50.7)	25(31.2)	12(25.5)	**0.009**
eGFR, ml/min/1.73m^2	92.4(72.7-105.7)	97.9(78.9-111.3)	88.2(73.5-97.9)	92.3(74.4-99.1	0.122
Pain-to-balloon time, min	183(125-347)	196(133-360)	166(110-314)	215(142-322)	0.058
Troponin*	1200(301-2776)	445(121-1179)	1342(547-3135)	1752(1131-6387)	**0.0001**

eGFR, estimated glomerular filtration rate *Magnitude of troponin release defined as the multiple of upper limit of normal reference

Table 2. Angiographic, procedural and physiology data

Variable	Overall	Group 1 (No significant CMD)	Group 2 (CMD with high IMR or MVO)	Group 3 (CMD with high IMR and MVO)	p-value
Infarct related artery					
LAD	98(49.5)	26(37.1)	42(51.9)	30(63.9)	**0.028**
LCX	21(10.6)	6(8.6)	8(9.9)	7(14.9)	
RCA	75(37.9)	36(51.4)	30(37.0)	9(19.1)	
Diagonal	4(2.0)	2(2.9)	1(1.2)	1(2.1)	
TIMI flow at baseline					
TIMI=0	148(74.7)	43(61.4)	66(81.5)	39(83.0)	**0.003**
TIMI=1	17(8.6)	6(8.7)	7(8.6)	4(8.5)	
TIMI=2	19(9.6)	9(12.8)	6(7.4)	4(8.5)	
TIMI=3	14(7.1)	12(17.1)	2(2.5)	0(0.0)	
TIMI flow post-PCI					
TIMI=0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	**<0.0001**
TIMI=1	3(1.5)	1(1.4)	0(0.0)	2(4.2)	
TIMI=2	24(12.1)	1(1.4)	10(12.2)	13(27.7)	
TIMI=3	171(86.4)	68(97.2)	71(87.8)	32(68.1)	
Stent length, mm	29.1±13.7	30.1±15.9	28.7±11.0	28.6±14.7	0.817
Stent diameter, mm	3.47±0.46	3.41±0.47	3.48±0.42	3.52±0.52	0.589
Post-PPCI Coronary physiology					
Pd/Pa	0.95±0.05	0.96±0.05	0.95±0.05	0.95±0.05	0.321

FFR	0.93±0.06	0.92±0.06	0.93±0.06	0.93±0.07	0.484
Mean transit time	0.41(0.27-0.73)	0.28(0.20-0.42)	0.35(0.28-0.57)	0.87(0.55-1.32)	**<0.0001**
CFR	1.58(1.18-2.28)	1.99(1.49-2.69)	1.70(1.30-2.29)	1.10(1.00-1.48)	**<0.0001**
IMR	31.7(20.0-50.5)	20.0(14.6-31.0)	28.7(21.0-44.02)	69.6(46.2-100.2)	**<0.0001**

CFR, coronary flow reserve; FFR, fractional flow reserve; IMR, index of microcirculatory resistance; LAD, left anterior descending artery; LCX, left circumflex artery; Pa, aortic pressure; Pd, distal coronary pressure; RCA, right coronary artery; TIMI, thrombolysis in myocardial infarction.

Table 3. Cardiovascular magnetic resonance data within 48 hours from STEMI and at 6 months

	Group 1 (No significant CMD)	**Group 2 (CMD with high IMR or MVO)**	**Group 3 (CMD with high IMR and MVO)**	**p-value (overall)**	**p-value Group1 vs Group2**	**p-value Group1 vs Group3**	**p-value Group2 vs Group3**
CMR within 48 hours							
Time from PPCI, hours	39.6(23.5-46.8)	41.0(28.3-48.7)	31.2(22.6-43.9)	0.121	0.661	0.484	0.121
EDVi, ml/m^2	77.0±19.2	77.6±18.9	87.3±18.5	**0.010**	0.983	**0.020**	**0.025**
ESVi, ml/m^2	37.7±12.6	42.6±15.7	49.9±14.9	**<0.0001**	0.117	**<0.0001**	**0.028**
LVEF, %	51.4±8.5	46.0±10.1	43.2±8.8	**<0.0001**	**<0.0001**	**<0.0001**	0.114
IS%	17.1±10.1	29.9±12.9	35.5±11.8	**<0.0001**	**<0.0001**	**<0.0001**	**0.008**
MVO%	0(0-0)	1.0(0.0-3.7)	4.0(2.2-8.6)	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
CMR at 6 months							
EDVi, ml/m^2	76.0±15.5	81.4±17.8	94.3±24.1	**<0.0001**	0.326	**<0.0001**	**0.008**
ESVi, ml/m^2	31.6±10.8	39.4±14.7	51.2±21.1	**<0.0001**	**0.036**	**<0.0001**	**0.003**
LVEF, %	59.0±7.7	52.9±8.8	45.8±10.5	**<0.0001**	**0.001**	**<0.0001**	**<0.0001**
IS%	9.7±9.4	20.8±11.2	26.9±10.7	**<0.0001**	**<0.0001**	**<0.0001**	**0.006**

On regression analysis, LAD territory infarct (OR 2.53, 95% CI 1.14-5.66, p=0.023), diabetes (OR 3.20, 95% CI 1.05-9.72, p=0.040) and impaired TIMI flow at baseline (OR 0.53, 95% CI 0.28-0.99, p=0.049) were independently associated with CMD (high IMR and/or MVO) (Supplementary Table 2). Predictors of Groups 2 and 3 are presented in Supplementary Table 3-4.

Association between post-ischaemic CMD and the extent of infarct size and systolic function impairment

Significant differences in post-PPCI LVEF% and extent of infarct size were observed across the 3 groups. Group 2 had lower LVEF% and larger IS% acutely and at 6 months compared with Group 1 with no significant CMD (Figure 1; Table 3), but smaller IS% at 48 hours and at 6 months and higher LVEF% at 6 months when compared with patients with Group 3 with severe CMD (Figure 1; Table 3).

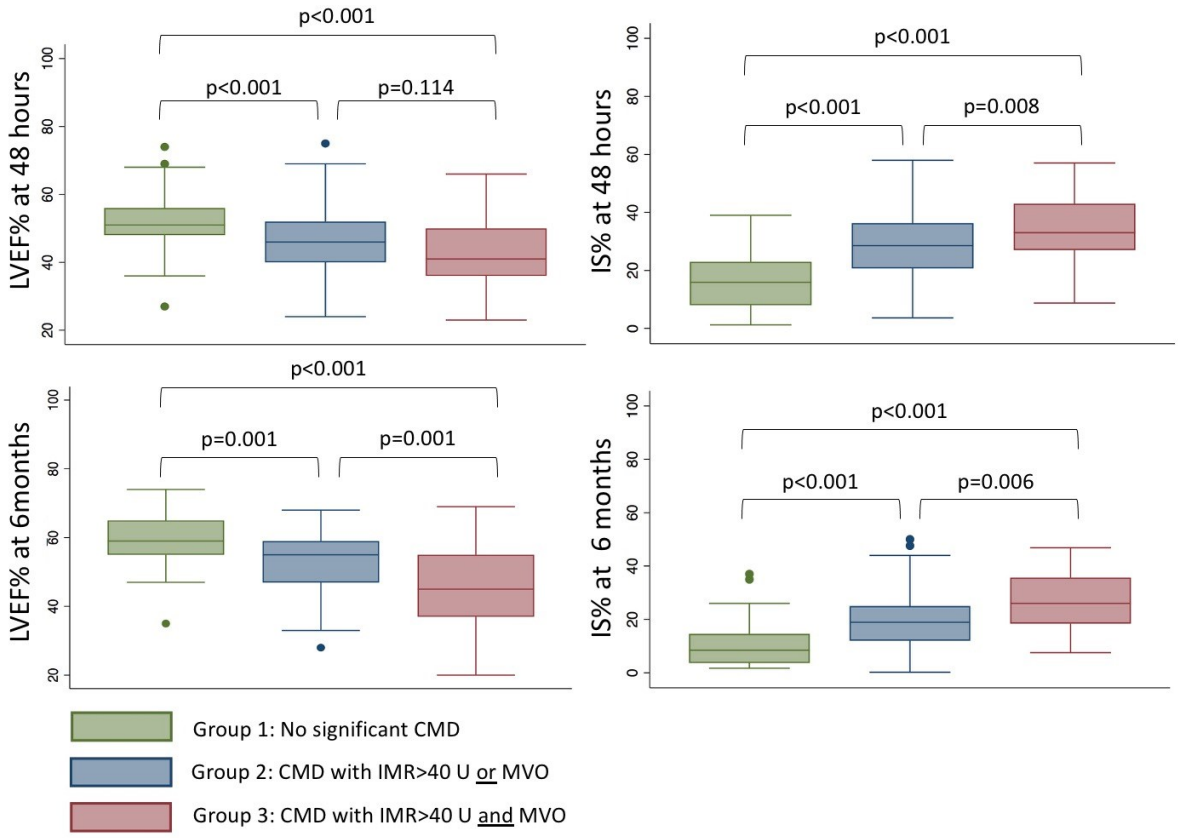


Figure 1. Left ventricular ejection fraction and Infarct size in patients stratified according to IMR and MVO. Box plots of LVEF% and IS% at 48 hours and 6 months in patients stratified according to IMR and MVO.

Table 4. Adverse clinical events at follow up

	Overall	Group 1 (No significant CMD)	Group 2 (CMD with high IMR or MVO)	Group 3 (CMD with high IMR and MVO)	p-value
No. Patients	198(100.0)	70(35.4)	81(40.9)	47(23.7)	
Primary endpoint	34(17.2)	4(5.7)	18(22.2)	12(25.5)	**0.016**
All cause death	15(7.6)	4(5.7)	8(9.9)	3(6.4)	0.080
Cardiac death	6(3.0)	1(1.4)	3(3.7)	2(4.2)	0.459
Heart failure	27(13.6)	1(1.4)	15(18.5)	11(23.4)	**0.003**
VT/VF	11(5.5)	0(0.0)	4(4.9)	7(14.9)	**0.001**
ICD	5(2.5)	0(0.0)	2(2.5)	3(6.4)	0.153

ICD, implantable cardiac defibrillator; VF, ventricular fibrillation; VT, ventricular tachycardia.

Association between post-ischaemic CMD and the primary clinical outcome

At a median follow up time of 40.1 (12.8-73.8) months, the primary outcome occurred in 34 (17.2%) patients (Table 4).

Group 3 with severe CMD had a significantly higher risk of adverse events compared with patients with no significant CMD (Group 1) at 1 year (HR=12.6, 95%CI 1.6-100.6, p=0.017) and at long term follow up (HR=5.2, 95%CI 1.7-16.2, p=0.004, Figure 2, Table 5).

Group 2 with either high IMR or MVO showed no significant difference in the primary endpoint at 1 year of follow-up when compared with Group 1 with no significant CMD. However, the risk of long-term adverse events was significantly higher at long term follow up (Figure 2, Table 5).

Notably, whilst Group 2 CMD with either high IMR or MVO demonstrated a 4.2-fold increase in long-term risk when compared to Group 1 with no significant CMD, this risk was similar to Group 3 with severe CMD (HR=0.81, 95%CI 0.39-1.68, p=0.575, Figure 2).

Table 5. Cox Regression analysis of the primary endpoint

	Univariate		**Multivariate**	
Variable	**HR (95%CI)**	**p-value**	**HR (95%CI)**	**p-value**
Age, years	1.04(1.01-1.07)	**0.018**	1.04(1.01-1.08)	**0.037**
Male sex	0.65(0.28-1.50)	0.319	0.82(0.30-2.26)	-
Smoker	0.58(0.26-1.29)	0.184	1.22(0.50-2.99)	-
Diabetes	1.35(0.61-2.99)	0.462	1.54(0.68-3.52)	-
LAD	1.27(0.65-1.50)	0.483	1.15(0.53-2.49)	-
Ischemic time	1.00(1.00-1.01)	**0.041**	1.00(0.99-1.01)	0.144
Troponin (peak)§	1.00(0.99-1.01)	0.721	1.00(0.99-1.01)	-
Post-PCI TIMI flow	0.39(0.22-0.68)	**0.001**	0.74(0.37-1.49)	0.405
Group2 (IMR>40U or MVO)	4.24(1.43-12.52)	**0.009**	4.69(1.36-16.15)	**0.014**
Group3 (IMR>40U and MVO)	5.22(1.68-16.21)	**0.004**	6.80(1.83-25.22)	**0.004**

HR, hazard ratio; LAD, left anterior descending artery; IMR, index of microcirculatory resistance; MVO, microvascular obstruction; TIMI, thrombolysis in myocardial infarction.
§Magnitude of troponin release defined as the multiple of upper limit of normal reference

In Group 2 CMD with either high IMR or MVO, there was no significant difference in the long-term clinical outcome between those patients with low IMR with MVO vs. patients with high IMR but without MVO (HR 0.82, 95%CI 0.29-2.34, p=0.715) (Figure 3).

CMD defined as high IMR and/or MVO was associated with the primary endpoint independently of post-PPCI TIMI flow and CMR-based infarct size (Supplementary Table 5). Cox regression analysis adjusted for clinical confounders is presented in Table 5.

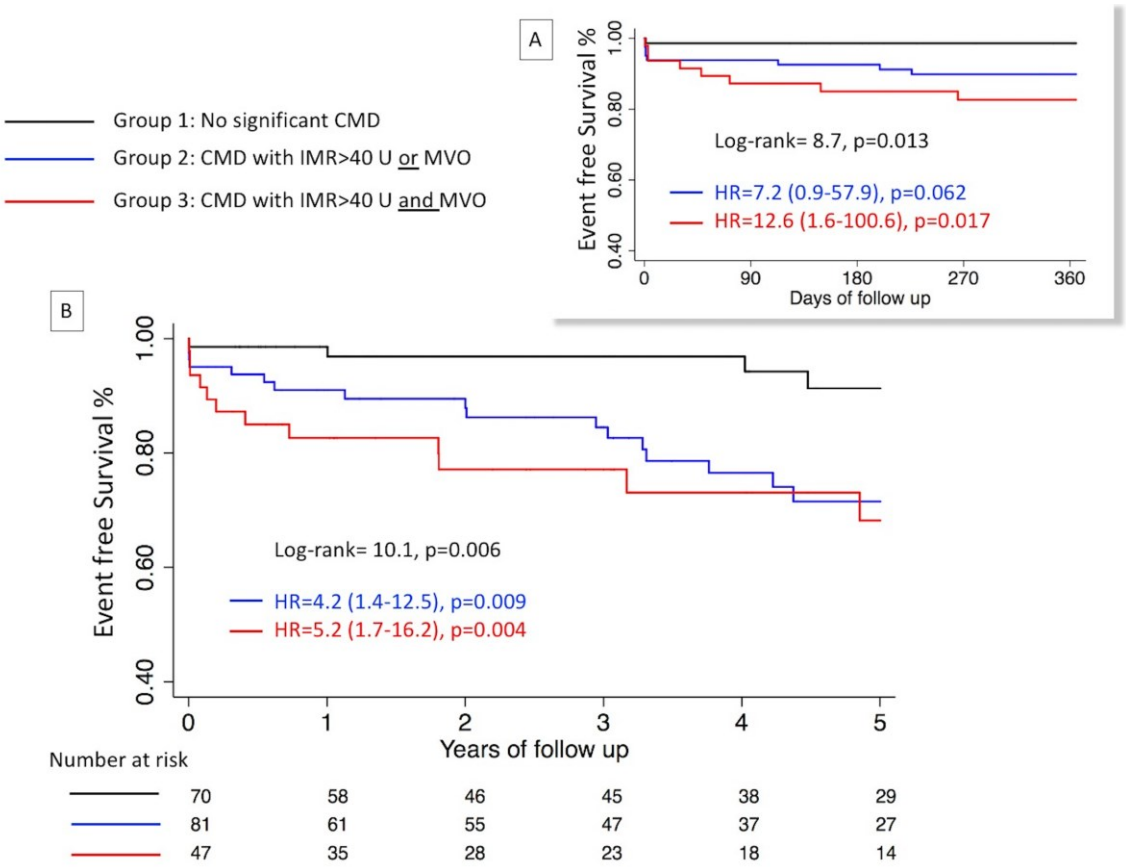


Figure 2. Survival analysis of the primary endpoint
Kaplan-Meier curves of patients stratified according to IMR and MVO at 1 year (a) and at long term (b). Hazard ratios for patients with IMR >40 U and MVO (Group 3) and for those with either high IMR or MVO (Group 2) are provided in comparison with patients with no significant CMD (Group 1).

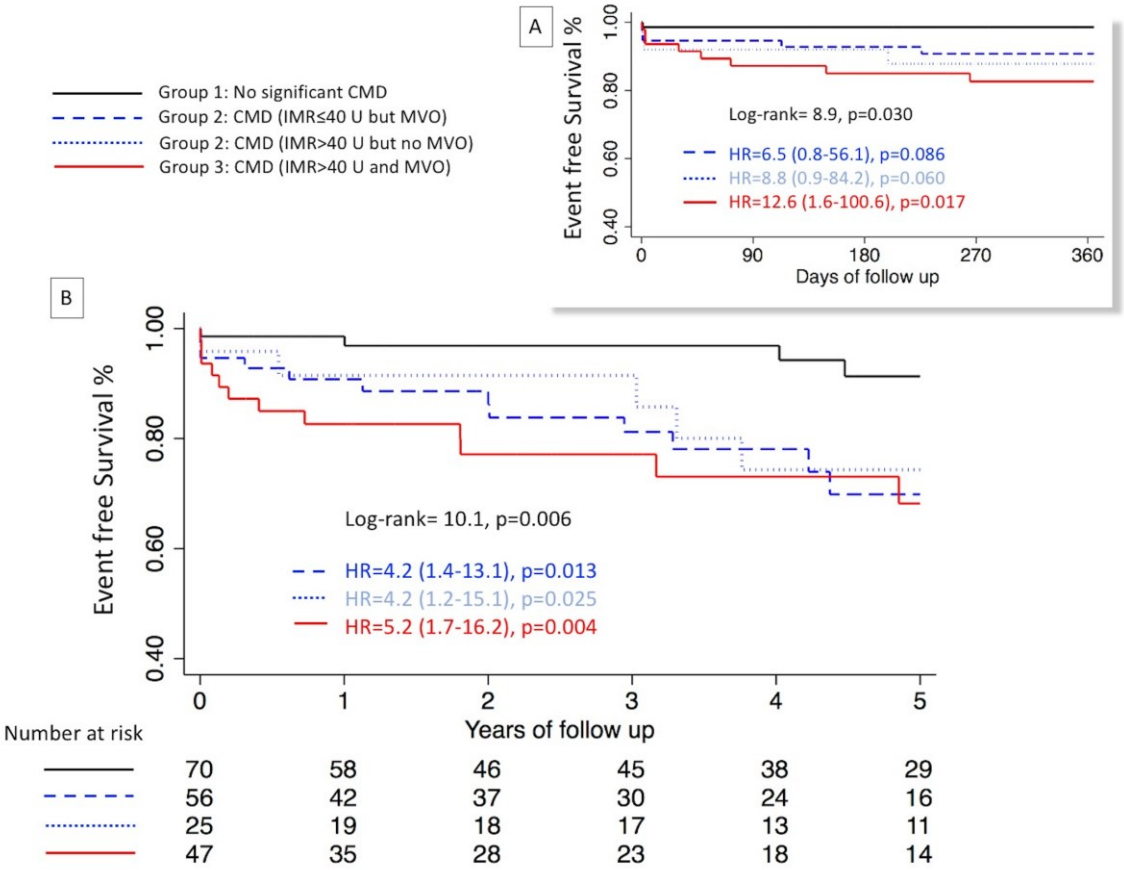


Figure 3. Survival analysis of the subgroups with either elevated IMR or MVO
Kaplan-Meier curves of patients stratified according to IMR and MVO at 1 year (a) and at long term(b). Hazard ratios for patients are provided in comparison with patients with no significant CMD (Group 1).

Association between IMR, MVO and adverse outcomes

IMR and MVO were both associated with the primary outcome. In particular, patients with high IMR demonstrated significantly higher risk of composite adverse events (HR 2.07, 95% CI 1.06-4.07, p=0.03), heart failure (HR 2.82, 95% CI 1.23-6.46, p=0.01) and malignant ventricular arrhythmias and/or ICD implantation (HR 19.2, 95% CI 2.45-150.16, p=0.005) (Supplementary Figure 3). Patients with MVO demonstrated significantly higher risk of composite adverse events (HR 2.46, 95% CI 1.17-5.18, p=0.02) and heart failure (HR 3.37, 95% CI 1.32-8.60, p=0.01). (Supplementary Figure 4).

All-cause mortality

All-cause mortality occurred in 15 (7.6%) cases during the study period. No significant difference was observed among patients stratified according to IMR and MVO (Supplementary figure 3). Age (HR=1.14, 95% CI 1.06-1.23, p<0.0001) and LVEF% (HR=0.93, 95% CI 0.88-0.97, p=0.004) were independently associated with all-cause mortality on Cox regression analysis (Supplementary Table 6).

Heart failure

Overall, 27 (13.6%) patients developed HF during the study period. Patients in Group 3 with severe CMD (HR=17.4, 95%CI 2.2-136.5, p=0.006) and Group 2 CMD with either high IMR or MVO (HR=12.6, 95%CI 1.6-96.6, p=0.015) demonstrated higher risk of developing HF compared with patients in Group 1 (Supplementary Figure 4).

Longer ischemic time (HR=1.01, 95% CI 1.00-1.02, p=0.042), LVEF% (HR=0.91, 95% CI 0.86-0.96, p<0.0001) and being in Group 3 with severe CMD (HR=17.4 95% CI 2.2-136.1, p=0.006) or Group 2 CMD with either high IMR or MVO (HR=12.6, 95% CI 1.6-96.6, p=0.015) were associated with HF at Cox regression analysis (Supplementary Table 7).

Recurrent Myocardial Infarction

Myocardial infarction occurred in 11 (5.5%) cases during the study period. No significant difference in the risk of recurrent infarction was observed when patients were stratified according to IMR and MVO (Supplementary figure 5).

The presence of diabetes (HR=8.23, 95% CI 2.05-32.95, p=0.003) and lower thrombus burden (HR=0.53, 95% CI 0.34-0.82, p=0.005) were associated with recurrent infarction on Cox regression analysis. (Supplementary Table 8).

DISCUSSION

In this study, the pathophysiology of post-ischaemic CMD and its long-term prognostic implications were analysed in patients with revascularized STEMI. We used a multimodality approach, comparing IMR and MVO, in the same patients. The principal findings are as follows:

1. When assessed with IMR and CMR, 35% had no evidence of significant CMD (Group 1) and had significantly better clinical outcomes than patients with CMD.
2. Severe CMD with high IMR and MVO (Group 3) was present in 24% of the study cohort and these patients had a larger infarct size and lower LVEF when compared to patients with CMD with either high IMR or MVO (Group 2) or patients with no evidence of CMD (Group 1). Patients in Group 3 with severe CMD had the highest risk of adverse clinical outcome at 1 year.
3. 41% of our cohort had abnormality of only one of the indices (Group 2) (either IMR >40 U or MVO). While the risk of adverse events at 1 year was not different compared with patients with no significant CMD (Group 1), in the longer term, these patients had similar outcomes to patients with the highest risk (Group 3 CMD with both high IMR and MVO). Ultimately, they exhibited a > 4-fold higher risk of adverse outcome at long-term when compared with patients with no significant CMD at initial assessment (Group 1).

Coronary microvascular dysfunction is reported in a significant proportion of patients undergoing PPCI and it is a major determinant of adverse outcome in STEMI[4,5]. CMD is associated with suboptimal myocardial recovery and adverse LV remodelling, predisposing to both HF and ventricular arrhythmias[27, 28]. Importantly, CMD has important prognostic implications even when post-procedure TIMI flow is normal [29]. Our study found that more than 65% of patients had post-ischaemic CMD, defined by either or both IMR and CMR and that it was adversely prognostic.

Post-ischaemic CMD is a heterogenous entity and can be assessed with multiple tools. MVO on CMR and elevated IMR are the most commonly used indices of post-ischaemic CMD.

A 1% increase in MVO size is associated with a 14% relative increase in mortality and an 8% increase in HF at 1 year of follow-up[5]. IMR has been extensively validated to predict infarct size and clinical outcomes including mortality and hospitalization for HF in patients with STEMI[14, 16].

However, it is unclear which of either CMR or invasive physiology or even both should be used to risk stratify patients with CMD early after STEMI and how it should alter the clinical management. Our study is the first to show that combining MVO and IMR, may offer incremental long-term risk stratification compared to assessments of MVO and IMR in isolation. Although this will require further investigations to be confirmed, implementing this approach in the clinical setting could represent an important step towards personalized precision medicine. In particular, patients identified with CMD may benefit from being followed up regularly in order to aggressively optimize medical therapy and promptly detect and treat the onset of heart failure (Central Figure). The effectiveness of this approach and its implications on health care systems will need to be tested in adequately powered studies.

In a proportion of patients with STEMI, IMR (≤40 or >40 U) can be discordant from the presence (or absence) of MVO at CMR[7]. In a previous study, we showed that patients with both MVO and IMR>40 U presented an 11.9-fold increased risk of having IS larger than 25% of the myocardial mass at 6 months follow up[7]. Similarly, patients with either IMR>40 or MVO showed a larger IS at 6 months compared with patients with no MVO and preserved IMR ≤40 U[7]. Moreover, IMR ≤40U appeared associated with a favourable reduction of the infarct size at six months, irrespective of MVO, suggesting the possibility that IMR could offer complementary information to CMR, in assessing the severity of post-ischemic CMD[7].

In the present study, we observed that, when compared to patients with either IMR>40 or MVO (Group 2), patients with IMR >40 and MVO (Group 3) have larger infarcts both acutely and at 6 months. However, this study, looking at long term events, shows that the patients with either high IMR or MVO, defined here as Group 2 CMD, also had adverse clinical outcomes similar to Group 3 with severe CMD (with both high IMR and MVO) at a median follow up of 40 months (3.3 years).

This implies that the multi-faceted pathophysiology of CMD, when detected by either IMR or MVO after PPCI for STEMI, may continue to drive adverse changes

in the myocardium and cardiac function, with lasting effects translating into adverse clinical events beyond 6 months.

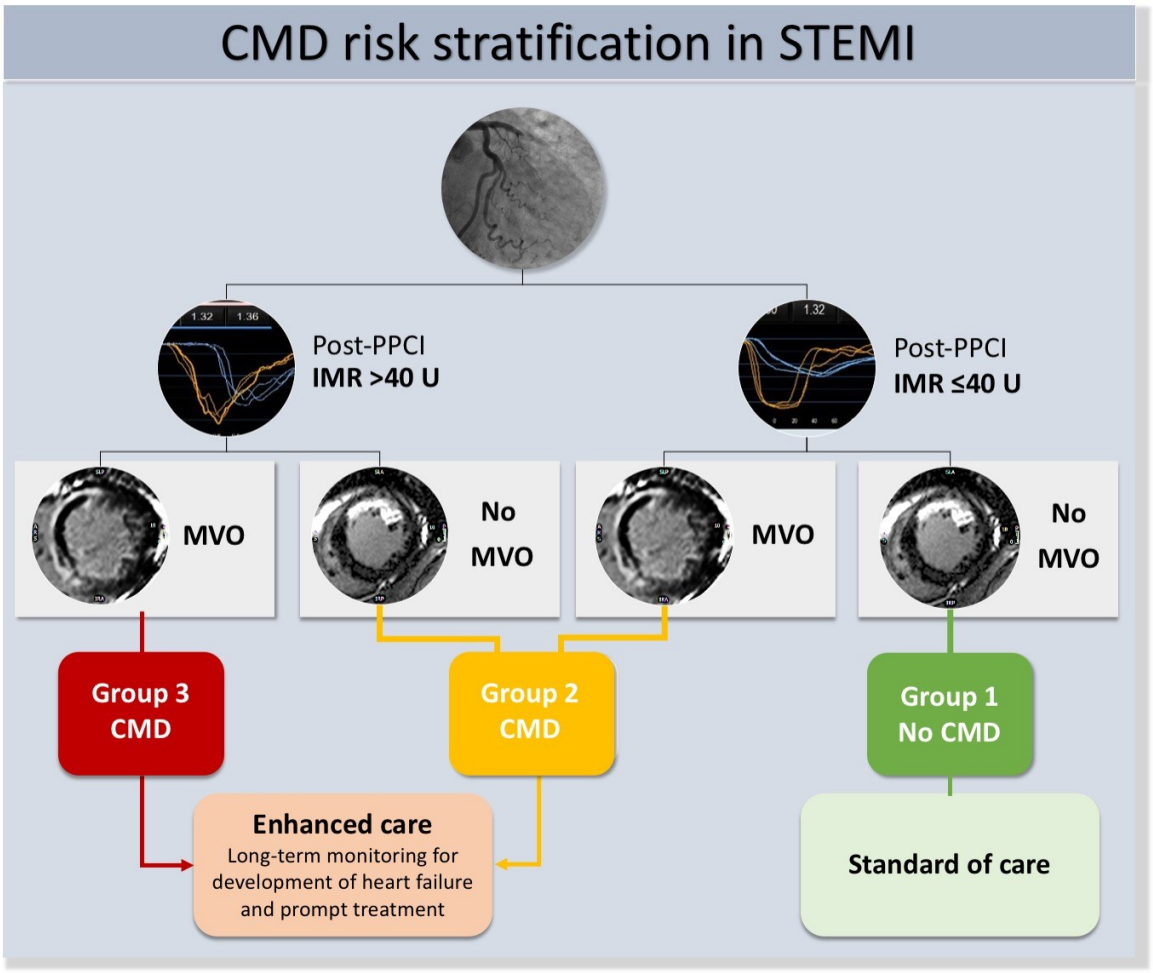
The difference in the primary endpoint between groups was mainly driven by the development of HF at follow up and there was no difference in all-cause mortality. These observations on long-term clinical outcomes further confirm and extend the previously reported effect of combined IMR and MVO on IS and left ventricular remodelling at 6 months post-STEMI[7]. However, contrary to previous speculation that patients with discordant IMR and MVO could represent a group of patients with moderate CMD and therefore at intermediate risk, this study suggests that this is only partially true. This is because patients in this group tend to develop HF after the first year and, eventually, at long term, a prognosis not dissimilar from those of patients with both IMR>40 and MVO at completion of PPCI. Our data suggest that these patients should be carefully followed-up and considered at risk of developing late HF.

We previously observed that in patients where the IMR is preserved, even in presence of MVO, a significant regression of the infarct size over time is possible, whereas in patients with an IMR above 40 Units, the microvasculature in the infarct zone appeared to be irreversibly damaged[7]. Inevitably, IMR and MVO are dynamic processes, and the extent of any abnormality will regress after the acute phase of STEMI. Consideration of our data suggests that MVO represents a severe perfusion defect and a profound marker of microvascular injury, especially when associated with intramyocardial haemorrhage.

On the other hand, IMR measured acutely is probably a combination of reversible stunning the of the microcirculation and irreversible damage related to the ischemia and reperfusion injury and/or distal embolization. Notably, IMR and MVO can reflect residual CMD and the disintegrity of the watershed zones adjacent to the infarct core, which are ultimately responsible for the final extent of the infarct size. It is also possible that an elevated IMR measured post infarct could contain a proportion of pre-existing CMD and it may not be entirely be related to the acute ischemic injury[11].

By offering a good compromise between ease of use and diagnostic accuracy, IMR is becoming the preferred method for the assessment of microvascular status in the

catheterization laboratory. Based on our study, IMR should be measured immediately post PPCI to detect clinically significant CMD. Our study also supports the use of CMR to detect MVO. If either or both are found, the patient can be diagnosed with clinically significant and prognostically important post-ischemic CMD. In such cases, close long-term follow up should be initiated to detect and treat the onset of heart failure (Central illustration).



Central illustration. Proposed risk stratification pathway post PPCI

IMR is measured post PCI in the catheterization laboratory. CMR is performed before hospital discharge. If both IMR>40 U and MVO are present patient should be considered at high risk of adverse outcomes both in the short and long terms. If either are present, they should be considered at high risk in the long term. Both of these groups (2 and 3) should be given 'enhanced care'; with close and regular clinical follow up, optimal medical therapy and prompt treatment for heart failure upon detection.

CONCLUSIONS

The presence of CMD post-myocardial infarction, predicts a more than 4-fold increase in long-term risk of adverse outcomes, which is mainly driven by the occurrence of heart failure. Importantly, patients with abnormality of either one of the CMD indices (Group 2: IMR >40 U or MVO) also presented a similar long-term risk of adverse outcome compared with patients with neither elevated IMR and MVO (Group 1).

Limitations

This is a single centre study with a relatively small sample size. Importantly, further large dedicated studies are warranted to confirm these observations. Nevertheless, this is the largest report available in which post-ischemic CMD has been assessed using a multimodality approach in the same patient, including invasive physiology and CMR assessment of MVO. Secondly, the survival analysis was conducted using a time-to-first-event approach. Therefore, the risk of having subsequent multiple events was not analysed in this study. Furthermore, the OxAMI study was not designed to detect differences in mortality between subgroup of patients stratified according to IMR and MVO.

In this study only HF with reduced ejection fraction has been considered, as systolic dysfunction is the predominant phenomenon after STEMI and due to the overwhelming evidence that post-ischemic CMD is contributory. However, it is possible that pre-existing CMD may have caused worse hemodynamic profile and high filling pressures in our patients with STEMI as previously reported in patients with HF with preserved ejection fraction (HFpEF)[30].

SUPPLEMENTARY MATERIAL

1. Cardiovascular magnetic resonance (CMR) protocol

CMR was performed using a 3.0 Tesla magnetic resonance scanner (either MAGNETOM TIM Trio or MAGNETOM Verio; Siemens Healthcare, Erlangen, Germany) within 48 hours after PPCI and at 6-month follow-up.

The scan protocol comprised Steady-State Free Precession (SSFP) for functional images, Shortened Modified Look-Locker Inversion recovery (ShMOLLI) native T1-mapping for area at risk characterization (1), T2* mapping and T2-weighted (T2-prepared SSFP) for intramyocardial hemorrhage assessment, and late gadolinium enhancement (LGE) for infarct size and MVO quantification.

Typical acquisition parameters for steady-state free precession (SSFP) retrospectively gated cine images were TE / TR =1.4/3.2 ms; flip angle 50°; voxel size: 2.4 x 1.8 x 8.0 mm.

T2W was performed using a T2-prep-SSFP single shot sequence with surface coil correction (TE/TR = 1/4.1 msec; effective TE = 60 msec; flip angle 90°; voxel size: 2.1 x 1.6 x 8.0 mm).

ShMOLLI T1 maps were generated from 5-7 SSFP images with variable inversion preparation S2 time as described previously (1). Typical acquisition parameters were: TE/TR = 1.07/2.14 msec, flip angle=35°, FOV=340×255mm, matrix size=192×144, 107 phase encoding steps, actual experimental voxel size = 1.8 × 1.8 × 8 mm, interpolated reconstructed voxel size = 0.9 x 0.9 x 8 mm, GRAPPA = 2, 24 reference lines, cardiac delay time TD = 500 msec and 206 msec acquisition time for single image, phase partial Fourier 6/8.

T2* maps were obtained using a gradient echo sequence. Typical imaging parameters were: flip angle 20°; voxel size 1.8 x 1.8 x 8 mm.

LGE was performed with a T1-weighted segmented inversion recovery gradient echo-phase sensitive-inversion recovery (GRE_PSIR) sequence (TE/TR = 2.5 msec/5 msec, voxel size =1.8 x 1.4 x 8.0 mm, flip angle 20°). LGE images were collected 10-15 min after the administration of 0.1 mmol/kg contrast agent (Dotarem, Guerbet, Villepinte, France). The inversion time was adjusted for optimal nulling of remote normal myocardium.

References

1. Piechnik SK, Ferreira VM, Dall'Armellina E et al. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. J Cardiovasc Magn Reson. 2010;19:12-69.

Supplementary Table 1. Clinical and procedural characteristics of patients lost at follow up

Variable	**Patients lost at follow-up (n=8)**	**Patients included in the analysis (n=198)**	**p-value**
Age, years	66.5(57.2-74.5)	61.0(53.0-68.0)	0.14
Sex male, %	6(75.0)	170(85.9)	0.32
Hypertension, %	6(75.0)	88(44.9)	0.15
Diabetes, %	1(12.5)	39(19.8)	0.51
Smoker, %	1(12.5)	72(36.7)	0.26
Ischemic time, min	285(194-386)	183(125-347)	0.14
Systolic blood pressure on admission, mmHg	123(110-160)	128(110-150)	0.73
Heart rate on admission, bpm	82(66-92)	80(68-90)	0.77
Culprit vessel			0.84
LAD	3(37.5)	98(49.5)	
LCX	1(12.5)	21(10.6)	
RCA	4(50.0)	75(37.9)	
Diagonal	0(0.0)	4(2.0)	
TIMI pre			0.42
0-1	5(62.5)	165(83.3)	
2-3	3(37.5)	33(16.7)	

TIMI post			0.12
0-1	1(12.5)	3(1.5)	
2-3	7(87.5)	195(98.5)	
Post-PPCI FFR	0.95(0.84-1.00)	0.93(0.90-0.98)	0.78
Post-PPCI CFR	1.59(1.43-2.51)	1.58(1.18-2.28)	0.64
Post-PPCI IMR	36.8(23.8-40.2)	31.7(20.0-50.5)	0.87
LVEF% within 48h	50(42-58)	49(40-53)	0.64
IS% within 48h	26.0(2.0-33.0)	24.6(15.5-34.0)	0.68
MVO%	0.0(0.0-8.0)	0.5(0.0-3.14)	0.98

Supplementary Table 2. Predictors of CMD defined as high IMR and/or MVO

Univariate logistic regression analysis		
Variable	**OR (95%CI)**	**p-value**
Age	1.01 (0.99-1.04)	0.355
Male sex	1.22 (0.53-2.77)	0.639
LAD	2.31 (1.27-4.22	0.006
Smoker	0.40 (0.22-0.73)	0.003
Hypertension	0.99 (0.55-1.80)	0.995
Hypercholesterolemia	1.50 (0.77-2.95)	0.236
Diabetes	2.10 (0.93-4.72)	0.074
Ischemic time	1.00 (0.99-1.01)	0.650
Clopidogrel	1.35 (0.62-2.96)	0.451
Ticagrelor	0.68 (0.33-1.39)	0.294
IIbIIIa	1.20 (0.59-2.43)	0.617
Thrombus score	1.79 (1.31-2.44)	<0.0001
Predilatation	1.28 (0.34-4.75)	0.714
Postdilatation	0.99 (0.39-2.53)	0.990
Number of stents	0.70 (0.40-1.26)	0.212
Stent volume	1.01 (0.99-1.01)	0.690
TIMI pre	0.52 (0.38-0.73)	<0.0001
TIMI post	0.20 (0.06-0.68)	0.010
MBG	0.69 (0.48-0.98)	0.041

SBP on admission	1.01 (0.99-1.02)	0.115
DBP on admission	1.01 (0.99-1.03)	0.131
Multivariate Logistic Regression analysis		
Variable	**OR (95% CI)**	**p-value**
LAD	2.53 (1.14-5.66)	0.023
Smoker	0.47 (0.21-1.06)	0.070
Diabetes	3.20 (1.05-9.72)	0.040
Thrombus score	1.41 (0.87-2.27)	0.158
TIMI pre	0.53 (0.28-0.99)	0.049
TIMI post	0.50 (0.14-1.82)	0.291
MBG	0.64 (0.36-1.13)	0.125
Hosmer-Lemeshow goodness-of-fit test: Chi-square=6.80, p=0.558).		

Supplementary Table 3. Predictors of Group 2 defined as the presence of high IMR or MVO

Univariate logistic regression analysis		
Variable	**OR (95%CI)**	**p-value**
Age	1.02 (0.99-1.05)	0.148
Male sex	0.77 (0.34-1.72)	0.522
LAD	1.21 (0.69-2.14)	0.503
Smoker	0.67 (0.36-1.22)	0.187
Hypertension	1.42 (0.80-2.51)	0.234
Hypercholesterolemia	1.32 (0.70-2.46)	0.389
Diabetes	1.96 (0.96-3.97)	0.063
Ischemic time	0.99 (0.99-1.00)	0.284
Clopidogrel	1.75 (0.76-4.05)	0.189
Ticagrelor	0.66 (0.32-1.38)	0.272
IIbIIIa	0.90 (0.45-1.77)	0.756
Thrombus score	1.37 (1.01-1.86)	0.045
Predilatation	6.75 (0.83-54.73)	0.074
Postdilatation	1.36 (0.54-3.44)	0.515
Number of stents	1.08 (0.62-1.90)	0.776

Stent volume	1.00 (0.99-1.01)	0.636
TIMI pre	0.68 (0.48-0.96)	0.030
TIMI post	1.36 (0.64-2.86)	0.423
MBG	0.64 (0.36-1.13)	0.125
SBP on admission	1.01 (0.99-1.02)	0.352
DBP on admission	1.01 (0.99-1.03)	0.168
Multivariate Logistic Regression analysis		
Variable	**OR (95% CI)**	**p-value**
Diabetes	1.89 (0.82-4.38)	0.135
Thrombus score	1.15 (0.75-1.76)	0.530
TIMI pre	0.64 (0.35-1.17)	0.146
Predilatation	6.89 (0.83-57.07)	0.073
Hosmer-Lemeshow goodness-of-fit test: Chi-square=6.82, p=0.234).		

Supplementary Table 4. Predictors of Group 3 defined as the presence of high IMR and MVO

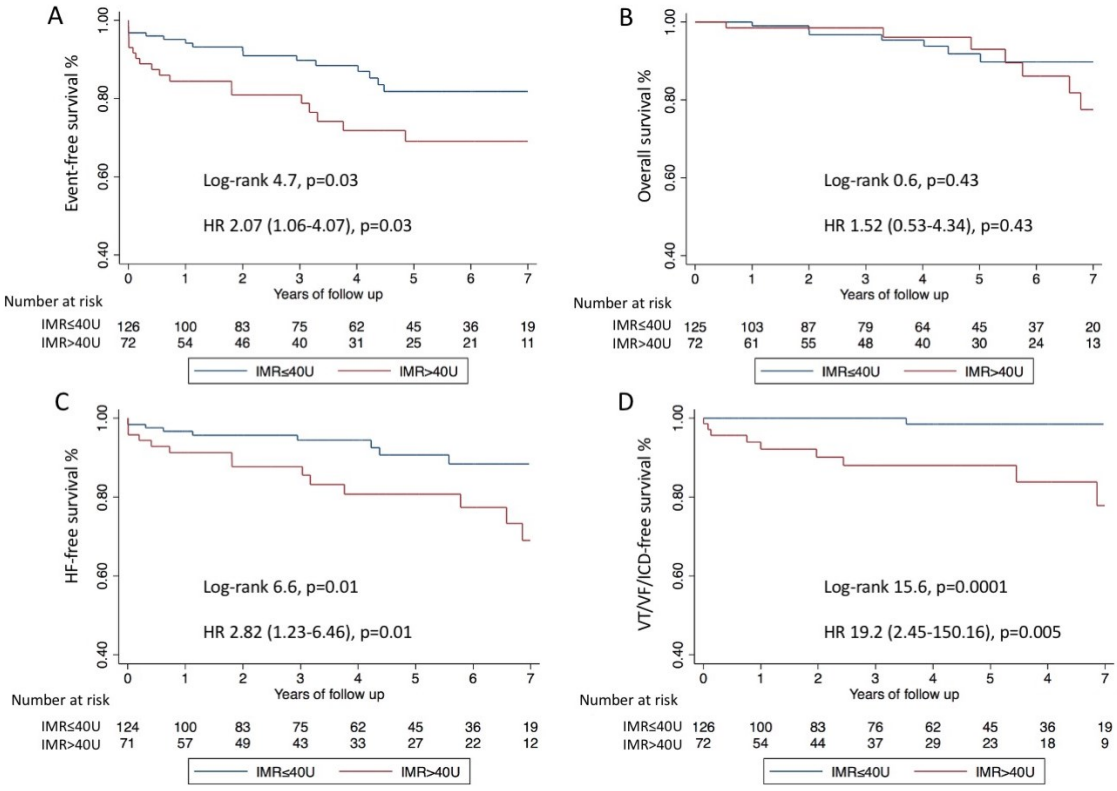
Univariate logistic regression analysis		
Variable	**OR (95%CI)**	**p-value**
Age	0.99 (0.96-1.02)	0.523
Male sex	2.03 (0.67-6.19)	0.212
LAD	2.21 (1.12-4.35)	**0.021**
Smoker	0.51 (0.24-1.06)	**0.071**
Hypertension	0.62 (0.32-1.22)	0.170
Hypercholesterolemia	1.12 (0.55-2.28)	0.757
Diabetes	0.95 (0.41-2.17)	0.898
Ischemic time	1.00 (0.99-1.01)	**0.084**
Clopidogrel	0.75 (0.32-1.76)	0.506
Ticagrelor	1.04 (0.46-2.32)	0.931
IIbIIIa	1.43 (0.67-3.05)	0.354

Thrombus score	1.47 (1.01-2.16)	**0.046**
Predilatation	0.30 (0.08-1.09)	**0.067**
Postdilatation	0.68 (0.26-1.82)	0.447
Number of stents	0.46 (0.19-1.13)	**0.090**
Stent volume	1.00 (0.99-1.01)	0.289
TIMI pre	0.63 (0.40-0.99)	**0.049**
TIMI post	0.23 (0.10-0.50)	**<0.0001**
MBG	0.48 (0.32-0.71)	**<0.0001**
SBP on admission	1.01 (0.99-1.02)	0.455
DBP on admission	1.01 (0.98-1.02)	0.903
Multivariate Logistic Regression analysis		
Variable	**OR (95% CI)**	**p-value**
LAD	1.31 (0.51-3.32)	0.574
Smoker	0.36 (0.12-1.07)	0.067
Ischemic time	1.01 (1.00-1.02	0.013
Thrombus score	0.96 (0.55-1.67)	0.887
TIMI pre	0.51 (0.20-1.33)	0.171
TIMI post	0.67 (0.21-2.18)	0.508
MBG	0.42 (0.21-0.84)	0.014
Number of stents	0.76 (0.26-2.23)	0.618
Hosmer-Lemeshow goodness-of-fit test: Chi-square 11.5, p=0.176).		

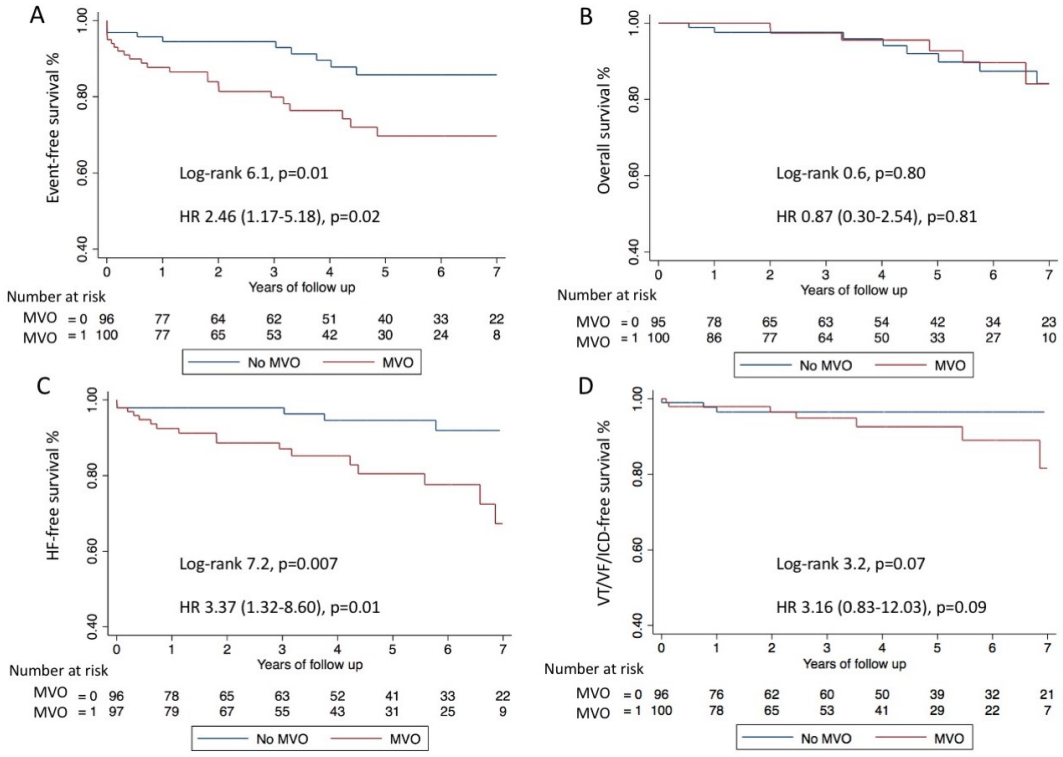
Supplementary Table 5. Cox regression analysis including CMD, TIMI flow and infarct size

Models	HR (95%CI)	p-value
CMD (high IMR and/or MVO)	1.76 (1.09-2.85)	0.022
Post-PPCI TIMI Flow	0.51 (0.28-0.95)	0.033
CMD (high IMR and/or MVO)	1.74 (1.02-2.98)	0.042
Infarct size at 48 hours	1.02 (0.99-1.05)	0.230
Group 2 (IMR>40 or MVO)	3.82 (1.28-11.35)	0.016
Group 3 (IMR>40 and MVO)	4.04 (1.24-13.20)	0.021
Post-PPCI TIMI Flow	0.50 (0.27-0.92)	0.027
Group 2 (IMR>40 or MVO)	4.91 (1.38-17.52)	0.014
Group 3 (IMR>40 and MVO)	4.93 (1.23-19.71)	0.024
Infarct size at 48 hours	1.01 (0.99-1.04)	0.325

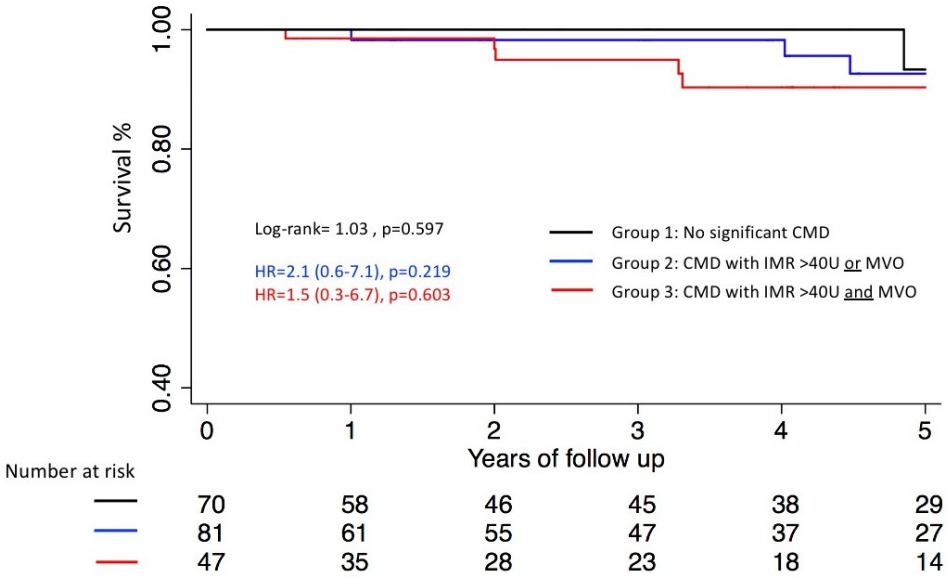
Supplementary Figure 1. Association of high IMR (>40 U) with the primary and secondary outcomes



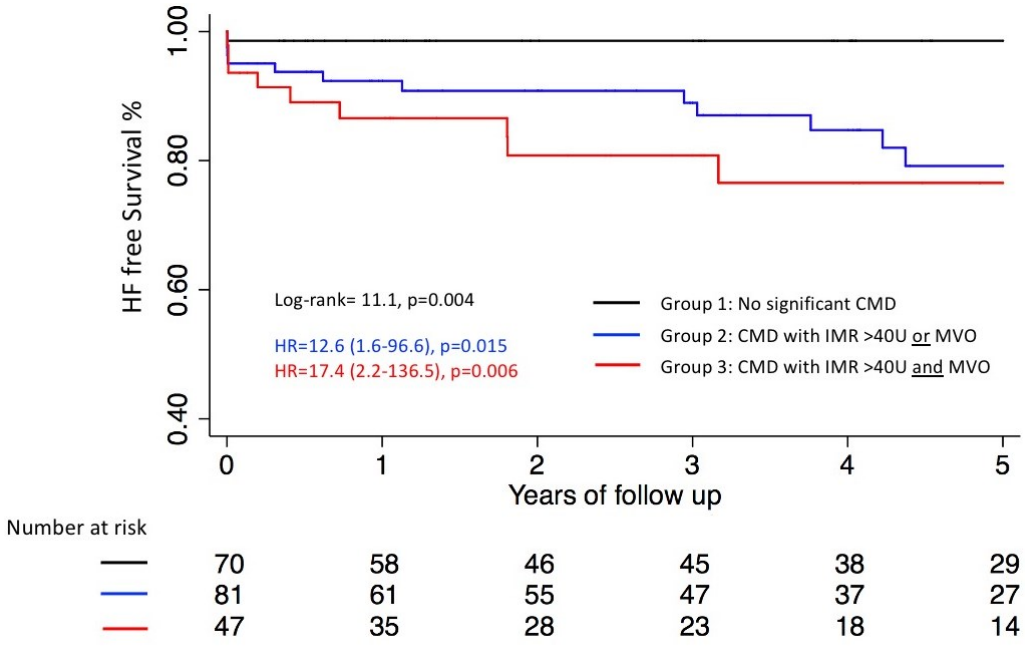
Supplementary Figure 2. Association of MVO with the primary and secondary outcomes



Supplementary Figure 3. Association between CMD Groups and all-cause mortality



Supplementary Figure 4. Association between CMD Groups and heart failure



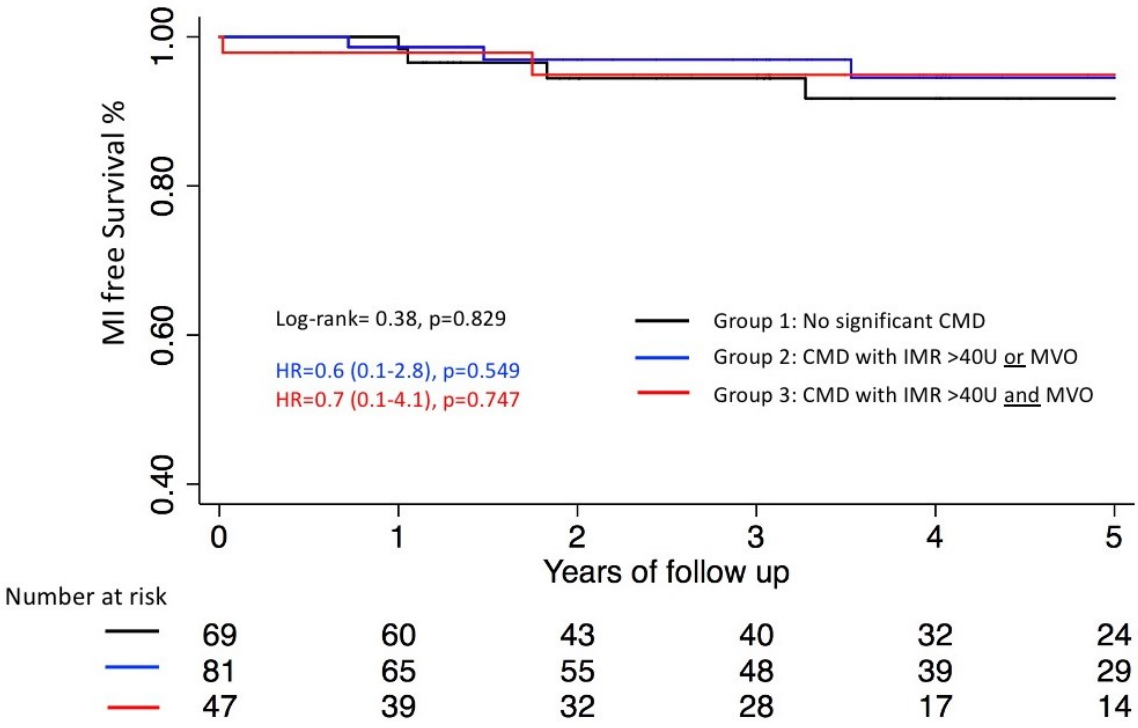
Supplementary Table 6. Predictors of all-cause mortality

Univariate			**Multivariate**	
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age, years	1.09 (1.03-1.16)	**0.003**	1.14 (1.06-1.23)	**<0.0001**
Sex male	0.45 (0.14-1.44)	0.182	-	
Smoking	0.52 (0.14-1.85)	0.311	-	
Hypertension	0.83 (0.28-2.48)	0.742	-	
Diabetes	1.88 (0.59-6.06)	0.286	-	
Insulin-dependent diabetes	3.85 (0.86-17.25)	0.078	-	
Previous MI	1.32 (0.29-5.93)	0.713	-	
Creatinine on admission	1.01 (0.99-1.03)	0.089		
Pain-to-balloon time	1.01 (0.99-1.02)	0.791	-	
Troponin (peak)	1.00 (0.99-1.01)	0.696	-	
IIb-IIIa	1.02 (0.31-3.35)	0.970	-	
N.vessel disease	1.14 (0.44-2.92)	0.787	-	
Thrombus score	1.15 (0.62-2.12)	0.648	-	
Thrombus aspiration	0.58 (0.17-1.96)	0.385	-	
TIMIpre	1.01 (0.59-1.74)	0.961	-	
TIMIpost	1.84 (0.26-12.9	0.539	-	
MBG	1.24 (0.59-2.64)	0.569	-	
post-PCI FFR	44.6 (0.01-59.6)	0.523	-	
post-PCI tTmn	1.92 (0.71-5.18)	0.196	-	
post-PCI CFR	0.77 (0.36-1.65)	0.507	-	
post-PCI IMR	1.01 (0.99-1.01)	0.648	-	
post-PCI IMR>40 U	1.76 (0.62-5.03)	0.290	-	
CMR				
EDV	0.99 (0.98-1.01)	0.656	-	
ESV	1.01 (0.98-1.02)	0.713	-	
LVEF%	0.98 (0.92-1.03)	0.423		
IS%	1.01 (0.97-1.04)	0.718	-	
MVO%	0.99 (0.87-1.14)	0.934	-	
MVO>0	0.93 (0.32-2.69)	0.890	-	
EDV at 6 months	1.01 (0.99-1.01)	0.870	-	
ESV at 6 months	1.01 (0.99-1.02)	0.248	-	
LVEF% at 6 months	0.94 (0.89-0.99)	**0.029**	0.93 (0.88-0.97)	**0.004**
IS% at 6 months	1.04 (0.99-1.08)	0.110	-	
IMR-MVO groups				
IMR>40 and MVO	1.78 (0.52-6.12)	0.355	-	
IMR>40 or MVO	1.43 (0.32-6.40)	0.639	-	
* *excluded from the multivariate analysis because of collinearity*				

Supplementary Table 7. Predictors of heart failure				
Univariate			**Multivariate**	
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age, years	1.03 (0.99-1.07)	0.157	-	
Sex male	1.04 (0.36-3.00)	0.945	-	
Smoking	0.82 (0.36-1.89)	0.647	-	
Hypertension	1.98 (0.91-4.31)	0.087	-	
Diabetes	1.10 (0.41-2.94)	0.847	-	
Insulin-dependent diabetes	1.38 (0.18-10.4)	0.753	-	
Previous MI	2.42 (0.79-7.46)	0.122	-	
eGFR	0.99 (0.97-1.02)	0.474	-	
Creatinine on admission	0.99 (0.97-1.02)	0.876		
Pain-to-balloon time	1.01 (1.00-1.02)	**0.011**	1.01 (1.00-1.02)	**0.042**
Troponin (peak)	1.00 (0.99-1.01)	0.100	-	
ACEi	1.14 (0.40-3.20)	0.801	-	
Beta-blockers	1.03 (0.30-3.57)	0.962	-	
Statin	2.17 (0.84-5.60)	0.110	-	
N.vessel disease	1.33 (0.74-2.39)	0.333	-	
Infarct-related artery (LAD)	1.27 (0.59-2.71)	0.536		
Thrombus score	1.37 (0.85-2.23)	0.199	-	
Thrombus aspiration	2.55 (0.76-8.57)	0.130	-	
TIMIpre	0.70 (0.41-1.18)	0.181	-	
TIMIpost	0.35 (0.19-0.67)	**0.002**	1.16 (0.44-3.11)	0.761
MBG	0.63 (0.41-0.98)	**0.041**	0.79 (0.44-1.41)	0.417
post-PCI FFR	0.86 (0.01-702.35)	0.966	-	
post-PCI tTmn	1.67 (0.85-3.25)	0.125	-	
post-PCI CFR	0.54 (0.29-1.00)	**0.052**	*	
post-PCI IMR	1.01 (1.00-1.02)	**0.006**	*	
post-PCI IMR>40 U	2.61 (1.21-5.62)	**0.014**	*	
CMR				
EDV	1.01 (1.00-1.03)	**0.001**	*	
ESV	1.02 (1.01-1.04)	**<0.0001**	*	
LVEF%	0.89 (0.85-0.93)	**<0.0001**	0.91 (0.86-0.96)	**<0.0001**
IS%	1.05 (1.02-1.08)	**0.002**	*	
MVO%	1.11 (1.05-1.18)	**0.001**	*	
MVO>0	3.97 (1.58-9.95)	**0.003**	*	
EDV at 6 months	1.01 (1.00-1.02)	**0.001**	*	
ESV at 6 months	1.02 (1.01-1.03)	**<0.0001**	*	
LVEF% at 6 months	0.90 (0.86-0.93)	**<0.0001**	*	
IS% at 6 months	1.06 (1.03-1.10)	**<0.0001**	*	
IMR-MVO groups				
IMR>40 and MVO	17.40 (2.20-136.51)	**0.006**	2.10 (1.14-3.89)	**0.017**

IMR>40 or MVO	12.61	(1.60-96.65)	**0.015**
** excluded from the multivariate analysis because of collinearity*			

Supplementary Figure 5. Association between CMD Groups and recurrent myocardial infarction



Supplementary Table 8. Predictors of recurrent myocardial infarction					
Univariate			**Multivariate**		
Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**		**p-value**
Age, years	0.98 (0.93-1.05)	0.678	-		
Sex male	1.40 (0.17-11.22)	0.749	-		
Smoking	1.01 (0.25-4.06)	0.983	-		
Hypertension	0.63 (0.16-2.50)	0.508	-		
Diabetes	8.23 (2.05-32.95)	**0.003**	4.90 21.24)	(1.11-	**0.035**
Insulin-dependent diabetes	10.14 (2.10-48.97)	**0.004**	*		
Previous MI	1.05 (0.13-8.37)	0.965	-		
eGFR	0.99 (0.97-1.03)	0.887	-		
Pain-to-balloon time	1.01 (0.99-1.01)	0.118	-		
Troponin (peak)	1.03 (0.99-1.01)	0.179	-		
ACEi	0.82 (0.17-3.93)	0.801	-		
Beta-blockers	0.77 (0.09-6.18)	0.808	-		
Statin	3.13 (0.84-11.67)	0.089	-		
N.vessel disease	1.59 (0.71-3.58)	0.261	-		
Infarct-related artery (LAD)	0.84 (0.22-3.13)	0.797			
Thrombus score	0.53 (0.34-0.82)	**0.005**	0.64 1.02)	(0.40-	0.06
Thrombus aspiration	1.06 (0.22-5.13)	0.940	-		
TIMIpre	1.58 (0.93-2.68)	0.092	-		
TIMIpost	0.39 (0.12-1.28)	0.121			
MBG	0.71 (0.33-1.57)	0.403			
post-PCI FFR	0.96 (0.93-1.01)	0.106	-		
post-PCI tTmn	1.04 (0.28-3.83)	0.957	-		
post-PCI CFR	0.20 (0.04-1.06)	0.058	*		
post-PCI IMR	1.01 (0.99-1.01)	0.869	*		
post-PCI IMR>40 U	0.48 (0.10-2.32)	0.364	*		
Post-PCI RRR					
CMR					
LVEF%	0.99 (0.92-1.06)	0.732			
IS%	1.02 (0.97-1.07)	0.403	*		
MVO%	1.09 (0.94-1.24)	0.238	*		
MVO>0	1.19 (0.32-4.44)	0.793	*		
LVEF% at 6 months	1.05 (0.96-1.15)	0.268	*		
IS% at 6 months	0.99 (0.92-1.06)	0.694	*		
IMR-MVO groups					
IMR>40 and MVO	0.75 (0.14-4.13)	0.747			
IMR>40 or MVO	0.63 (0.14-2.83)	0.549			
** excluded from the multivariate analysis because of collinearity*					

CHAPTER 2.

Towards the simplification of CMD assessment: Pressure-bounded coronary flow reserve in patients with STEMI.

ABSTRACT

Aims. Assessment of microvascular function in patients with ST-elevation acute myocardial infarction (STEMI) may be useful to determine treatment strategy. The possible role of pressure-bounded coronary flow reserve (pb-CFR) in this setting has not been determined.

Methods and Results. Thermodilution-pressure-wire assessment of the infarct-related artery was performed in 148 STEMI patients before stenting and/or at completion of primary percutaneous coronary intervention (PPCI). The extent of the myocardial injury was assessed with cardiovascular magnetic resonance imaging at 48-hours and 6-months after STEMI. Post-PPCI pb-CFR was impaired (<2) and normal (>2) in 69.9% and 9.0% of the cases respectively. In the remaining 21.1% of the patients, pb-CFR was "indeterminate".

In this cohort, pb-CFR correlated poorly with thermodilution-derived coronary flow reserve (k=0.03, p=0.39). The index of microcirculatory resistance (IMR) was significantly different across the pb-CFR subgroups. Similarly, significant differences were observed in microvascular obstruction (MVO), myocardium area-at-risk and 48-hours infarct-size (IS). A trend towards lower 6-month IS was observed in patients with high (>2) post-PPCI pb-CFR. Nevertheless, pb-CFR was inferior to IMR in predicting MVO and the extent of IS.

Conclusions. Pb-CFR can identify microvascular dysfunction in patients after STEMI and provided superior diagnostic performance compared to thermodilution-derived CFR in predicting MVO. However, IMR was superior to both pb-CFR and thermodilution-derived CFR and consequently, IMR was the most accurate in predicting all of the studied CMR endpoints of myocardial injury after PPCI.

INTRODUCTION

Coronary physiology is a useful tool to assess the extent of coronary microvascular dysfunction in patients with ST-elevation acute myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI)[3].

The presence of microvascular obstruction (MVO) or high values of index of microcirculatory resistance (IMR) have been associated with poor myocardial reperfusion after PPCI, larger infarct size and worse long-term clinical outcome[3, 7, 14]. Moreover, STEMI patients with high IMR are at increased risk of post-procedural and in-hospital complications compared with patients with low post-PPCI IMR[17].

However, the use of coronary physiology to assess the extent of microvascular dysfunction in STEMI patients remains limited in routine clinical practice, partly because of the complexity of the available techniques to assess coronary flow and coronary resistance in the catheterization laboratory[31].

Recently, pressure-bounded coronary flow reserve (pb-CFR) has been proposed to estimate CFR using standard pressure-wire technology, obviating the need for intracoronary thermodilution or doppler-velocity measurements[32]. pb-CFR demonstrated a good correlation with Doppler and thermodilution-derived CFR although its value to predict clinical outcomes remains uncertain[32-34].

The diagnostic accuracy of pb-CFR in detecting the extent of coronary microvascular dysfunction and predicting myocardial injury has not been assessed in patients with STEMI. In this study we aimed to compare pb-CFR with thermodilution derived physiology including IMR and CFR_{thermo} in a consecutive series of patients enrolled in the Oxford Acute Myocardial Infarction (OxAMI) study. Moreover, we aimed to assess the presence of MVO and myocardial injury on cardiovascular magnetic resonance imaging (CMR) performed at 48 hours and 6 months in STEMI patients stratified according to pb-CFR.

METHODS

Patients with STEMI admitted to the Oxford Heart Centre for PPCI were prospectively considered for enrolment in the Oxford Acute Myocardial Infarction (OxAMI) Study (REC number 10/H0408/24). The study protocol was approved by

the local ethics committee and conducted in accordance with the Declaration of Helsinki. Details of the OxAMI study have been previously described[3]. The diagnosis of STEMI required chest pain lasting at least 30 min, within 12 h from onset of symptoms, and ST-segment elevation of >2 mm (0.2 mV) in at least 2 contiguous leads on ECG. Symptom duration >12 hours, presence of severe hemodynamic instability, severe left main disease, contraindications to adenosine infusion, balloon angioplasty without stent implantation and general contraindications to CMR were all exclusion criteria for this analysis. PPCI was performed in a standard fashion and decisions about direct stenting technique, thrombectomy and glycoprotein IIb/IIIa adoption were all left to operator's discretion. All patients were loaded with dual antiplatelet therapy. Weight-adjusted unfractionated heparin or bivalirudin was adopted as antithrombotic regimen. Angiographic thrombus score was graded from 0 to 5 after the passage of the guidewire, as previously described[24].

Coronary angiography

Coronary flow was graded using the standard TIMI criteria[23]. Myocardial blush grade at the end of the procedure was evaluated according to van't Hof[25]. Angiographic no-reflow was defined as TIMI flow grade <3 and/or TIMI flow grade 3 with myocardial blush grade <2 at completion of the procedure. Two interventional cardiologists blinded to clinical and outcome parameters performed the angiographic analyses, and differences were resolved by consensus.

Invasive coronary physiology measurements

Indices of coronary physiology of the infarct-related artery were assessed after flow restoration (before stenting) and/or at completion of PPCI. IMR was defined as the mean distal pressure multiplied by the mean transit time (Tmn) at hyperemia as previously described[3] using a coronary PressureWire (Abbott - St. Jude Medical, St. Paul, Minnesota). When measured before stent implantation, IMR value was corrected for collateral flow by coronary wedge pressure (Pw), measured during prolonged balloon inflation, as follows

$$Pa_{hyp} \times Tmn_{hyp} [(Pd_{hyp}-Pw) / (Pa_{hyp} - Pw)]$$

CFR_{thermo} was defined as the ratio of hyperemic to resting coronary flow and was calculated using the equation:

Tmn_{base} / $Tmn_{at\ hyperemia}$

Pressure-bounded coronary flow reserve

The concept of pb-CFR has been proposed to estimate CFR applying a fundamental fluid dynamics equation that quantifies the pressure-gradient induced across a lesion in an epicardial coronary vessel:

$$\Delta P = f * Q + s * Q^2$$

where ΔP is the pressure gradient across the lesion, Q is coronary flow, *f* is friction coefficient and *s* is separation coefficient. *f* and *s* are geometric and rheologic properties of the lesion and the vessel.

Pb-CFR assumes that, at one extreme, the lower bound of CFR is calculated as $\sqrt{\Delta P\ during\ hyperemia})/\sqrt{\Delta P\ at\ rest}$, assuming that all the energy losses across the stenosis may be explained by separation forces and, on the other extreme, the upper CFR bound is calculated as the ratio between ΔP at hyperemia and ΔP at rest, assuming that the energy losses may be due to friction across the lesion[32]. In other words, pb-CFR defines the interval between the minimum and the maximum possible CFR values as follows:

$$\sqrt{\left[\frac{\Delta Phyp}{\Delta Prest}\right]} \leq CFR \leq \frac{\Delta Phyp}{\Delta Prest} \quad (1)$$

As reported by Ahn at al., the equation can also be rewritten as:

$$\sqrt{\frac{1-\frac{Pd}{Pa}hyp}{1-\frac{Pd}{Pa}rest}} \leq CFR \leq \frac{1-\frac{Pd}{Pa}hyp}{1-\frac{Pd}{Pa}rest} \quad (2)$$

Since Pd/Pa was available in 100% of the cases we adopted equation (2) to derive pb-CFR (Supplementary Figure 1).

Pb-CFR was considered abnormal when both the upper and the lower bounds of PB-CFR were <2 and normal when both the upper and the lower bounds were >2. In all other cases PB-CFR was considered indeterminate as previously described[32,8]. Patients with resting Pd/Pa >0.98 were excluded from the analysis.

Cardiovascular magnetic resonance image protocol and analysis

CMR was performed using a 3.0 Tesla magnetic resonance scanner (either MAGNETOM TIM Trio or MAGNETOM Verio; Siemens Healthcare, Erlangen, Germany) within 48 hours after PPCI and at 6-month follow-up. CMR protocol has been previously reported[12]. Cvi42 image analysis software (Circle Cardiovascular Imaging Inc, Calgary, Canada) was used for image analysis.

Statistical analysis

Normally distributed variables are reported as mean SD, and the Student t test used for comparisons. Nonparametric distributions are reported as median (interquartile range), and the Mann-Whitney test used for unpaired data. Difference between groups were compared with one-way ANOVA or Kruskal-Wallis as appropriate. The Fisher exact chi-square test was used for binary variables. Correlation between variables was tested by Spearman-rho method. Choen's kappa coefficient method and % agreement were used to assess the agreement between pb-CFR and CFR_{thermo}. Receiver Operating Characteristic (ROC) curve analysis was used to test the diagnostic performance of physiological variables to predict the extent of microvascular dysfunction and myocardial injury after STEMI. In calculating ROC curves for IS, the cut-off value for the highest quartile was used to define the endpoint (IS% (48h)≥38.1% and IS% (6months) ≥30.0%). Areas under the ROC curve were compared using the Delong method. In cases with repeated pre- and post-stent physiological assessment, the variations of IMR were measured using non-parametric Wilcoxon's test and variations in pb-CFR were assessed using McNemar's test. Patients were classified in good responders or partial/poor responders to stenting according to the final IMR value ≥40U, as previously described[1]. For regression and ROC curve analysis, Pb-CFR was used a binary categorical variable in the analysis, excluding patients with indeterminate results. Statistical analysis was performed using SPSS, Version 25.0 (IBM Corp., Armonk, NY, USA) and MedCalc statistical software, version 15.8 (Mariakerke, Belgium). All tests were 2-tailed and a p-value <0.05 was considered statistically significant.

RESULTS

One-hundred-and-sixty-five patients presenting with STEMI underwent coronary physiological assessment of the infarct-related artery during PPCI as part of the OxAMI study. Pb-CFR was available in 148 patients (before and/or after PPCI) and was measured before stenting in 112 patients and at completion of PPCI in 123 patients. 87 patients had both pre- and post-stenting pb-CFR data. was CMR available in all the cases (100%) at 48 hours and in 109 (74%) patients at 6 months of follow-up.

Pb-CFR in the infarct-related artery before stenting (immediately after flow restoration)

After flow restoration pb-CFR was <2 in 89/112 (79.5%) patients, >2 in 5/112 (4.5%) patients and indeterminate in 18/112 (16.0%) patients (Supplementary Table 1). No significant difference in CFR_{thermo} was observed in patients stratified according to pb-CFR (Supplementary Table 2). Notably, significant differences in pre-stenting IMR were observed stratifying the patients according to pb-CFR (Supplementary Table 2).

Correlation between pre-stenting pb-CFR and the extent of myocardial injury after STEMI

Pre-stenting pb-CFR >2 was associated with smaller myocardial AAR% (Figure 1). Moreover, a trend towards smaller infarct size at 48 hours and 6 months was observed in patients with pre-stenting pb-CFR >2 (Figure 1; Supplementary Table 1).

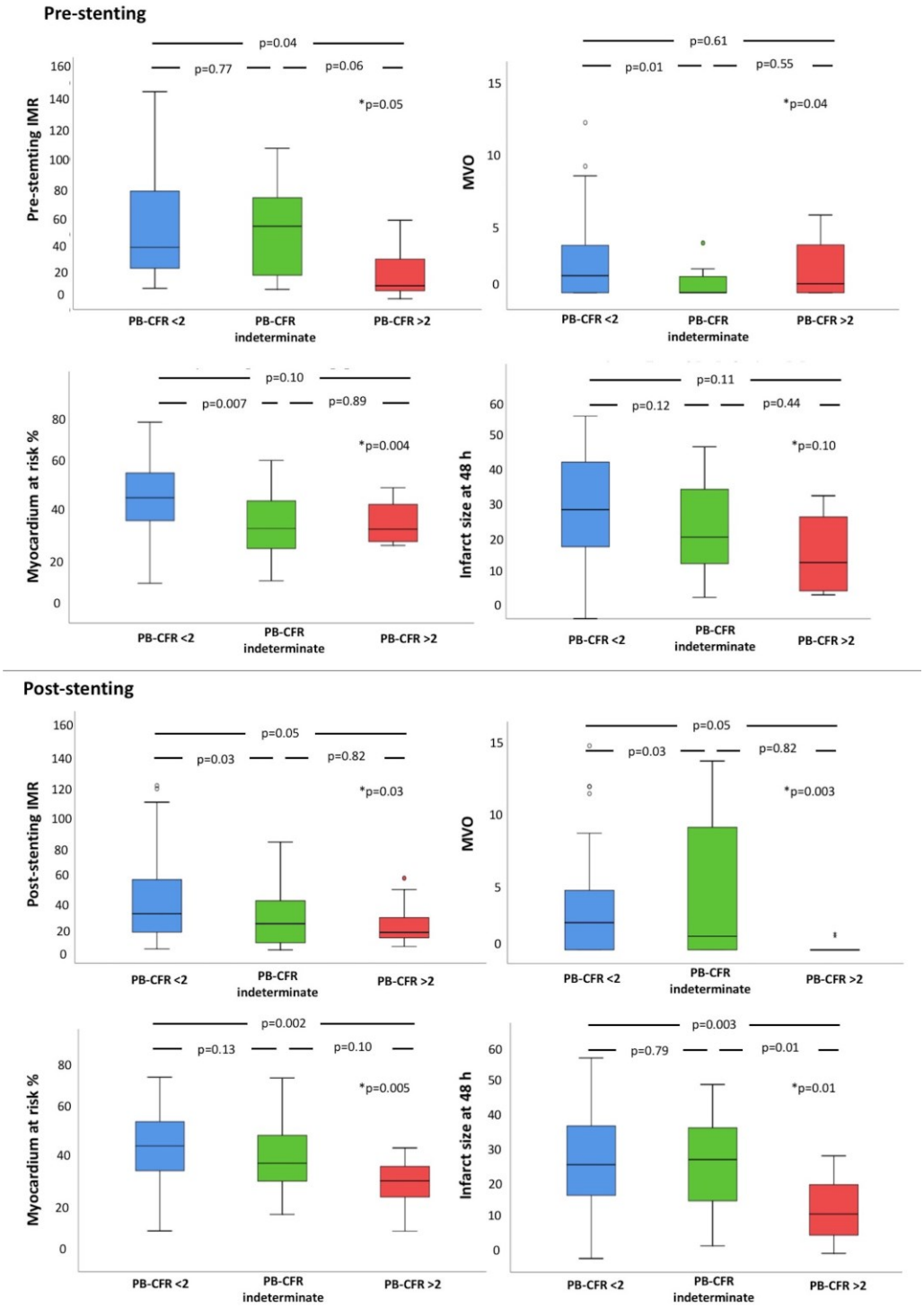


Figure 1. Upper panel: differences in IMR, MVO, myocardial AAR% and 48 hours IS% in patients stratified according to pre-stenting pb-CFR. *indicates overall p-value. Lower panel: differences in IMR, MVO, myocardial AAR% and 48 hours IS% in patients stratified according to pb-CFR measured at completion of PPCI. *indicates overall p-value.

At ROC curve analysis, pre-stenting pb-CFR demonstrated inferior but not statistically different diagnostic value compared to pre-stenting IMR in predicting the infarct size at 48 hours ($AUC_{lPB-CFR}$=0.53 [0.42-0.64] vs AUC_{IMR}=0.63 [0.52-0.73], p=0.12), the final infarct size at 6 month (AUC_{PB-CFR}=0.54 [0.42-0.67] vs AUC_{IMR}=0.64 [0.52-0.76]; p=0.17) and the presence of intramyocardial hemorrhage (AUC_{PB-CFR}=0.50 [0.32-0.68] vs AUC_{IMR}=0.60 [0.41-0.77]; p=0.35). Moreover, the performance of pb-CFR in predicting the presence of MVO was inferior but marginally non-statistically different compared with IMR (AUC_{pb-CFR}=0.52 [0.41-0.63] vs AUC_{IMR}= 0.64 [0.53-0.74], p for AUC comparison=0.052).

Table 1. Clinical and procedural characteristic of patients with STEMI stratified according to post-procedural PB-CFR

	PB-CFR <2	PB-CFR indeterminate	PB-CFR >2	p-value
Clinical data	*n=86*	*n=26*	*n=11*	
Age, years	61(54-67)	65(55-71)	54(48-68)	0.34
Sex, male	75(87)	21(81)	11(100)	0.28
Hypertension	46(53)	10(38)	8(72)	0.14
Dyslipidaemia	32(37)	17(65)	2(18)	0.10
Diabetes	36(42)	6(23)	3(27)	0.17
Smoking	39(45)	13(50)	5(45)	0.91
eGFR, ml/min/1.73m^2	94.2(78.9-106.8)	89.2(77.0-99.0)	83.0(75.0-121.1)	0.41
Pain-to-balloon time, min	193(125-379)	146(118-234)	195(167-380)	0.14
Peak troponin	32.1(8.3-67.8)	32.1(0.5-60.0)	2.5(1.99-2.5)	0.07
CMR imaging at 48 h				
EDV, ml	168.5(142.0-199.0)	148.0(125.5-180.5)	132.0(109.0-162.0)	0.005
ESV, ml	92.0(73.2-114.7)	76.0(58.0-95.0)	67.0(56.0-87.0)	0.005
LVEF%	46.5(40.0-50.7)	49.0(39.5-55.0)	48.0(46.0-55.0)	0.26
SV, ml	77.0(66.0-89.0)	66.0(55.0-84.5)	59.0(52.0-78.0)	0.044
AAR%	46.1(37.0-55.6)	39.7(31.5-53.0)	33.3(27.1-40.2)	0.005
IS% at 48h	28.7(20.4-39.3)	30.0(18.0-41.0)	15.5(8.3-25.0)	0.010

MVO, %	2.0(0.0-4.43)	1.0(0.0-10.0)	0.0(0.0-2.2)	0.003
CMR imaging at 6 months				
EDV, ml	172.5(147.5-197.5)	147.5(119.0-184.5)	159.0(120.5-165.0)	0.052
ESV, ml	78.5(57.7-103.0)	66.0(55.2-86.5)	69.0(44.0-72.0)	0.13
LVEF, %	52.5(43.0-60.0)	55.5(48.0-59.0)	57.0(55.5-65.0)	0.21
SV, ml	88.5(73.5-99.0)	81.5(59.0-93.5)	88.0(76.5-94.0)	0.37
IS at 6 m, %	21.8(13.3-30.3)	20.0(8.0-30.4)	12.9(5.5-21.8)	0.18
Salvage, %	34.5(23.5-48.8)	37.1(14.8-60.6)	42.9(32.7-64.4)	0.39

Pb-CFR in the infarct-related artery at completion of primary PCI

At completion of PPCI 86/123 (69.9%) patients presented a pb-CFR <2 and 11/123 (9.0%) patients had a pb-CFR >2. In the remaining 26/123 (21.1%) patients pb-CFR was indeterminate. Clinical, angiographic and imaging characteristics of patients stratified according to the post-PPCI pb-CFR are presented in Table 1 and Supplementary Table 3. Patients with pb-CFR >2 at completion of PPCI presented a trend toward lower frequency of LAD as culprit vessel, higher TIMI flow post-stenting and lower peak troponin level.

No significant difference was observed in CFR_{thermo} across the pb-CFR groups (Table 2). Moreover, a poor agreement was observed between pb-CFR and CFR_{thermo} (k=0.031, p=0.39; % agreement=65%; Supplementary Figure 2).

At completion of PPCI, IMR was significantly different across the pb-CFR groups, with pb-CFR>2 associated with lower IMR values (Table 2).

We found no interaction between binary Pb-CFR and culprit vessel (p for interaction=0.601).

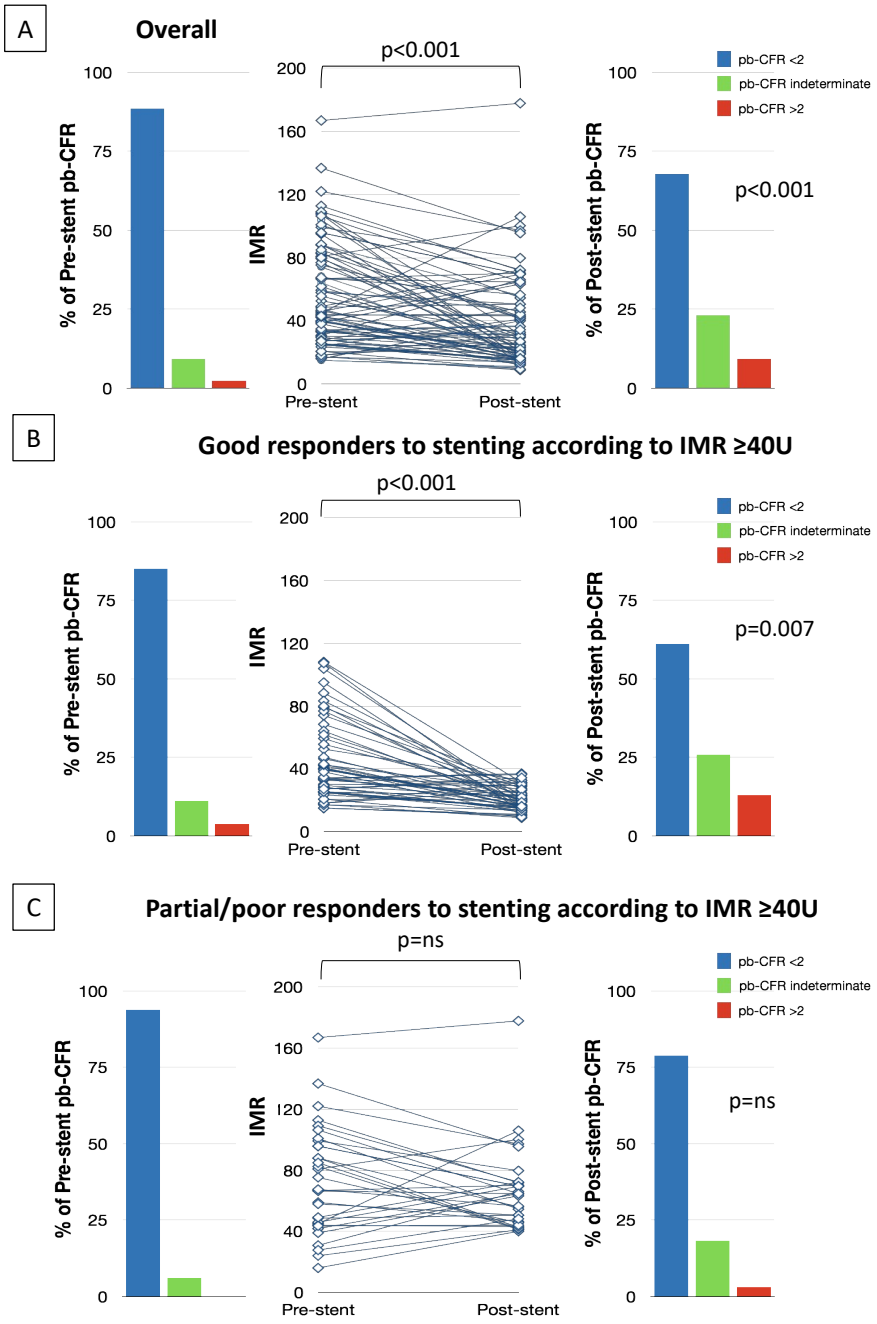


Figure 2. IMR and pb-CFR variations before and after stenting.
Patients with both pre- and post-stenting physiological measurements were classified, according to the final IMR, as good responders to stenting (post-PPCI IMR <40U) or partial/poor responders (post-PPCI IMR ≥40U). Pb-CFR improved significantly good responders but not in partial/poor responders to stenting.

Significant variations were observed in IMR values before and after stenting (44.0 [28.4-80.0] to 28.7 [16.7-50.6], p<0.001). Notably, an overall improvement was observed in pb-CFR values at completion of pPCI (p<0.001; Figure 2A; Supplementary material). 54/87 (62%) patients were classified as good responders to stenting according to final IMR value <40U. In this subgroup pb-CFR significantly improved at completion of PCI (p=0.007; Figure 2B; Supplementary material 8). Conversely no significant variations in pb-CFR were observed in patients categorized as partial or poor responders to stenting according to final IMR≥40U (Figure 2C). Notably, good responders to stenting presented smaller IS% at 48 hours and 6 months, less MVO% and greater myocardial salvage compared with partial/poor responders to stenting (Supplementary table 4; Supplementary Figure 3).

Table 2. Intracoronary physiology of patients with STEMI stratified according PB-CFR

	PB-CFR <2	PB-CFR indeterminate	PB-CFR >2	p-value
Post-Stenting				
Baseline Pd/Pa	0.93(0.91-0.95)	0.97(0.94-0.98)	0.98(0.97-0.98)	<0.001
FFR	0.91(0.89-0.94)	0.93(0.89-0.97)	0.91(0.88-0.93)	0.43
Delta PdPa-FFR,	0.01(0.0-0.03)	0.02(0.01-0.07)	0.07(0.04-0.10)	<0.001
Pd(Hyp), mmHg	75(68-88)	75(64-84)	76(67-85)	0.55
Tmn(Rest)	0.75(0.44-1.14)	0.64(0.39-1.01)	0.50(0.35-0.98)	0.23
Tmn(Hyp)	0.41(0.27-0.86)	0.39(0.20-0.55)	0.28(0.22-0.44)	0.10
CFR	1.50(1.10-2.18)	1.81(1.34-2.59)	1.96(1.35-2.46)	0.17
IMR	32.5(20.3-55.4)	26.0(13.3-41.0)	20.2(16.5-37.0)	0.03
RA pressure,				
mmHg	8(6-10)	8(2-12)	5(3-8)	0.42
LowerPB	1.10(1.00-1.22)	1.53(1.41-1.67)	2.45(2.01-2.83)	<0.001
UpperPB	1.22(1.00-1.45)	2.33(2.00-2.87)	6.00(4.00-8.00)	<0.001

Correlation between pb-CFR at completion of PPCI and the extent of myocardial injury after STEMI

Post-PCI pb-CFR was significantly associated with the myocardial AAR% and the extent of myocardial injury at 48 hours after STEMI (Figure 1). A trend towards smaller infarct size at 6 months was observed in patients with pb-CFR >2. Notably, IMR and CFR_{thermo} outperformed pb-CFR in predicting the extent of final IS (Table 3 and Supplementary Table 5-6).

Table 3. Regression analysis for pb-CFR, CFR and IMR in predicting physiological and CMR endpoints

	Pb-CFR		**CFR**		**IMR**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
IS% (48h)*	0.93 (0.32-2.66)	0.89	0.75 (0.45-1.27)	0.29	1.01 (1.00-1.02)	0.022
MVO**	0.08 (0.02-0.42)	0.003	0.71 (0.48-1.05)	0.09	1.02 (1.01-1.03)	0.008
Hemorrhage %	0.42 (0.04-4.32)	0.46	0.75 (0.43-1.31)	0.31	1.02 (1.01-1.04)	0.019
IS% (6months)*	0.92 (0.29-2.91)	0.89	0.54 (0.27-1.06)	0.07	1.02 (1.01-1.03)	0.017

IS, infarct size; MVO, microvascular obstruction.

*For infarct size (IS) at 48 hours and 6 months the cut-off value for the highest quartile was used to define the endpoint: IS% (48h)≥38.1% and IS% (6months) ≥30.0%

**The presence vs. absence of MVO at 48 hours CMRhas been used to define the endpoint MVO.

No significant differences were observed in the LV ejection fraction across pb-CFR groups 6 months after STEMI (Figure 3).

Post-PPCI pb-CFR was significantly associated with the presence of MVO (Table 1 and Figure 1). Using regression analysis, pb-CFR outperformed CFR_{thermo} in predicting the presence of MVO (OR=0.08; 95%CI: 0.02-0.42, p=0.003; Table 3) but presented only modest diagnostic accuracy at ROC curve analysis (AUC=0.63

[0.52-0.73], p=0.003). Nonetheless, a pb-CFR >2 demonstrated high sensitivity (96.7% [88.5%-99.6%]) and fair negative predictive value (81.8% [48.2%-97.7%]) in excluding the presence of MVO.

Pb-CFR was inferior compared to IMR in predicting the presence of hemorrhage and the extent of IS% at 48 hours and 6 months (Table 3 and Figure 3). Using alternate cutoffs other than 2 for Pb-CFR (1.5 or 2.5) to define microvascular dysfunction did not improve the prognostic role of the index regarding CMR endpoints (please see Supplementary Table 7).

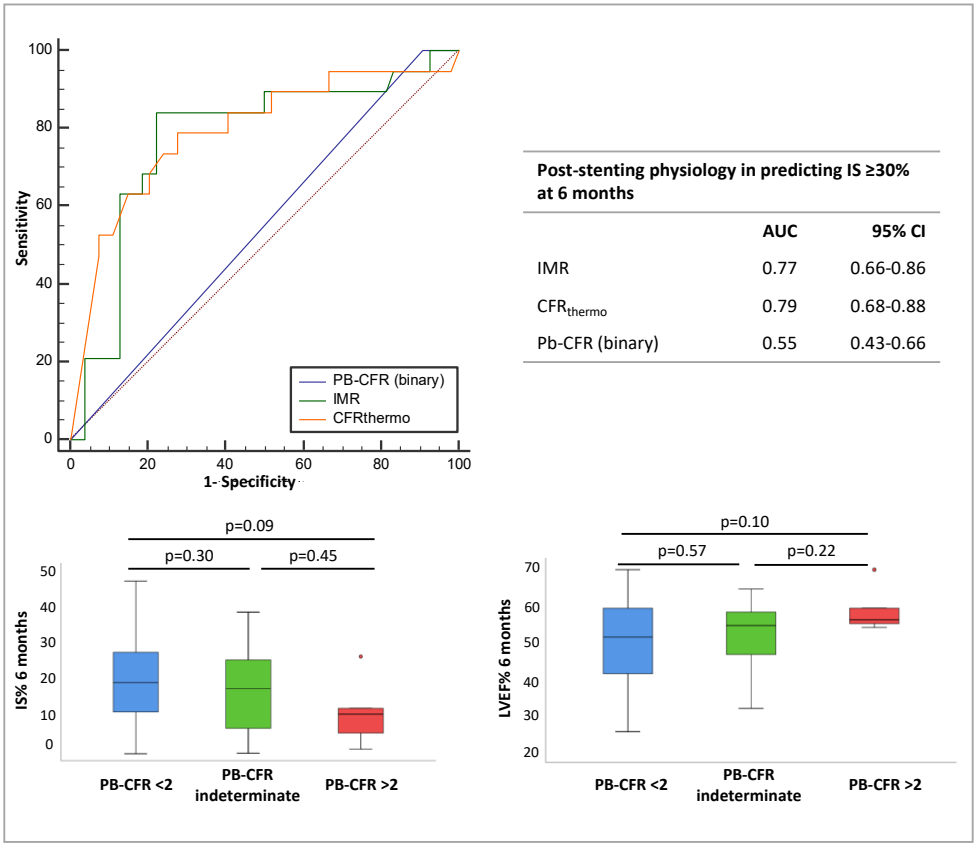


Figure 3. Diagnostic accuracy of physiological indices in predicting the final infarct size at 6 months after STEMI. IMR and CFRthermo presented a significantly higher AUC at ROC curve analysis compared with pb-CFR in predicting an IS ≥30.0% at CMR.

In the lower panel, a trend towards smaller IS% at 6 months was observed for patients with post-PPCI pb-CFR>2. Conversely, no difference in LV ejection fraction (LVEF%) was observed between the groups.

DISCUSSION

Pb-CFR measured before and after stent placement has poor correlation with CFR_{thermo} in this cohort of patients with STEMI undergoing PPCI. However, pb-CFR was associated with the extent of microvascular dysfunction assessed both in the catheterization laboratory using IMR and with MVO measured using CMR. In particular, pb-CFR was able to identify a subgroup of patients (pb-CFR >2) who experienced better reperfusion after PPCI with lower IMR, MVO and smaller acute myocardial injury after STEMI.

Unfortunately, despite the advantage of being an easy technique based on standard pressure-wire without additional measurements of transit time or other coronary flow surrogates, pb-CFR is a suboptimal index of microvascular dysfunction in the STEMI population, with inferior diagnostic metrics compared with IMR.

Prompt restoration of coronary flow in the infarct related artery by PPCI is the standard of care in patients presenting with STEMI. Nevertheless, a significant number of patients do not achieve complete myocardial reperfusion despite apparent satisfactory angiographic result in the epicardial vessel[3]. This is mainly related to microvascular injury after PPCI and it has been associated with larger infarct size, adverse LV remodelling and increased risk of heart failure and cardiovascular mortality[14, 20, 35, 36].

The identification of patients who are less likely to experience optimal reperfusion post-PPCI and may be candidates to adjunctive or alternative therapeutic strategies is a field of ongoing research[13]. Coronary physiological indices, and specifically CFR and IMR have been extensively investigated as potential tools to identify high-risk patients in the catheterization laboratory. In particular IMR emerged as an accurate index of microvascular function with good predictive value for adverse outcome after STEMI[15-17], as confirmed by this analysis (Figure 2 and supplementary table 4).

However, the use of physiological assessment in STEMI is still limited because of the additional technical complexity, the additional procedural time and requirement for dedicated equipment to measure coronary flow using either Doppler or thermodilution techniques[31].

Pb-CFR offers the advantage of avoiding thermodilution or Doppler velocity measurements and has been demonstrated to provide important information on the relationship between FFR and CFR[32, 34]. However, Ahn et al. showed that pb-CFR was not associated with clinical outcome in a large cohort of patients with stable coronary artery disease[34] and this result was recently confirmed by Wijntjens et al. at long-term follow-up[33].

The applicability of pb-CFR is also limited by the fact that it produces an indeterminate result (lower limit < 2 and upper limit > 2) in those cases that cannot be classified as normal (both limits > 2) or abnormal (both limits < 2)[32, 34]. In our study pb-CFR resulted "indeterminate" in 21.1% of the cases at completion of PPCI. Notably the proportion of cases categorized as "indeterminate" is lower than what observed by previous investigators[6-8]. It is of interest that this subgroup of patients presented intermediate risk characteristics compared with the low and high CFR groups, with lower IMR values and smaller AAR% compared with patients with pb-CFR <2, but larger IS% compared with patients with pb-CFR >2.

In this study pb-CFR measured in the infarct-related artery was impaired when measured post-stenting in the majority of the cases (86/123=69.9%) and, consequently, the value of pb-CFR <2 in identifying cases with high IMR (>40 U) or MVO is limited. However, a lower limit pb-CFR >2 (normal pb-CFR) was significantly associated with lower IMR, smaller MVO and IS% at 48 hours and 6 months after STEMI.

Limitations

Our study has several limitations. This is a retrospective analysis of the OxAMI study and pb-CFR has been calculated using pre-existing recorded physiological data.

Another limitation of our study is the relatively small sample size and the fact that the prognostic value of pb-CFR has been tested against thermodilution-derived indices and CMR parameters and not against clinical endpoint. Nevertheless, this is the first study to explore the value of pb-CFR in predicting the extent of microvascular dysfunction and myocardial damage in the setting of STEMI patients.

An additional inherent limitation of pressure-derived CFR is the required minimum resting pressure gradient. In fact, in cases with small ΔP at rest, the measurement of pb-CFR might become inaccurate, as the value of $\sqrt{\Delta P}$ at rest is in the range of the error of the pressure measurement itself[18-19]. To overcome this limitation, we excluded those patients with a final resting Pd/Pa >0.98 as previously described. In our series, the overall mean Pd/Pa and FFR were 0.94±0.04 and 0.92±0.05 respectively and even in the subgroup of patients with post-PPCI pb-CFR>2, the resting and hyperaemic gradient across the lesion allowed a reliable measurement of pb-CFR (Table 2). Nonetheless, we cannot exclude some degree of inaccuracy in the measurement of post-PCI pb-CFR, especially in those cases with high FFR.

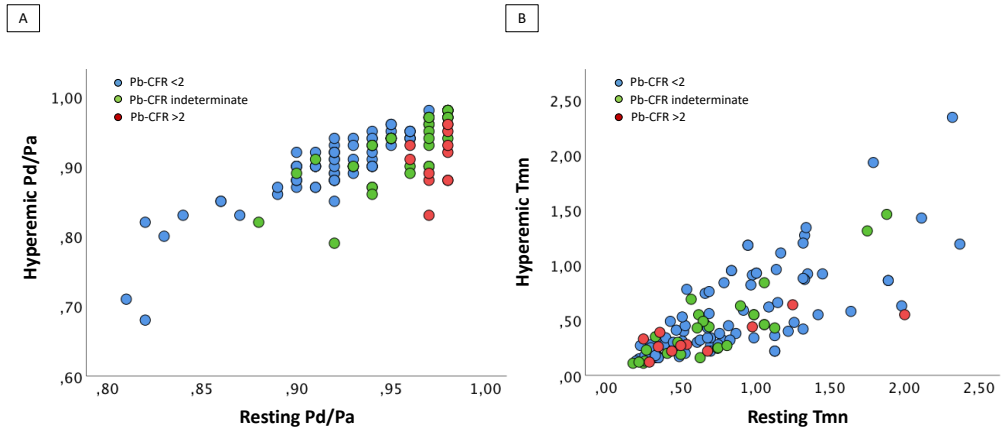
CONCLUSIONS

Pb-CF is a pressure-only derived index of coronary flow reserve. In our study pb-CFR was impaired (upper limit < 2) in 70% of the cases at completion of PPCI and it was modestly associated with the extent of microvascular dysfunction and myocardial injury after STEMI. Pb-CFR provided superior diagnostic performance compared to thermodilution-derived CFR in predicting MVO after STEMI. However, IMR was superior to both pb-CFR and thermodilution-derived CFR and consequently, IMR was the most accurate in predicting all of the studied CMR endpoints of myocardial injury after PPCI.

SUPPLEMENTARY MATERIAL

Supplementary Figure 1

In panel A, Pd/Pa at rest is plotted vs Pd/Pa measured at maximal hyperaemia. In panel B, transit time (Tmn) at rest is plotted vs Tmn at hyperaemia.



Supplementary Table 1

Table S1. Clinical and procedural characteristic of patients with STEMI stratified according to pre-stenting pb-CFR

	PB-CFR <2	PB-CFR indeterminate	PB-CFR >2	p-value
Clinical data	*n=89*	*n=18*	*n=5*	
Age, years	61(54-67)	61(46-68)	68(61-74)	0.26
Sex, male	83(93)	12(67)	5(100)	0.003
Hypertension	46(52)	12(67)	5(100)	0.06
Dyslipidemia	40(45)	8(44)	1(20)	0.55
Diabetes	36(40)	8(44)	2(40)	0.95
Smoking	43(48)	10(56)	0(0)	0.08
eGFR	95.7(82.0-106-8)	87.2(77.7-101.0)	77.7(65.7-89.2)	0.03
Pain-to-balloon time, min	190(129-360)	130(91-171)	231(157-478)	0.006
Procedural data				
LAD (Culprit)	57(64)	3(17)	0(0)	<0.001
Baseline TIMI flow 3	7(7.9)	2(11)	1(20)	0.45
Thrombus score (4&5)	52(58)	9(50)	4(80)	0.48
Thrombus aspiration	59(66)	13(72)	3(60)	0.84
Pre-dilatation	87(98)	17(94)	5(100)	0.68
Total stent length, mm	28(20-38)	28(20-42)	18(13-30)	0.19
Total stent diameter, mm	3.5(3.0-3.7)	4.0(3.0-4.0)	3.5(3.5-4.25)	0.14
Post-dilatation	66(74)	14(78)	4(80)	0.92
Final TIMI flow 3	79(89)	15(83)	5(100)	0.21
ST-resolution	62(70)	12(67)	4(80)	0.85
Final MBG>2	69(77)	12(67)	5(100)	0.28
CMR imaging at 48 h				
EDV, ml	170.5(142.0-193.7)	136.0(104.0-165.2)	200.0(138.0-220.5)	0.007
ESV, ml	90.0(65.7-114.0)	58.0(53.5-74.5)	104.0(63.0-129.5)	0.003
LVEF%	49(40-52)	54(46-61)	48(41-55)	0.078
SV, ml	78(63-93)	70(52-94)	78(75-100)	0.36
AAR%	44(36-53)	33(26-44)	33(28-45)	0.009
IS% at 48h	28.6(18.6-41)	21.3(13.6-34.1)	14.7(6.8-29.4)	0.10
MVO	2.0(0.0-6.0)	0.0(0.0-1.9)	1.0(0.0-7.4)	0.04
CMR imaging at 6 months				
EDV, ml	171.5(145.0-189.5)	140.0(116.5-157.5)	156.0(140.5-200.7)	0.03
ESV, ml	75.0(57.0-100.0)	59.0(39.5-79.0)	73.5(52.7-101.0)	0.11
LVEF%	52(43-61)	56(49-63)	52(46-66)	0.48

SV, ml	88(72-100)	71(61-90)	96(72-101)	0.17
IS at 6 m, %	22.3(12.3-34.5)	16.8(9.6-29.2)	14.3(3.6-24.2)	0.28
Salvage, %	32.8(18.2-50.0)	41.9(24.2-56.9)	44.6(16.4-80.2)	0.47

Supplementary Table 2

Supplementary Table 2. Intracoronary physiology of patients with STEMI stratified according pre-stenting pb-CFR

	Pb-CFR <2	Pb-CFR indeterminate	Pb-CFR >2	p-value
Baseline Pd/Pa	0.82(0.72-0.88)	0.93(0.87-0.97)	0.93(0.83-0.96)	<0.001
FFR	0.74(0.62-0.83)	0.82(0.66-0.92)	0.78(0.64-0.91)	0.17
Delta PdPa-FFR	0.06(0.02-0.11)	0.08(0.04-0.18)	0.08(0.03-0.25)	0.15
Pd(Hyp)	62(52-73)	66(46-70)	55(48-70)	0.69
Tmn(Rest)	0.94(0.64-1.39)	1.26(0.88-1.59)	0.77(0.21-1.10)	0.054
Tmn(Hyp)	0.78(0.47-1.24)	0.99(0.31-1.45)	0.37(0.26-0.82)	0.44
CFR	1.20(1.00-1.43)	1.31(1.18-2.35)	1.00(1.00-3.10)	0.055
IMR	45.9(31.9-82.9)	54.7(22.2-82.4)	17.9(13.7-51.2)	0.05
IMR corrected	41.0(27.0-78.9)	54.9(22.5-79.7)	15.6(9.7-46.1)	0.09
P Wedge, mmHg	20(16-27)	17(13-19)	22(18-25)	0.12
LowerPB	1.14(1.04-1.22)	1.58(1.49-1.71)	2.24(2.02-2.61)	<0.001
UpperPB	1.30(1.08-1.50)	2.50(2.23-2.93)	5.00(4.08-6.91)	<0.001

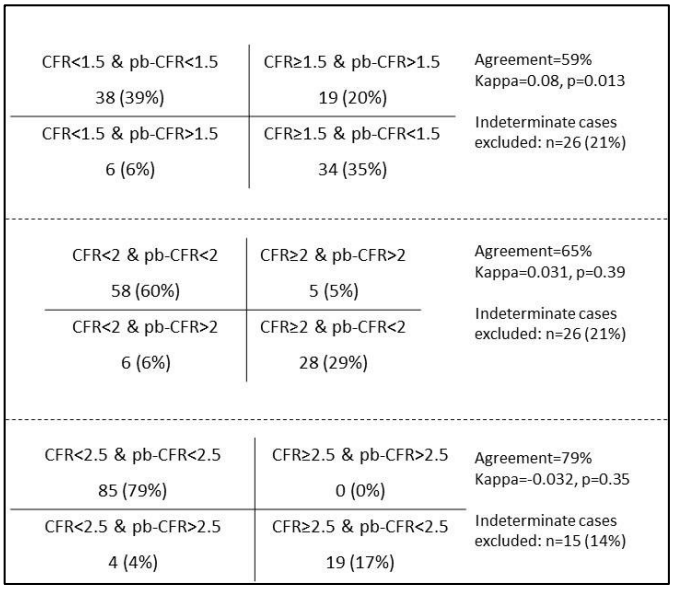
Supplementary Table 3

Supplementary Table 3. Procedural data of patients with STEMI stratified according to post-procedural pb-CFR

	Pb-CFR <2	*Pb-CFR indeterminate*	*Pb-CFR >2*	*p-value*
LAD (Culprit)	67(78)	14(54)	3(27)	0.001
Baseline TIMI flow 3	3(4)	3(11)	3(27)	0.16
Thrombus score (4&5)	55(64)	17(65)	5(45)	0.46
Thrombus aspiration	67(78)	15(58)	6(54)	0.06
Pre-dilatation	80(93)	25(95)	10(91)	0.79
total stent length, mm	24(20-32)	24(20-38)	24(18-38)	0.99
total stent diameter, mm	3.5(3.0-3.5)	3.5(3.0-4.0)	3.5(3.0-4.0)	0.27
Post-dilatation	59(69)	21(81)	9(82)	0.36
Final TIMI flow 3	74(86)	23(88)	10(91)	0.87
ST-resolution	60(70)	18(69)	8(73)	0.98
MBG	67(78)	23(88)	9(82)	0.49

Supplementary Figure 2. Agreement between pb-CFR and CFR_{thermo}

The agreement between pb-CFR and CFR_{thermo} has been tested using Cohen's kappa coefficient and % agreement.



Supplementary Table 4

Table S4. Comparison between post-pPCI pb-CFR, thermodilution-derived CFR and IMR in predicting CMR endpoints at 48 hours and 6 months after STEMI.

	Pb-CFR<2	**Pb-CFR>2**	**p-value**	**CFR<2**	**CFR≥2**	**p-value**	**IMR≥40**	**IMR<40**	**p-value**
LVEF% (48h)	46.5 (40.0-50.7)	48.0 (46.0-55.0)	0.13	46.5 (39.0-51.0)	47.0 (42.5-52.0)	0.67	45.0 (39.0-50.7)	47.0 (41.0-51.0)	0.67
AAR%	46.1 (37.0-55.6)	33.3 (27.1-40.2)	0.002	45.2 (34.5-53.8)	45.6 (37.0-56.1)	0.74	46.4 (38.0-56.1)	45.1 (34.0-52.0)	0.21
IS% (48h)	28.7 (20.4-39.3)	15.5 (8.3-25.0)	0.003	28.8 (18.3-40.9)	25.2 (17.1-33.7)	0.22	32.4 (23.0-44.0)	25.0 (12.6-33.6)	0.002
MVO %	2.0 (0.0-4.4)	0.0 (0.0-0.0)	0.001	1.8 (0.0-4.2)	0.9 (0.0-4.2)	0.37	3.7 (1.3-6.0)	0.5 (0.0-2.1)	<0.001
Hemorrhage%	0.0 (0.0-3.5)	0.0 (0.0-2.2)	0.49	0.0 (0.0-3.0)	0.0 (0.0-4.0)	0.81	1.0 (0.0-1.0)	0.0 (0.0-1.0)	0.018
Salvage%	34.5 (23.5-48.8)	42.9 (32.7-64.4)	0.16	32.6 (20.4-47.9)	38.8 (28.9-57.3)	0.06	27.8 (17.5-37.8)	42.0 (29.2-55.5)	<0.001
LVEF% (6mo)	52.5 (43.0-60.0)	57.0 (55.5-65.0)	0.09	52.0 (42.0-59.0)	55.5 (50.5-61.0)	0.09	47.0 (37.7-57.5)	55.0 (49.0-60.5)	0.024
IS% (6mo)	21.8 (13.3-30.3)	12.9 (5.5-21.8)	0.09	20.5 (12.6-35.2)	19.0 (16.0-26.7)	0.66	30.0 (21.4-37.7)	16.2 (8.9-22.8)	<0.001

LVEF, left ventricle ejection fraction; AAR, myocardial area at risk; IS, infarct size; MVO, microvascular obstruction.

Supplementary Table 5. Sensitivity analysis excluding cases with pre-stent resting Pd/Pa >0.96 and >0.95

Table S5. Sensitivity analysis of pre-pPCI pb-CFR accuracy according to resting Pd/Pa									
	Excluding cases with resting Pd/Pa>0.98			*Excluding cases with resting Pd/Pa>0.96*			*Excluding cases with resting Pd/Pa>0.95*		
	Pb-CFR<2	**Pb-CFR>2**	**p-value**	**Pb-CFR<2**	**Pb-CFR>2**	**p-value**	**Pb-CFR<2**	**Pb-CFR>2**	**p-value**
CFR_{thermo}	1.2 (1.0-1.4)	1.0 (1.0-3.1)	0.84	1.2 (1.0-1.4)	1.0 (1.0-3.1)	0.32	1.2 (1.0-1.4)	1.0 (1.0-3.1)	0.31
IMR	45.9 (31.9-82.9)	17.9 (13.7-51.2)	0.026	46.2 (31.3-83.1)	17.6 (11.9-55.8)	0.027	45.9 (30.8-82.5)	17.6 (11.9-55.8)	0.029
LVEF% (48h)	49.0 (40.0-52.2)	48.0 (41.0-55.0)	0.71	49.0 (40.0-52.2)	46.0 (39.5-58.5)	0.89	49.0 (40.0-52.7)	46.0 (39.5-58.5)	0.90
AAR%	44.3 (36.1-53.0)	33.1 (28.0-44.9)	0.10	43.6 (36.1-52.7)	36.0 (30.0-36.0)	0.31	44.3 (36.1-53.0)	36.0 (30.0-36.0)	0.31
IS% (48h)	28.6 (18.6-41.0)	14.7 (6.8-29.4)	0.11	28.5 (18.6-41.0)	21.0 (6.2-21.0)	0.35	28.5 (18.6-41.0)	21.0 (6.2-21.0)	0.35
MVO%	2.0 (0.0-6.0)	1.05 (0.0-7.4)	0.60	1.8 (0.0-5.4)	2.1 (0.0-2.1)	0.95	2.0 (0.0-5.6)	2.1 (0.0-2.1)	0.96
Salvage%	32.8 (18.3-50.0)	44.6 (16.4-80.2)	0.52	33.3 (18.2-50.0)	20.0 (15.1-20.0)	0.96	33.9 (18.4-50.8)	20.0 (15.1-20.0)	0.95
LVEF% (6mo)	52.0 (43.0-60.7)	52.0 (46.5-65.7)	0.71	52.0 (43.0-61.0)	48.0 (46.0-48.0)	0.91	52.0 (43.0-61.0)	48.0 (46.0-48.0)	0.91

IS% (6mo)	22.3 (12.3-34.5)	14.3 (3.6-24.2)	0.23	21.8 (12.2-34.0)	21.0 (2.0-21.0)	0.48	21.8 (12.2-34.0)	21.0 (2.0-21.0)	0.48

CFR_{thermo}, thermodilution-derived coronary flow reserve; IMR, index of microcirculatory resistance; LVEF, left ventricle ejection fraction; AAR, myocardial area at risk; IS, infarct size; MVO, microvascular obstruction.

Supplementary Table 6. Sensitivity analysis excluding cases with post-stent resting Pd/Pa >0.97 and >0.96

Table S6. Sensitivity analysis of post-pPCI pb-CFR accuracy according to resting Pd/Pa

	Excluding cases with resting Pd/Pa>0.98			*Excluding cases with resting Pd/Pa>0.97*			*Excluding cases with resting Pd/Pa>0.96*		
	Pb-CFR<2	**Pb-CFR>2**	**p-value**	**Pb-CFR<2**	**Pb-CFR>2**	**p-value**	**Pb-CFR<2**	**Pb-CFR>2**	**p-value**
CFR_{thermo}	1.5 (1.1-2.2)	2.0 (1.3-2.5)	0.23	1.5 (1.1-2.2)	1.8 (1.0-2.7)	0.93	1.5 (1.1-2.2)	1.4 (1.0-1.4)	0.50
IMR	32.5 (20.3-55.4)	20.2 (16.5-37.0)	0.05	32.3 (20.0-54.5)	17.0 (12.9-21.6)	0.017	33.1 (20.2-56.1)	18.6 (16.9-18.6)	0.22
LVEF % (48h)	46.5 (40.0-50.7)	48.0 (46.0-55.0)	0.13	45.0 (39.0-50.0)	51.0 (45.0-55.5)	0.11	45.5 (39.0-50.0)	47.0 (43.0-47.0)	0.61
AAR %	46.1 (37.0-55.6)	33.3 (27.1-40.2)	0.002	45.9 (37.0-55.6)	33.1 (19.1-36.5)	0.015	45.9 (36.8-56.3)	35.7 (34.2-35.7)	0.26
IS% (48h)	28.7 (20.4-39.3)	15.5 (8.3-25.0)	0.003	28.5 (20.0-39.3)	13.2 (5.3-22.2)	0.012	28.6 (20.2-40.3)	21.1 (13.2-21.1)	0.47

MVO %	2.0 (0.0-4.43)	0.0 (0.0-2.2)	0.001	2.0 (0.0-4.4)	0.0 (0.0-0.6)	0.02	2.0 (0.0-4.7)	0.0 (0.0-0.0)	0.08
Salvage%	34.5 (23.5-48.8)	42.9 (32.7-64.4)	0.16	34.5 (23.6-48.7)	54.7 (23.8-63.1)	0.34	34.5 (22.6-48.4)	38.7 (15.8-38.7)	0.93
LVEF % (6mo)	52.5 (43.0-60.0)	57.0 (55.5-65.0)	0.09	52.0 (43.0-60.0)	55.0 (55.0-60.0)	0.82	52.0 (43.0-60.2)	-	Na
IS% (6mo)	21.8 (13.3-30.3)	12.9 (5.5-21.8)	0.10	22.3 (12.9-30.2)	14.6 (11.8-21.0)	0.57	22.8 (12.9-31.5)	-	Na

CFR_{thermo}, thermodilution-derived coronary flow reserve; IMR, index of microcirculatory resistance; LVEF, left ventricle ejection fraction; AAR, myocardial area at risk; IS, infarct size; MVO, microvascular obstruction.

Supplementary Table 7. Comparison between pb-CFR and CFR_{thermo}: ROC curve analysis

Supplemental Table S7. Comparison between different cutoffs for pb-CFR in discriminating major cardiac MRI endpoints

	Pb-CFR (cut-off=2)		**Pb-CFR (cut-off=1.5)**		**Pb-CFR (cut-off=2.5)**	
	AUC (95%CI)	**p-value**	**AUC (95%CI)**	**p-value**	**AUC (95%CI)**	**p-value**
MVO**	0.37 (0.24-0.50)	0.045	0.37 (0.25-0.498)	0.047	0.46 (0.33-0.59)	0.50
IS% (6months)*	0.45 (0.31-0.60)	0.55	0.41 (0.27-0.55)	0.23	0.48 (0.35-0.62)	0.81

IS, infarct size; MVO, microvascular obstruction.

*For infarct size (IS) at 6 months the cut-off value for the highest quartile was used to define the endpoint: IS% (6months) ≥30.0%

**The presence vs. absence of MVO at 48 hours cardiac MRI has been used to define the endpoint MVO.

Supplementary Table 8. Comparison of areas under the curve (AUC) for Pb-CFR (binary) and CFR in predicting CMR endpoints

Table S8. Comparison of areas under the curve (AUC) for Pb-CFR (binary) and CFR in predicting CMR endpoints						
	Pre-stent			**Post-stent**		
	AUC (95%CI) Pb-CFR	**vs. CFR**	**p-value**	**AUC (95%CI) Pb-CFR**	**vs. CFR**	**p-value**
IS% (48h)*	0.53 (0.42-0.64)	0.53 (0.42-0.63)	0.94	0.58 (0.47-0.68)	0.69 (0.58-0.78)	0.29
MVO**	0.52 (0.41-0.63)	0.53 (0.42-0.64)	0.87	0.63 (0.52-0.73)	0.62 (0.51-0.72)	0.87
Hemorrhage %	0.50 (0.32-0.68)	0.58 (0.39-0.75)	0.46	0.53 (0.38-0.67)	0.55 (0.41-0.70)	0.80
IS% (6m)*	0.54 (0.42-0.67)	0.52 (0.39-0.64)	0.74	0.55 (0.43-0.66)	0.79 (0.68-0.88)	0.004
IS, infarct size; MVO, microvascular obstruction. *For infarct size (IS) at 48 hours and 6 months the cut-off value for the highest quartile was used to define the endpoint: IS% (48h)≥38.1% and IS% (6months) ≥30.0%						

IMR and pb-CFR variations before and after stenting.

Patients with both pre- and post-stenting physiological measurements were classified, according to the final IMR, as good responders to stenting (post-PPCI IMR <40U) or partial/poor responders (post-PPCI IMR ≥40U).

Repeated measurements of IMR and pb-CFR before and after stenting were available in 87 (58.8%). Overall, IMR decreased significantly after stenting (from 44.0 (28.4-80.0) to 28.7 (16.7-50.6), p<0.001 (Figure 2).

Similarly, a significant overall variation was observed in pb-CFR after stenting of the infarct related artery. In particular, pre-stent pb-CFR was *<2* in 88.5% of the cases, *indeterminate* in 9.2% and *>2* in 2.3%. After stenting pb-CFR was *<2* in 67.8%, *indeterminate* in 23% and *>2* in 9.2% of the cases (p<0.0001).

54 out of 87 (62%) of the patients were classified as good responders to stenting based on post-pPCI IMR <40U (Figure 2B). In this subgroup IMR significantly decreased after stenting (36.5 [25.5-59.9] vs 19.1 [15.4-26.4], p<0.001). Consistently, pb-CFR significantly improved after stenting (Pre-stent pb-CFR was <2 in 85.2%, *indeterminate* in 11.1% and >2 in 3.7% of the patients. Post-stent pb-CFR was <2 in 61.1%, *indeterminate* in 25.9% and >2 in 13.0% of the cases [p=0.007]).

33 out of 87 (38%) of the patients were classified as partial or poor responders to stenting based on post-pPCI IMR ≥40U (Figure 2C). In this subgroup IMR did not vary significantly after stenting (67.7 [44.5-97.5] vs 64.4 [45.9-71.9], p=ns). Similarly, pb-CFR did not significantly improve after stenting (Pre-stent pb-CFR was <2 in 94.0%, *indeterminate* in 6.0% and >2 in 0% of the patients. Post-stent pb-CFR was <2 in 78.8%, *indeterminate* in 18.2% and >2 in 3% of the cases [p=ns]).

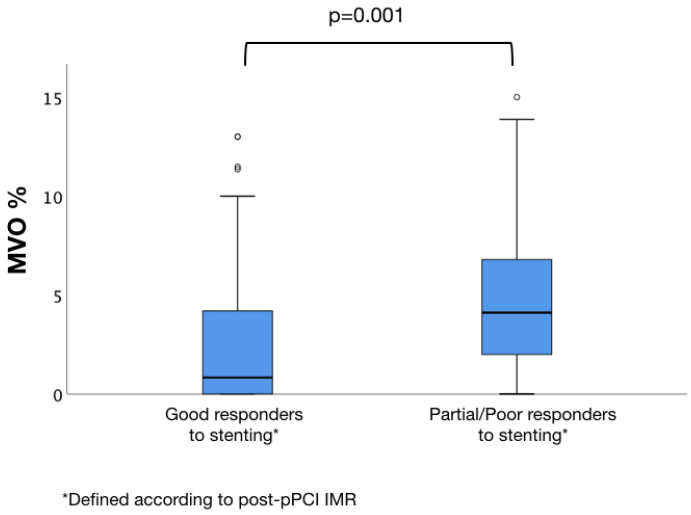
Table S7 summarizes the cardiac MRI endpoints stratified according to microcirculatory response to stenting categorized according to post-stenting IMR < or ≥40 U.

Supplementary Table 9. CMR endpoints according to microcirculatory response to stenting defined according to post-pPCI IMR ≥40 U

	Good responders	**Partial/Poor responders**	**p-value**
AAR%	44.3(34.1-52.0)	44.7(36.9(55.0)	0.37
LVEF%	49.0(43.0-54.0)	46.0(39.0-51.0)	0.36
IS% 48h	25.2(15.6-34.2)	32.0(21.4-43.2)	0.041
MVO %	0.8(0.0-4.6)	4.1(1.8-7.0)	0.001
Hemorrhage%	0.0(0.0-4.5)	4.0(0.0-12.0)	0.046
Salvage%	40.7(23.7-55.5)	21.8(14.5-38.8)	0.011
IS% 6mo	17.5(9.5-25.9)	30.4(24.3-41.4)	<0.0001

Supplementary figure 3.

Patients categorized as good responders to stenting (post-PPCI IMR <40U) presented significant less microvascular obstruction (MVO) at 48 hours cardiac MRI compared with partial/poor responders (post-PPCI IMR ≥40U)



CHAPTER 3.

Angiography-derived index of microcirculatory resistance as a novel, pressure-wire-free tool to assess coronary microcirculation in STEMI

ABSTRACT

Aims: Immediate assessment of coronary microcirculation during treatment of ST elevation myocardial infarction (STEMI) may facilitate patient stratification for targeted treatment algorithms. Use of pressure-wire to measure the index of microcirculatory resistance (IMR) is possible but has inevitable practical restrictions. We aimed to develop and validate angiography-derived index of microcirculatory resistance (IMR_{angio}) as a novel and pressure-wire-free index to facilitate assessment of the coronary microcirculation.

Methods and Results: 45 STEMI patients treated with primary percutaneous coronary intervention (pPCI) were enrolled. Immediately before stenting and at completion of pPCI, IMR was measured within the infarct related artery (IRA). At the same time points, 2 angiographic views were acquired during hyperaemia to measure quantitative flow ratio (QFR) from which IMR_{angio} was derived. In a subset of 15 patients both IMR and IMR_{angio} were also measured in the non-IRA. Patients underwent cardiovascular magnetic resonance imaging (CMR) at 48 hours for assessment of microvascular obstruction (MVO). IMR_{angio} and IMR were significantly correlated (rho: 0.85, $p<0.001$). Both IMR and IMR_{angio} were higher in the IRA rather than in the non-IRA ($p= 0.01$ and $p= 0.006$, respectively) and were higher in patients with evidence of clinically significant MVO (>1.55% of left ventricular mass) ($p=0.03$ and $p=0.005$, respectively). Post-pPCI IMR_{angio} presented and area under the curve (AUC) of 0.96 (CI95% 0.92-1.00, $p<0.001$) for prediction of post-pPCI IMR>40U and of 0.81 (CI95% 0.65-0.97, $p<0.001$) for MVO>1.55%.

Conclusion: IMR_{angio} is a promising tool for the assessment of coronary microcirculation. Assessment of IMR without the use of a pressure-wire may

enable more rapid, convenient and cost-effective assessment of coronary microvascular function.

INTRODUCTION

Coronary microvascular injury remains an important determinant of poor prognosis and an unsolved challenge in the management of patients with ST elevation myocardial infarction (STEMI). The index of microcirculatory resistance (IMR) has been proposed to provide information about the status of coronary microvasculature and it is based on the combined application of thermodilution technique and of coronary pressure-wire[1]. It has been validated against cardiovascular magnetic resonance imaging (CMR)[2] and against major clinical outcomes. Measured at the completion of the procedure, a post-pPCI IMR $\geq$ 40U is associated with a higher rate of mortality and readmission for heart failure in STEMI patients[3]. Moreover, IMR has been showed to provide information about the status of the microvasculature before stenting[4], and either alone or in combination with other clinical and anatomical parameters it can provide an immediate indicator of patients at high risk of suboptimal reperfusion[5,6].

Despite encouraging preliminary results of studies showing the potential efficacy of IMR-guidance in triaging novel therapies in STEMI[7], IMR is still perceived as a research tool and its application within clinical practice remains extremely limited. Probable reasons for a lack of clinical penetration include the additional procedural time /complexity, increased procedural cost and the potential challenge of pressure wire manipulation in the infarct related artery (IRA) in STEMI patients.

Quantitative flow ratio (QFR) is a novel angiography-based index derived from application of computational flow dynamics to three-dimensional modelling of the coronary artery[8]. QFR has been shown to have a good correlation with invasive fractional flow reserve (FFR) and it appears to be superior to angiography in assessing the ischemic potential of angiographically intermediate coronary stenosis[9]. QFR does not rely on pressure-wire use, but it remains an index for characterization of coronary epicardial segment and does not provide direct assessment of coronary microcirculation.

By measuring QFR in the IRA, we aimed to derive and validate a novel index, the angiography-derived index of microcirculatory resistance (IMR_{angio}), to provide a pressure-wire-free alternative to IMR for the assessment of coronary microvasculature.

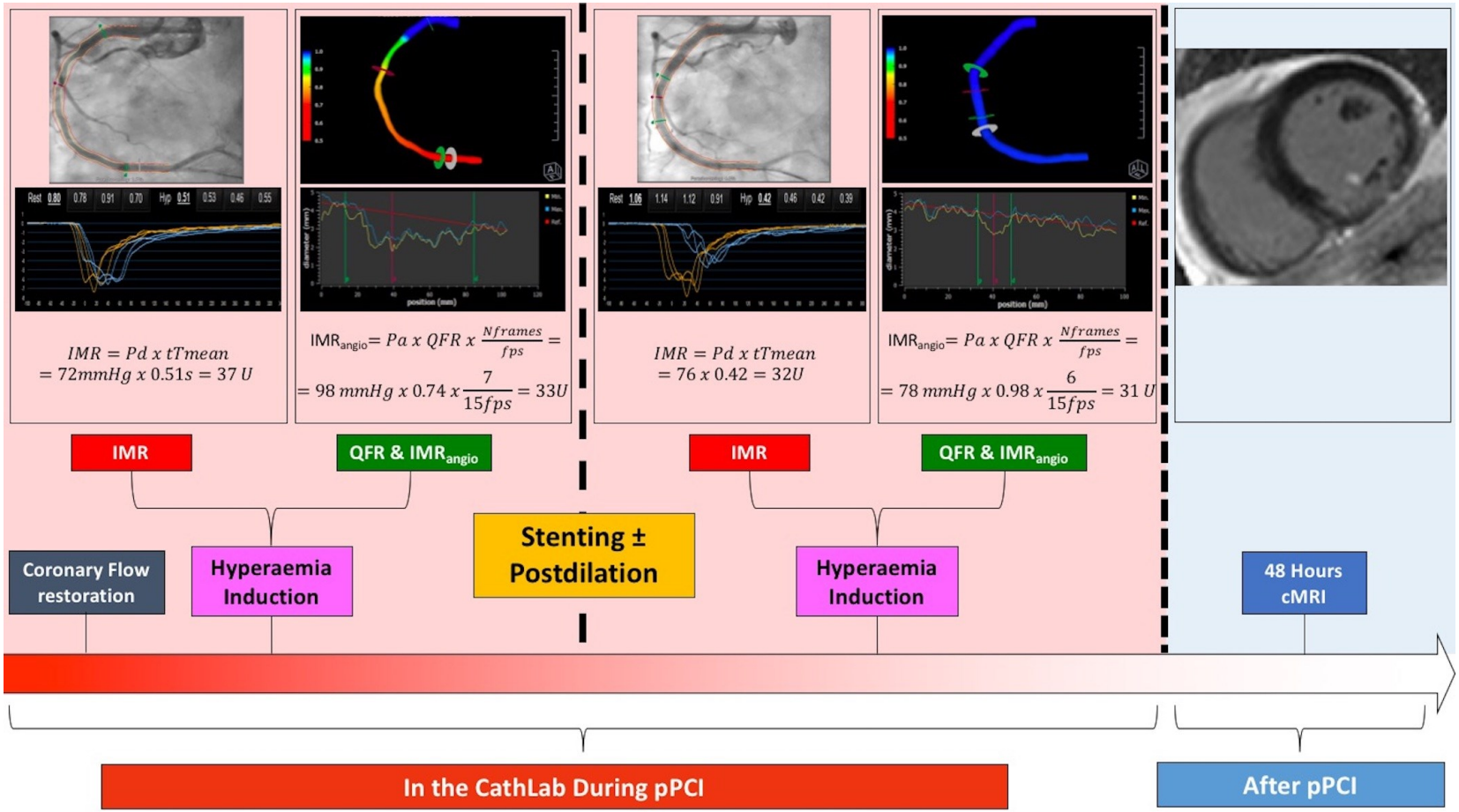
METHODS

Patients with STEMI admitted to the Oxford Heart Centre for pPCI between September 2018 and August 2019 were prospectively considered for enrolment in the OxAMI (Oxford Acute Myocardial Infarction) study. Details about OxAMI study have been previously described[10]. The OxAMI study protocol was approved by the local ethics committee (REC number 10/H0408/24) and conducted in accordance with the Declaration of Helsinki.

STEMI was defined as the occurrence of ongoing chest pain for at least 30 minutes associated with ST-segment elevation >2 mm in at least two contiguous leads. Enrolled patients were excluded for IMR_{angio} and IMR assessment in case of haemodynamic instability, evidence of angiographic left main disease, anticipated plain old balloon angioplasty without stent implantation or unsuitability for CMR assessment.

Figure 1 summarizes the study-methods as described in detail within the next sections.

Figure 1. Study Methods Flow Chart.



Index of microcirculatory resistance measurement

IMR was measured using thermodilution technique on the CoroFlow system (Coroventis, Uppsala Sweden) as previously described, immediately before stenting and at completion of pPCI[4]. Briefly, a standard pressure wire (PressureWire X, Abbott, Santa Clara, CA) was calibrated, equalized and advanced towards the distal third of the IRA. After intracoronary injection of 250 μg isosorbide dinitrate, mean aortic pressure (Pa), mean distal pressure (Pd) and mean transit time (tTmean) were measured both at baseline and at hyperaemia, achieved with intravenous infusion of adenosine at a rate of 140 μg/kg/min. Mean transit time was calculated as the average of three transit time measurements during three separate injections of 3ml of room temperature 0.9% saline solution. IMR was then calculated as follows:

$$IMR = Pd_{(hyperaemia)} \; x \; tTmean_{(hyperaemia)}$$

When assessed before stenting, IMR was measured either according to the above formula and also corrected for coronary wedge pressure, to account for residual collateral flow:

$$IMR = \mathrm{Pa}_{(\mathrm{hyperaemia})} \times \mathrm{tTmean}_{(\mathrm{hyperaemia})} \times \frac{Pd(hyperaemia) - Pcorwedge}{Pa(hyperaemia) - Pcorwedge}$$

Coronary wedge pressure was measured by the pressure sensor of the pressure-wire during prolonged angioplasty-balloon inflation.

In a subset of patients, IMR was measured also in one of the two non-IRAs. The selection of which non-IRA to assess was left to operator's discretion.

Quantitative flow ratio measurement

At the same time points when IMR was measured, and only when measurement of IMR was completed, angiographic images were acquired at 15 frame/second with manual injection of contrast dye during maximal hyperaemia, using a monoplane radiographic system (Siemens Healthcare, Germany). Pre-specified projections were agreed with the radiographer to guarantee views at least 25° apart.

Three-dimensional quantitative coronary angiography (3D-QCA) and then QFR were measured off-line using QAngio® XA 3D software (Medis, Leiden, the Netherlands) by two independent operators (RS, MS) blinded to clinical, IMR and CMR data. Contrast-flow QFR (cQFR) and fixed-flow QFR (fQFR) were provided. Cases of disagreement were resolved by consensus.

Since IMR is measured during maximal hyperaemia, we elected to assess IMR_{angio} under hyperaemic conditions, as well. For this reason, QFR was assessed using the angiographic views taken at peak hyperaemia during adenosine infusion. Pressure-wire was left in place during angiographic acquisition to allow calculation of QFR exactly at the site of the distal pressure/temperature transducer.

As per IMR, in a subset of patients, QFR was measured also in one of the two non-IRAs.

Angiography-derived Index of Microcirculatory Resistance

IMR_{angio} was derived starting from the formula for calculation of IMR.

$$IMR = Pd_{(hyperaemia)} \text{ x } tTmean_{(hyperaemia)}$$

where $Pd_{(hyperaemia)}$ is distal pressure at hyperaemia and $tTmean_{(hyperaemia)}$ is mean transit time at hyperaemia. By multiplying and dividing by hyperaemic aortic pressure ($Pa_{(hyperaemia)}$), the formula becomes:

$$IMR = Pa(hyperaemia)x\ \frac{Pd(hyperaemia)}{Pa(hyperaemia)}x\ tTmean(hyperaemia)$$

Since QFR is a surrogate of Pd(hyperaemia)/Pa(hyperaemia) ratio, (QFR ~ $\frac{Pd(hyperaemia)}{Pa(hyperaemia)}$), QFR can be used to replace $\frac{Pd(hyperaemia)}{Pa(hyperaemia)}$ in the formula. Similarly, $tTmean_{(hyperaemia)}$ can be expressed as the ratio between the number of frames (Nframes) for contrast dye to travel, during hyperaemia, from the guiding catheter to a distal reference (corresponding to the position of the distal marker of the pressure wire) divided by the acquisition rate (fps).

In this way the formula becomes:

$$IMR_{angio} = Pa_{(hyperaemia)}\, x\, QFR\, x\, \frac{Nframes_{(hyperaemia)}}{fps}$$

being fps set at 15 frame/second for QFR measurement.

IMR_{angio} was derived in the IRA at the same time points when IMR was measured, and in the non-IRAs where IMR assessment was performed per protocol.

Cardiovascular magnetic resonance imaging

CMR scans were performed at 48 hours after pPCI using a 3.0 Tesla scanner (either MAGNETOM TIMTrio or MAGNETOM Verio, Siemens Healthcare, Germany). Sequence acquisition was performed as previously described[11].

Microvascular obstruction (MVO) was defined as hypointense area within the hyperenhancement region on the late gadolinium enhancement images and was manually contoured[11]. We considered an MVO>1.55% of left ventricle mass as prognostically significant based on de Waha et al[12].

Statistical analysis

After verifying normal distribution by Shapiro-Wilk's test, variables were expressed as mean and (±) standard deviation (SD) or as median accompanied by interquartile range (IQR), as appropriate. Frequencies were compared using Chi square test or Fisher's exact test, as appropriate. Continuous variables were compared using T test or analysis of variance (ANOVA) with Scheffe's post-hoc comparisons, as appropriate. Non-normally distributed continuous variables were compared using Mann-Whitney's test or Kruskall Wallis' test, as appropriate. T test or Wilcoxon test were used as appropriate for paired samples. Correlations between variables were expressed using Pearson r or Spearman rho coefficients as appropriate.

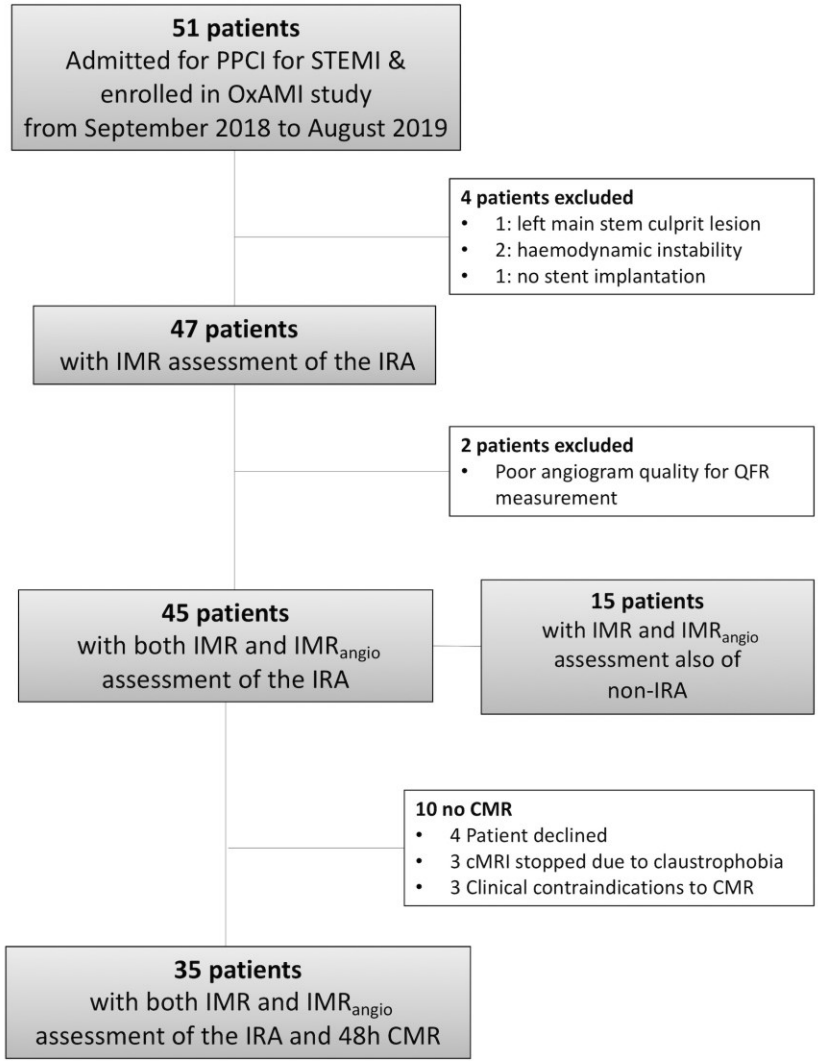
Inter-rater reliability was assessed by interclass coefficient (ICC) and corresponding 95% confidence interval.

The concordance between IMR_{angio} and IMR was assessed by Bland-Altman plot and the diagnostic efficiency of IMR_{angio} in predicting IMR ≥ 40U and MVO>1.55% was assessed by the area under the receiver-operating characteristic

curve. Youden index analysis was used to identify best cut-off of IMR_{angio} for prediction of post-pPCI IMR ≥ 40U.

Statistical analysis was performed using SPSS 24.0 (SPSS, Inc Chicago, Illinois) and a p value <0.05 was considered statistically significant.

Figure 2. Patients Flow Chart.



RESULTS

Clinical and procedural characteristics

A total of 45 STEMI patients were included in the current analysis (Figure 2). Clinical and procedural characteristics are presented for the whole cohort (Table 1) and stratified according to IMR_{angio} above or below 40 U (Supplementary Table 1&2). The cut-off of 40U for IMR_{angio} was derived from ROC analysis (see section "Correlations between IMR and IMR_{angio}").

Table 1. Overall clinical, angiographic and procedural characteristics

Clinical data	n=45
Age, years	61.5(54.7-71.0)
Male *(%)*	35(77.8)
Hypertension *(%)*	28(62.2)
Hypercholesterolemia *(%)*	19(42.2)
Active Smoker *(%)*	26(57.0)
Diabetes *(%)*	8(17.7)
Family history of CAD *(%)*	14(31.1)
Ischemic time, minutes *(IQR)*	196.0(127.5-425.5)
Culprit vessel	
LAD (%)	22(48.8)
LCx (%)	6(13.3)
RCA (%)	17(37.9)
TIMI flow at presentation	
0 (%)	26(57.8)
1 (%)	4(8.9)
2 (%)	10(22.2)
3 (%)	5(11.1)
Periprocedural Medication	
Aspirin (%)	45(100.0)
Clopidogrel (%)	45(100.0)
Heparin (%)	21(46.7)
Bivalirudin (%)	24(53.3)
GPIIbIIIa inhibitors (%)	3(6.6)
Angiographic and procedural data	

Thrombus aspiration *(%)*	10(22.2)
Predilation *(%)*	45(100)
Total Stent length, mm	24.0(20.0-38.0)
Stent Diameter, mm	3.5(3.0-4.0
Postdilation *(%)*	38(84.4)
Final TIMI flow	
0 (%)	0(0.0)
1(%)	2(4.4)
2 (%)	3(6.7)
3 (%)	40(88.9)
Thrombus Score ≥4	23(51.1)
Haemodynamics	
Pre-stenting	
Hyperemic Pd/Pa	0.75(0.61-0.85)
CFR	1.27(1.11-1.67)
IMR	48.6(25.5-60.3)
cQFR	0.76(0.64-0.86)
fQFR	0.74(0.57-0.84)
IMR_{angio}	37.3(23.7-50.2)
Post-pPCI	
Hyperemic Pd/Pa	0.95(0.90-0.98)
CFR	1.81(1.51-2.26)
IMR	30.9(16.5-52.9)
cQFR	0.95(0.88-0.98)
fQFR	0.95(0.89-0.99)
IMR_{angio}	30.0(19.3-43.9)

Correlations between IMR and IMR_{angio}

Satisfactory inter-rater reliability was detected for QFR (ICC 0.83 (CI95% 0.61-0.93), F=6.37, p<0.001) and IMR_{angio} (ICC 0.93 (CI95% 0.84-0.97), F=14.02, p<0.001).

Good correlation was observed between FFR and QFR (Supplementary Figure 1). IMR and IMR_{angio} were significantly correlated in the overall sample of 92 lesions (37 IRA pre-pPCI, 40 IRA post-pPCI and 15 non IRA) (rho= 0.85, p<0.001). Correlation between the two variables was maintained when analysis was restricted

to only IRA pre-pPCI (rho= 0.73, p< 0.001), IRA post-pPCI (rho= 0.88, p<0.001) and to the non-IRA (rho= 0.64, p= 0.009) (Figure 3).

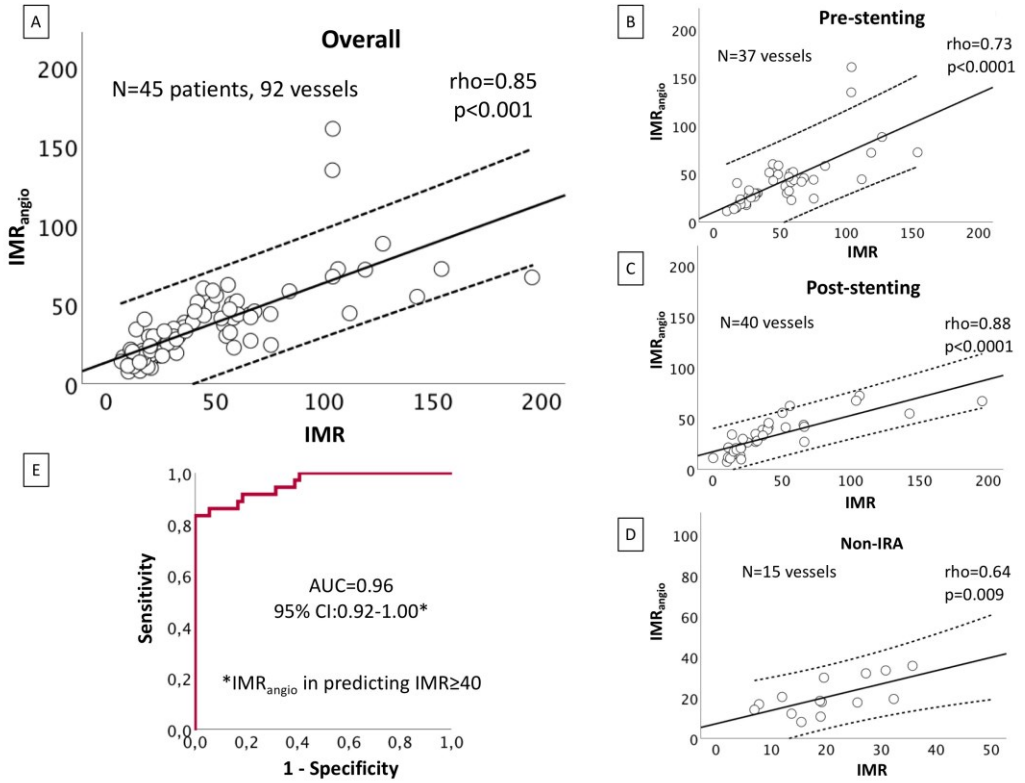


Figure 3. IMR_{angio} and IMR correlations in acute STEMI patients. Scatter plots summarise significant correlations between IMR_{angio} and IMR in the overall cohort of 92 lesions assessed (panel A) and then split into IRA before stent implant (panel B), IRA after stent implant (Panel C) and non-IRA (panel D). Dotted lines represent 95% Confidence interval. Panel E reports ROC curve analysis for IMR_{angio} in predicting IMR≥40U in the whole cohort of 92 lesions.

Pre-pPCI IMR_{angio} was also significantly correlated with pre-pPCI IMR corrected by coronary wedge pressure (rho=0.80, p=0.03).

Notably, both IMR_{angio} and IMR were significantly lower in the non-IRA compared to IRA (IMR_{angio} = 17.8U (12.2-29.9) vs 30.0U (20.5-44.3), p= 0.006; IMR= 19.0U (12.5-27.5) vs 31.0 (16.8-55.2), p= 0.01) (Supplementary Figure 2).

ROC curve analysis showed an excellent diagnostic performance of IMR_{angio} in predicting an IMR≥ 40 U (AUC= 0.96 (CI95% 0.92-1.00, p< 0.001; Figure 3e). The optimal cut-off of IMR_{angio} for prediction of IMR≥ 40 U was 40U (sensitivity 83.0%, specificity 100%, negative predictive value 90.2%, positive predictive value 96.8%, diagnostic accuracy 92.4%).

Bland Altman analysis further confirmed concordance between IMR_{angio} within the whole sample and across subgroups (IRA pre-pPCI, IRA post-pPCI and non IRA) (Figure 4). Only seven discordant cases were identified when a threshold of 40U was applied for both IMR and IMR_{angio}. Binary logistic regression analysis could not identify any clinical or procedural factors associated with IMR/IMR_{angio} discordance (Supplementary Table 3).

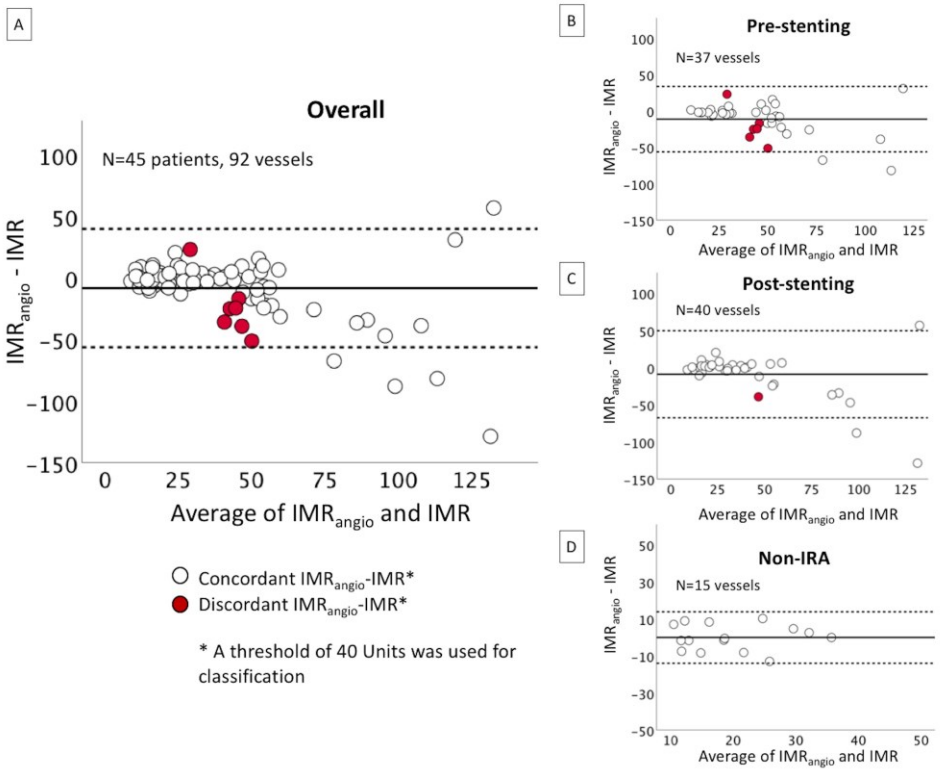


Figure 4. IMR_{angio} and IMR concordance. Bland-Altman plots summarise concordance between IMR_{angio} and IMR in the overall cohort of 92 lesions (panel A) and then split into IRA before stent implant (panel B), IRA after stent implant (Panel C) and non-IRA (panel D).

Variation of IMR and IMR_{angio} after pPCI

Assessment of both IMR_{angio} and IMR before and after stenting was available in 33 out of 45 patients. Both IMR_{angio} and IMR decreased significantly after stenting in the IRA (IMR_{angio} from 40.7U (25.0-50.2) to 28.2 (20.2-41.7), p= 0.048; IMR from 48.6U (25.5-64.4) to 31.0 (16.9-51.7), p= 0.048) (Figure 5). Variation in IMR_{angio} mirrored the one observed for IMR when patients were labelled as good or partial/poor responders to stenting, based on post-pPCI IMR≥ or <40U, respectively. In good responders IMR_{angio} went from 32.4U (23.7-48.2) to 21.3U (14.9-31.7) (p=0.002) and IMR from 41.9U (22.6-58.9) to 20.3U (15.0-28.0) (p=0.001). In partial/poor responders IMR_{angio} went from 44.3U (25.0-57.6) to 44.8U (41.2-64.3) (p=0.18) and IMR from 57.5U (34.4-102.8) to 66.2U (43.1-105.9) (p=0.21). Using the threshold of 40U, post-pPCI IMR categorized 63.6% of patients as good responders, whilst post-pPCI IMR_{angio} categorized 69.7% of patients as good responders (p= 0.69). IMR_{angio} presented a 3% misclassification rate for response to stenting, with only 1 out of the 33 patients misclassified as "good responder" by IMR_{angio} and labelled as "poor responder" according to IMR variation post pPCI.

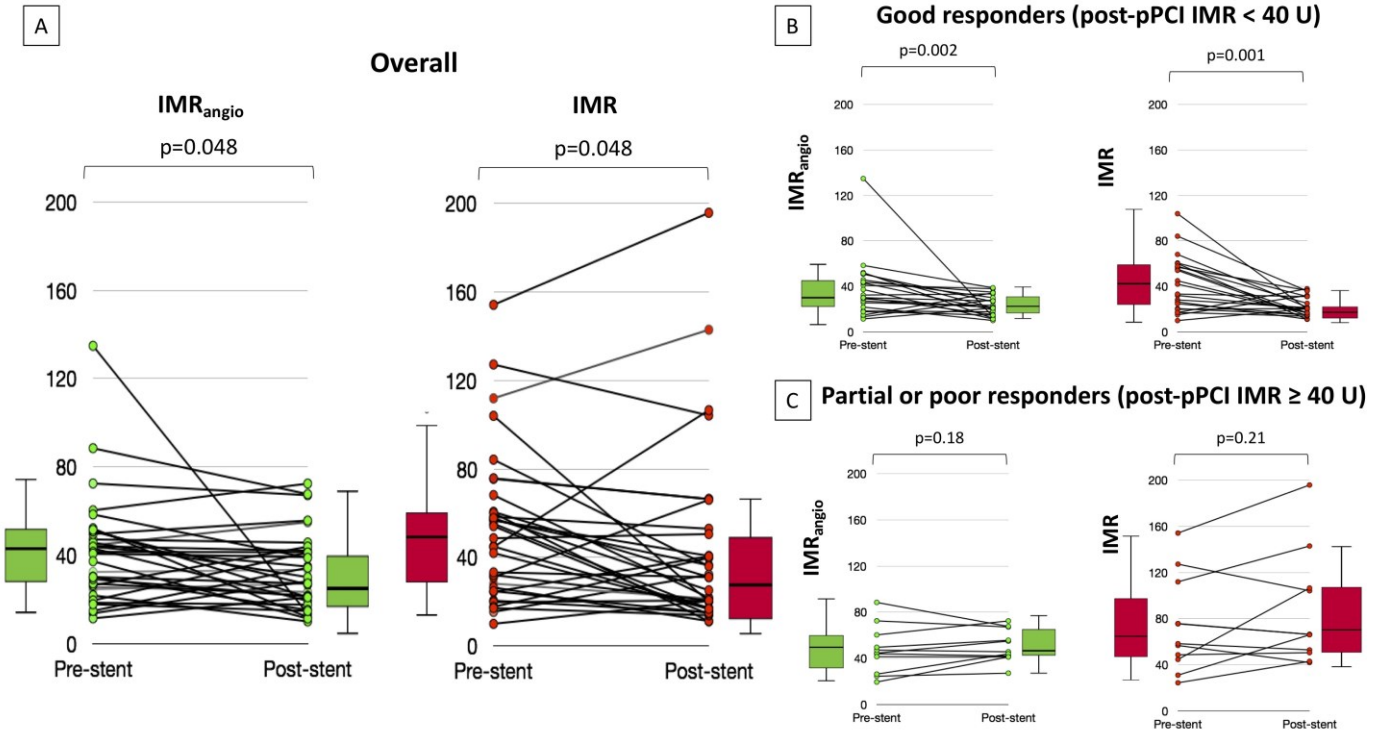


Figure 5. IMR_{angio} and IMR variations before and after stent implant. IMR_{angio} and IMR reduce after stent implantation (Panel A). The change in IMR_{angio} consistently mirrored the change in IMR; the relationships persist when patients were divided into 'good' (Panel B) or 'partial-poor' (Panel C) responders to stent implant.

Correlation between IMR_{angio} and MVO

CMR data are summarised in Table 2 and stratified according to post-pPCI IMR_{angio} above or below 40U.

IMR_{angio} was significantly higher in patients with MVO>1.55% (48.1U (29.3-68.9) vs 22.6U (13.7-39.0), p= 0.005). Post-pPCI IMR_{angio} presented a satisfactory efficiency for prediction of MVO>1.55% (AUC= 0.81 (CI95% 0.65-0.97), p= 0.006) (Figure 6). At the pre-specified cut-off of 40U, IMR_{angio} presented a 60.0% sensitivity, 80.0% specificity, 83.3% negative predictive value, 60.0% positive predictive value and 76.5% diagnostic accuracy).

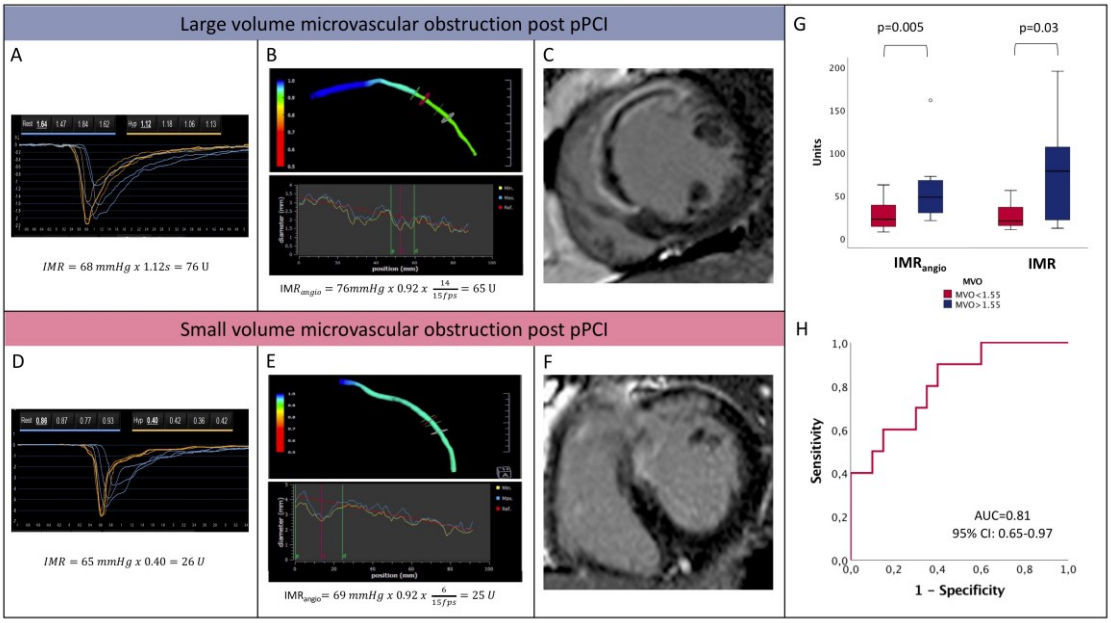


Figure 6. IMR_{angio} and MVO. The figure depicts two STEMI cases with IMR (Panels A&D), IMR_{angio} (Panels B&E) assessment and corresponding short axis CMR images with presence (Panel C) and absence (Panel F) of MVO. The correlation between IMR_{angio} and IMR with the occurrence of clinically relevant MVO (>1.55% of left ventricle mass) is summarised by the box plots (Panel G). Panel H depicts the ROC curve analysis of post-pPCI IMR_{angio} in predicting MVO>1.55.

Table 2. CMR at 48h assessment stratified according to post-pPCI IMR_{angio} ≥40U

Variable	post-pPCI IMR_{angio}<40U	post-pPCI IMR_{angio}≥40U	p-value
Number of patients	21(67.7)	10(32.3)	
LVEDV(ml)	151(126-179)	166 (146-201)	0.19
LVESV(ml)	80 (56-108)	83 (67-121)	0.67
LVEF(%)	49(40-54)	50 (41-57)	0.70
Infarct Size(g)	18 (13-27)	22 (15-30)	0.86
Infarct Size(%)	22(18.0-30)	25(19-31)	0.77
MVO >1.55%	4(19)	6(60)	**0.03**

DISCUSSION

In the current study, we have derived and validated IMR_{angio} as a novel and pressure-wire-free index for the assessment of coronary microcirculation in STEMI patients. We have specifically observed that:

1) IMR_{angio} is significantly correlated with IMR both in the IRA and in the non-IRA of STEMI patients
2) Both IMR and IMR_{angio} are significantly higher in the IRA than in the non-IRA
3) A value of 40 U appears the best threshold of IMR_{angio} to predict an abnormal IMR (≥ 40 U) in STEMI patients
4) The correlation between IMR_{angio} and IMR is maintained when these variables are measured before or after pPCI
5) IMR_{angio} variation before and after pPCI mirrors the same variation that is observed in IMR
6) IMR_{angio} measured at the end of pPCI is higher in patients with significant MVO and can predict the occurrence of significant MVO (>1.55% of left ventricle mass).

The availability and performance of pPCI have changed the prognosis for patients presenting with STEMI. However, up to 25-33% of STEMI patients will develop heart failure within five years of treatment, despite contemporary therapy[13]. Extensive coronary microvascular injury results in suboptimal reperfusion and this

portends a larger infarct size and a higher risk of adverse remodelling[12]. Identifying, minimising and potentially reversing microvascular injury in STEMI is an unmet clinical need.

In addressing this challenge, assessing the status of coronary microvasculature within the catheter laboratory at the time of STEMI is pivotal since it has the potential to triage patients who might benefit from additional therapy. Early diagnosis/identification of "high risk' individuals is essential and IMR measurement using pressure-wire can offer a reasonable compromise between practicality and diagnostic accuracy. However, measuring IMR increases procedural time, cost and has an intrinsic (but small) risk related to additional wire manipulation of the IRA (Central illustration).

Within routine interventional practice, novel angiography-based indices are becoming available to address the limitations of pressure-wire-based measurement of FFR, using computational flow dynamics to model the coronary artery[8]. Amongst these indices, QFR is the one with the largest amount of data cumulated so far[9,14]. QFR has been used extensively in routine practice to predict FFR, and its application to derive an angiography-based, pressure-wire-free parameter to depict the status of coronary microcirculation is now emerging[15].

This study demonstrates that IMR derived from QFR, labelled as IMR_{angio}, can be measured in STEMI patients in the vast majority of cases in a standard catheter laboratory (95.7% of lesions were successfully analysed for QFR and IMR_{angio}). Comparisons showed a significant correlation between IMR and IMR_{angio}, as confirmed by the ROC curve analysis. Previously, a post-pPCI IMR≥ 40U has been shown to be prognostically relevant[3] and notably in our data, IMR_{angio} showed a similar upper cut-off of 40U to predict abnormal IMR. When applying this threshold of 40U for both IMR and IMR_{angio}, they were concordant in 92% of cases, especially when the assessment was performed at the end of pPCI or in the non-IRA compared to assessment in the IRA before stent implant.

This result is further confirmed by the Bland-Altman analysis showing that IMR_{angio} and IMR are not numerically different for IMR values below 75U. Above 75U, IMR_{angio} can be instead either higher or lower than IMR. This observation emphasises that the absolute numerical values of the two variables are less related

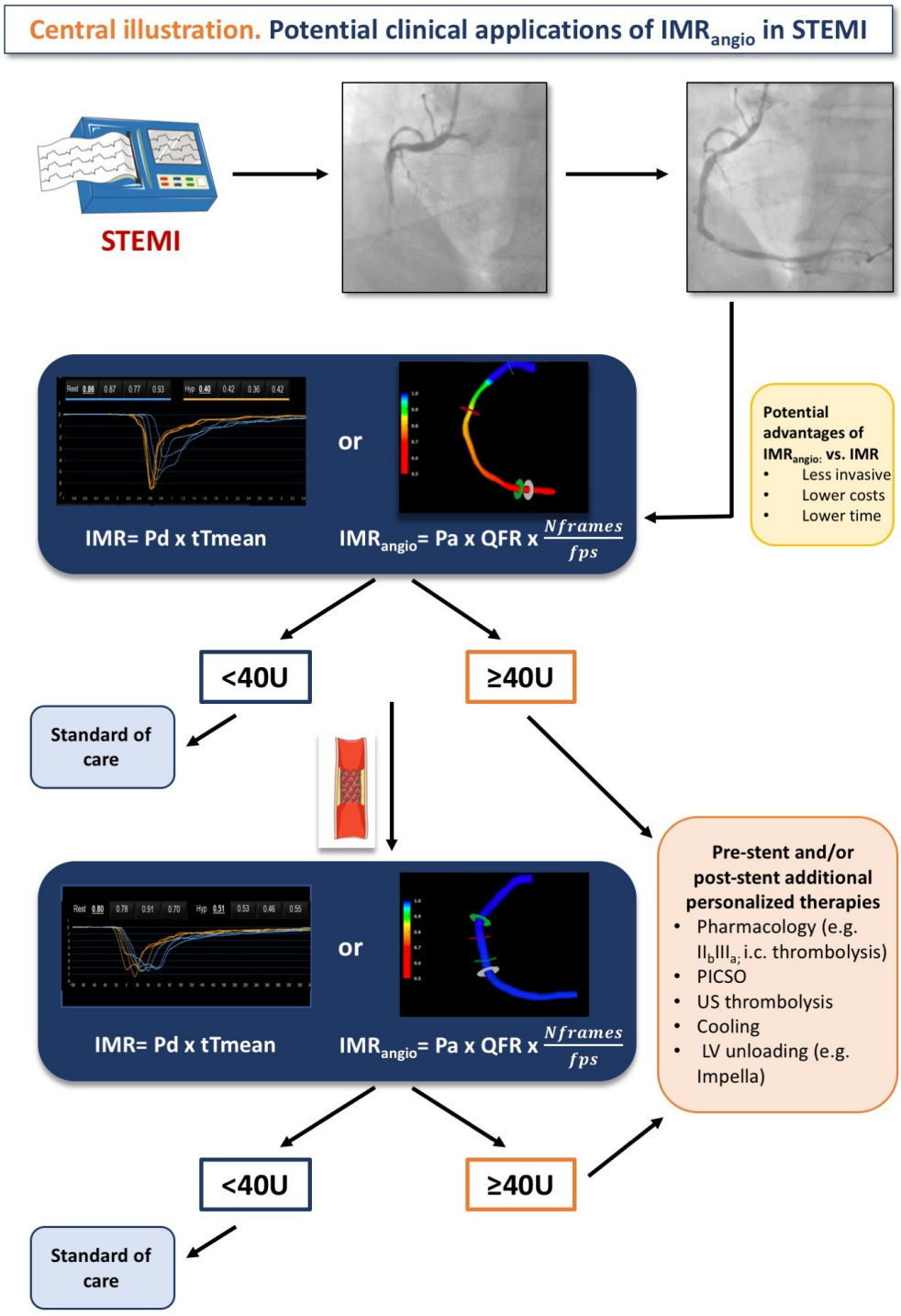
in cases of extreme (very high IMR) microvascular dysfunction (Figure 4). This may reflect the previous suggestion that agreement between QFR and FFR is negatively affected by the presence of severe microvascular impairment[14]. However, even though the difference between IMR and IMR_{angio} values tends to widen with the severity of microvascular impairment, it remains a clinically meaningful concordance between the two measures. Indeed, both IMR and IMR_{angio} measurements are within the adverse range (> 40U) in cases of extreme microvascular dysfunction, with no cases of severely abnormal IMR presenting a normal IMR_{angio} and vice versa. Notably, the few cases of discordance were clustered around the threshold of 40U (Figure 4).

Interestingly, in the subset of 15 patients with multivessel assessment, IMR_{angio} and IMR were correlated both in the IRA as in the non-IRA. Moreover, both IMR and IMR_{angio} appeared to be significantly higher in the IRA. This is in line with previous observations that microvascular impairment in the non-IRA, when present, is usually not severe and the observed values of IMR are not significantly different from those measured in patients with stable coronary artery disease[16].

In our study IMR and IMR_{angio} were measured at two time points (before and after stenting). We have previously described that, overall, IMR tends to improve after stenting, as a consequence of flow-mediated dilation of the microvascular bed[4]. However, a proportion of patients appear to experience a suboptimal response to stent implant, ending with a final IMR≥40 U as consequence of post-pPCI IMR increase or incomplete reduction below the desired threshold of 40 U[4]. The same trends were observed for IMR_{angio} in this study, with a similar rate of poor or partial responders to stenting when classification was based either on final IMR_{angio} or IMR.

Recently de Waha et al have reported, in a pooled cohort of 1688 STEMI patients undergoing post-pPCI CMR, that MVO>1.55% of left ventricle mass was associated with higher rates of mortality and heart failure at one year[12]. In our study, post-pPCI IMR_{angio} appeared significantly elevated in patients with evidence of clinically significant MVO (>1.55 % of left ventricle mass) on CMR. This observation echoes that by McGeoch et al who reported higher IMR values in STEMI patients with MVO[2].

Notably, whilst IMR and IMR_{angio} were correlated with the presence of MVO, neither of them presented a strong correlation with the extent of MVO and infarct size. This discrepancy is consistent with previous studies[2]. Potential explanations include the difference in the timing of IMR/ IMR_{angio} measurement and CMR scanning and the fact that IMR/ IMR_{angio} provides a functional assessment of coronary microcirculatory injury, whilst CMR an anatomical one[17].



Central illustration. Potential clinical implications of IMR_{angio} in STEMI. IMR or IMR_{angio} can be used to assess microvascular function in patients with STEMI undergoing pPCI before stenting (after flow restoration in the IRA) and at completion of pPCI.

Limitations

The relatively small sample size represents a limiting factor to keep into account when interpreting the results of the current study. A second observation is that QFR and IMR_{angio} were both measured offline. This accounts for a small proportion of lesions that had to be discarded for IMR_{angio} assessment because of suboptimal quality of angiographic views. One of the advocated benefits of QFR in management of patients with stable coronary disease is that accuracy is maintained in predicting FFR, irrespective of the use of adenosine to achieve maximal vasodilation. The so called "contrast-QFR" represents an index that is pressure-wire and adenosine-free[8]. In our study, in order to replicate IMR, QFR (and thus IMR_{angio}), was derived from angiographic views acquired at maximal hyperaemia achieved during intravenous adenosine infusion. In fact, the assessment of microvascular function in STEMI appears to be more reliable and consistent at maximal hyperaemia, since it is less prone to the heterogeneity of the same measurements obtained under resting conditions[18]. Whether IMR_{angio} might maintain the same diagnostic accuracy in predicting IMR and MVO also under non-hyperaemic conditions needs to be evaluated in future studies.

CONCLUSIONS

IMR_{angio} is a pressure-wire-free index with the potential to provide an easier and routine assessment of coronary microcirculation in the emergency setting of STEMI. Ultimately, even though further prospective validation is necessary in STEMI and across the spectrum of coronary artery disease, IMR_{angio} can be an easy, quick and cost-effective point-of-care test for routine assessment of microvascular function in the catheter lab with the ultimate goal of facilitating prognostic stratification and early triage of ad-hoc/personalised therapies.

SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Correlation between QFR and FFR

Scatter plot in (a) shows the correlation between the hyperaemic thermodilution-derived transit time and the total frame count (TCF) divided by the angiographic acquisition frame rate (TFC/15 fps).

Scatter plot in (b) shows the correlation between distal coronary pressure (Pd) measured with pressure wire and derived using QFR analysis. QFR-based Pd was derived according to the following formula:

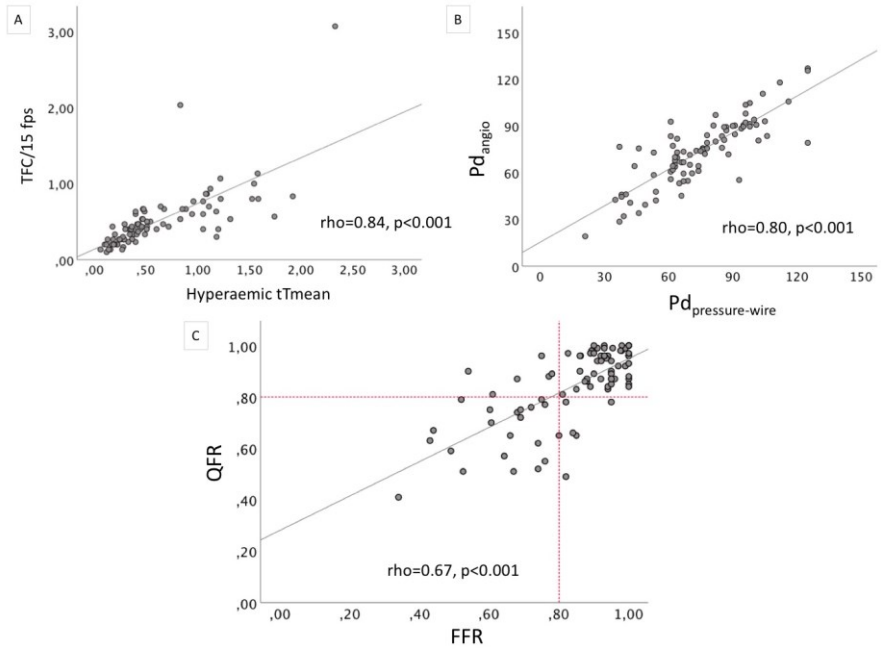
FFR=Pd/Pa (1)

FFR ~ QFR (2)

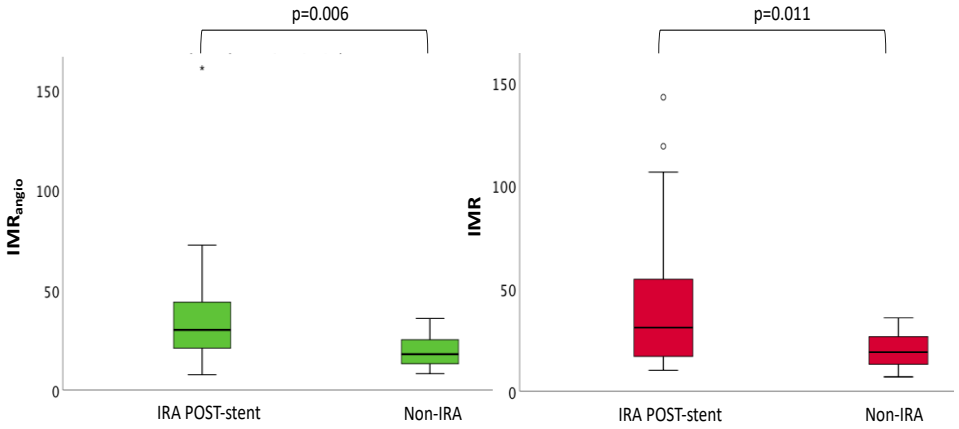
Solving (1) and (2): QFR ~ Pd/Pa (3)

Pd = QFR x Pa (4).

Scatter plot in (c) shows the correlation between QFR and FFR in the study cohort. Red dotted lines identified QFR and FFR conventional cut-offs (≤0.80).



Supplementary Figure 2. IMR_{angio} and IMR in IRA versus non-IRA. Box plots show how both IMR_{Angio} (Panel A) and IMR (Panel B) are significantly higher in IRA than in non-IRA. Box plot represent median and interquartile range. Whiskers identify maximum and minimum observations within the upper and lower fences.



Supplementary Table 1. Clinical, angiographic and procedural characteristics stratified according to post-pPCI IMRangio≥40U

Variable	IMR_{angio}<40U	IMR_{angio}≥40U	p-value
	(N=27)	(N=13)	
Age, years	64.0(54.4-69.5)	57.5(54.2-71.7)	0.76
Male *(%)*	21(78)	11(85)	0.48
Hypertension *(%)*	13(52)	9(75)	0.28
Hypercholesterolemia *(%)*	12(48)	5(42)	0.50
Active Smoker *(%)*	14(56)	8(68)	0.40
Diabetes *(%)*	5(20)	2(17)	0.59
Family history of CAD *(%)*	9(36)	3(25)	0.71
Ischemic time, minutes *(IQR)*	196.0(128.2-480.5)	246.0(90.0-614.0)	0.84
Angiographic and procedural data			
Culprit vessel			
LAD (%)	14(52)	5(38.5)	
LCx (%)	3(11)	3(23)	0.41
RCA (%)	10(37)	5(38.5)	
TIMI flow at presentation			
0 (%)	15(60)	10(83)	
1 (%)	2(8)	0(0)	0.32
2 (%)	4(16)	2(17)	
3 (%)	4(16)	0(0)	
Periprocedural Medication			
Aspirin (%)	22(96)	12(100)	0.66
Clopidogrel (%)	12(54)	5(50)	1.00
Heparin (%)	10(42)	7(58)	0.48
Bivalirudin (%)	15(60)	5(42)	0.48
GPIIbIIIa inhibitors (%)	0(0)	3(25)	0.03
Predilation *(%)*	100	100	1.00
Total Stent length, mm	24.0(19.0-38.0)	24.0(20.0-29.5)	0.75
Stent Diameter, mm	3.5(3.0-4.0)	3.2(3.0-3.8)	0.73
Postdilation *(%)*	23(96)	10(91)	0.54
Final TIMI flow			
0 (%)	0(0)	0(0)	
1(%)	1(4)	0(0)	0.006
2 (%)	0(0)	4(36)	
3 (%)	23(96)	7(64)	
Thrombus Score ≥4	13(48)	9(69)	0.31
Post-pPCI haemodynamic data			

Hyperemic Pd/Pa	0.94(0.90-0.98)	0.95(0.91-1.00)	0.64
IMR	20.1(14.6-31.0)	85.1(51.1-115.9)	<0.001
CFR	1.9(1.5-2.3)	1.5(1.2-2.5)	0.12
QFR	0.95(0.87-0.98)	0.95(0.89-1.00)	0.48
IMR_{angio}	21.5(15.0-30.0)	55.7(42.9-69.9)	<0.001

CAD, coronary artery disease; CFR, coronary flow reserve; IMR, index microcirculatory resistance; IMR_{angio}, angiography-derived index of microcirculatory resistance; QFR, quantitative flow reserve; TIMI, thrombolysis in myocardial infarction.

Supplementary Table 2. Clinical, angiographic and procedural characteristics stratified according to pre-stenting IMR_{angio}≥40U

Variable	**IMR_{angio}<40U**	**IMR_{angio}≥40U**	**p-value**
	(N=19)	(N=18)	
Age, years	63.0(55.7-67.2)	64.5(53.7-71.7)	0.57
Male *(%)*	13(68.4)	14(77.8)	0.71
Hypertension *(%)*	9(47.5)	11(61.1)	0.31
Hypercholesterolemia *(%)*	9(47.5)	7(38.8)	0.74
Active Smoker *(%)*	13(68.4)	8(44.4)	0.29
Diabetes *(%)*	4(21.0)	3(16.6)	0.57
Family history of CAD *(%)*	4(21.0)	5(27.7)	0.43
Ischemic time, minutes *(IQR)*	170.0(102.5-299.5)	309.0(193.5-624.0)	0.03
Angiographic and procedural data			
Culprit vessel			
LAD (%)	10(52.6)	9(50.0)	
LCx (%)	3(15.8)	1(5.6)	0.52
RCA (%)	6(31.6)	8(44.4)	
TIMI flow at presentation			
0 (%)	12(63.2)	10(55.6)	
1 (%)	2(10.5)	2(11.1)	0.96
2 (%)	4(21.1)	5(27.8)	

3 (%)	1(5.3)	1(5.6)	
Periprocedural Medication			
Aspirin (%)	37(100)	37(100)	1.00
Clopidogrel (%)	37(100)	37(100)	1.00
Heparin (%)	7(36.8)	9(50.0)	0,3
Bivalirudin (%)	12(63.2)	9(50.0)	0.20
GPIIbIIIa inhibitors (%)	0(0.0)	2(5.4)	0.21
Predilation *(%)*	37(100)	37(100)	1.00
Total Stent length, mm	24.0(20.0-38.0)	23.5(16.5-20.5)	0.25
Stent Diameter, mm	3.5(3.0-4.0)	3.5(3.0-3.8)	0.96
Postdilation *(%)*	18(94.7)	13(72.2)	0.44
Final TIMI flow			
0 (%)	0(0.0)	0(0.0)	0.04
1(%)	0(0.0)	2(11.1)	
2 (%)	0(0.0)	2(11.1)	
3 (%)	19(100)	14(77.8)	
Thrombus Score ≥4	10(52.6)	10(55.6)	1.00
Pre-stenting haemodynamic data			
Hyperemic Pd/Pa	0.74(0.61-0.82)	0.75(0.60-0.88)	0.52
IMR	26.6(19.7-54.1)	59.2(47.7-89.1)	<0.001
CFR	1.27(1.06-1.95)	1.26(1.11-1.53)	0.71
QFR	0.72(0.55-0.870	0.77(0.69-0.86)	0.30
IMR_{angio}	24.5(17.7-30.1)	50.2(44.0-59.2)	<0.001

CAD, coronary artery disease; CFR, coronary flow reserve; IMR, index microcirculatory resistance; IMR_{angio}, angiography-derived index of microcirculatory resistance; QFR, quantitative flow reserve; TIMI, thrombolysis in myocardial infarction.

Supplementary Table 3. Predictors of IMR/IMR_{angio} disagreement

Variable	OR (95% CI)	p-value
Age, years	1.01 (0.92-1.11)	0.84
Sex male	0.67 (0.07-6.47)	0.73
Smoker	0.36 (0.05-2.44)	0.30
Hypertension	0.41 (0.06-2.73)	0.35
Diabetes	1.07 (0.10-11.13)	0.95
Pain time	0.99 (0.99-1.00)	0.39
LAD vs. non LAD	2.20 (0.33-14.79)	0.42
Thrombus score	0.44 (0.08-2.53)	0.36
TIMI flow	0.93 (0.37-2.32)	0.88
MVO	0.75 (0.41-1.37)	0.34
IMR value	1.00 (0.98-1.03)	0.88

LAD, left anterior descending artery; IMR, index of microcirculatory resistance; IMR_{angio}, angiography-derived index of microcirculatory resistance; MVO, microvascular obstruction; TIMI, thrombolysis in myocardial infarction.

CHAPTER 4

Angiography-derived index of microcirculatory resistance (IMR_{angio}) in acute coronary syndromes and stable coronary artery disease

ABSTRACT

Aims. To investigate the diagnostic accuracy of 1) hyperaemic angiography-derived index of microcirculatory resistance (IMR_{angio}) in defining coronary microvascular dysfunction (CMD) across patients with acute coronary syndromes (ST-elevation myocardial infarction [STEMI]; non-ST elevation acute coronary syndrome [NSTE-ACS]) and stable chronic coronary syndrome [CCS]) and 2) the accuracy of non-hyperaemic IMR_{angio} (NH-IMR_{angio}) to detect CMD in STEMI.

Methods. 145 patients (STEMI=66; NSTEMI=43; CCS=36) were enrolled. 246 pressure-wire IMR measurements were made in 189 coronary vessels. IMR_{angio} and NH-IMR_{angio} was derived using quantitative flow ratio (QFR). In patients with STEMI, cardiac magnetic resonance (CMR) was performed to quantify microvascular obstruction (MVO).

Results. IMR_{angio} was correlated with IMR (overall rho=0.78, p<0.0001; STEMI, rho=0.85 p<0.0001; NSTE-ACS and rho=0.72, p<0.0001; CCS, rho=0.70, p<0.0001) and demonstrated good diagnostic performance in predicting high IMR (STEMI AUC_{ROC}=0.93 [0.88-0.98]; NSTE-ACS AUC_{ROC}=0.77 [0.63-0.92]; CCS AUC_{ROC}=0.88 [0.79-0.97]). Agreement between the two indices was evident on Bland Altman analysis.

In STEMI, NH-IMR_{angio} was also well correlated with IMR (rho=0.64, p<0.0001), with good diagnostic accuracy in predicting high invasive IMR (AUC_{ROC}=0.82 [0.74-0.90]). Both IMR_{angio} (AUC_{ROC}=0.74 [0.59-0.89]) and NH-IMR_{angio} (AUC_{ROC}=0.76 [0.54-0.87]) were significantly associated with MVO in STEMI.

Conclusions. IMR_{angio} is a valid alternative to invasive IMR to detect CMD in patients with acute and stable coronary syndromes, whilst NH-IMR_{angio} has a good diagnostic accuracy in STEMI where it could be become is user-friendly diagnostic tool as it is adenosine-free.

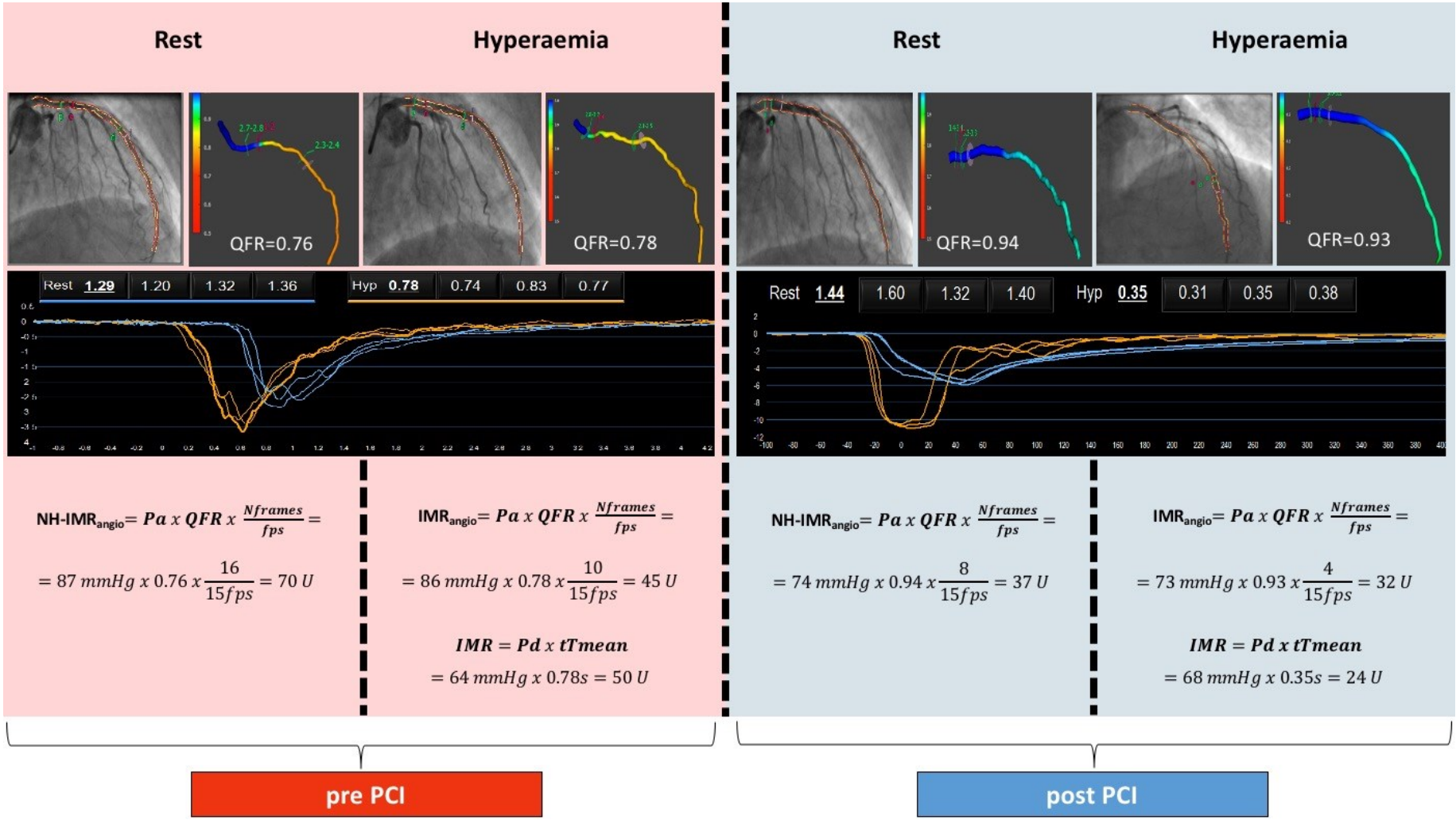
INTRODUCTION

Coronary microvascular dysfunction (CMD) often remains under-diagnosed in patients with coronary artery disease, despite its well reported clinical and prognostic implications[37]. Various methods have been proposed to aid the diagnosis of CMD in the catheterization laboratory[38]. Among them, the index of microcirculatory resistance (IMR) has gained particular attention[39]. It has been validated in patients with ST-elevation myocardial infarction (STEMI), in whom an elevated IMR (>40U) has been associated with adverse clinical outcome and more extensive myocardial injury[3, 14]. IMR has also been adopted to define the degree of CMD in patients with stable chronic coronary syndrome (CCS) with or without obstructive coronary disease[40,41]. However, the application of invasive coronary physiology to assess the extent of CMD remains very limited in routine clinical practice. This is partly due to the required additional procedural time, costs and technical complexity mainly related with pressure-wire manipulation and the need of adenosine infusion to achieve maximal hyperaemia. We have recently presented a novel pressure-wire-free and angiography-based index of microcirculatory resistance (IMR_{angio}), to assess coronary microvascular function in patients with STEMI based on computational flow analysis[42]. We investigated whether the utility of IMR_{angio} can be broadened across the spectrum of coronary syndrome, by assessing its diagnostic performance also in patients with non-ST-elevation acute coronary syndrome (NSTE-ACS) and CCS compared to pressure-wire-derived IMR. Furthermore, we assessed whether IMR_{angio} could retain its diagnostic accuracy also in non-hyperaemic conditions ($NH\text{-}IMR_{angio}$), thus overcoming the inherent limitation of adenosine-dependence of IMR. Moreover, we investigated the relationship of IMR_{angio} and $NH\text{-}IMR_{angio}$ with microvascular obstruction (MVO) cardiac magnetic resonance (CMR), as a structural index of CMD and known to be related with IMR[43].

METHODS

Patients admitted to the Oxford Heart Centre from September 2018 until February 2020, for a clinically indicated invasive coronary angiography were prospectively consented for enrolment into the OxAMI (Oxford Acute Myocardial Infarction) study[3]. Exclusion criteria are reported in Supplementary Materials. OxAMI study was approved by the Oxford University Hospitals ethics committee and conducted in accordance with the Declaration of Helsinki (REC number 10/H0408/24). All patients provided informed consent for participation to the study. Enrolled patients were divided into 3 groups according to the clinical presentation (STEMI, NSTE-ACS and CCS), defined according to the most recent recommendations (Supplementary material). In patients with STEMI undergoing primary PCI, invasive coronary physiology assessment of the infarct related artery (IRA) was performed after flow restoration with thrombus aspiration and/or balloon dilatation (e.g. immediately before stenting) and/or at completion of primary PCI, as previously described[3] (Figure 1).

Figure 1. Derivation of NH-IMR$_{angio}$ and IMR$_{angio}$



In patients with NSTE-ACS or CCS, invasive coronary physiology assessment was performed before and/or at completion of revascularization. In a subset of patients with STEMI and NSTE-ACS, IMR was also measured in one of the non-IRAs. The identification of the IRA was based on the combination of 1) lesion angiographic appearance compatible with plaque instability or presence of thrombus, 2) electrocardiographic and 3) echocardiographic findings.

In patients with CCS, microvascular angina was defined as a condition of increased IMR (>25U) in the absence of both angiographic and functional (fractional flow reserve (FFR) > 0.8) significant stenosis[40].

Index of microcirculatory resistance and Microvascular vasodilatory capacity

IMR was measured in a standard fashion using thermodilution technique and pressure wire (Abbott, Santa Clara CA) on the CoroFlow system (Coroventis, Uppsala Sweden) as previously reported[4].

Resistive reserve ratio (RRR) was calculated in all patients at the same time-points when IMR was assessed, as previously described[18] (For details see Supplementary material). RRR was measured to assess if the coronary microvascular vasodilatory capacity was associated with the diagnostic performance of IMR_{angio} and NH-IMR_{angio} in different clinical settings.

Quantitative flow ratio measurement

Three-dimensional quantitative coronary angiography (3D-QCA) and then QFR were measured off-line using QAngio® XA 3D software (Medis, Leiden, the Netherlands) by two independent certified operators (RS, MS) blinded to clinical, IMR and CMR data. At each time-point, QFR was assessed both at resting and at maximal hyperaemia. See supplementary material for details.

Angiography-derived Index of Microcirculatory Resistance

IMR_{angio} was derived from QFR as previously described[42] and reported in details in the Supplementary material.

Briefly, IMR_{angio} was calculated as:

$$IMRangio = Pa(hyperaemia)\ x\ QFR(hyperaemia)\ x\ \frac{Nframes(hyperaemia)}{fps}$$

being $Pa_{(hyperaemia)}$ mean aortic pressure at hyperaemia, Nframes the number of frames for contrast dye to travel from the guiding catheter to a distal reference (corresponding to the position of the distal marker of the pressure wire) and the fps is frame-acquisition rate, set at 15 frames/second.

$NH\text{-}IMR_{angio}$ was derived using the same formula but replacing the hyperaemic parameters with the resting ones as follows:

$$NH - IMRangio = Pa(resting)\ x\ QFR(resting)\ x\ \frac{Nframes(resting)}{fps}$$

IMR_{angio} and $NH\text{-}IMR_{angio}$ were derived for all the time points when invasive IMR was measured.

QFR and IMR_{angio} were analyzed by 2 independent operators in 29 vessels, in order to assess interobserver variability. Given the satisfactory interclass coefficient (see Results), the remaining 217 vessels included in the analysis was assessed by either of the two operators, blinded to the clinical characteristics including invasive coronary physiology data (FFR, IMR or CFR).

Cardiac magnetic resonance imaging in patients with acute myocardial infarction

In STEMI patients, CMR scans were performed following primary PCI but before discharge from hospital using a 3.0 Tesla scanner (either MAGNETOM TIMTrio or MAGNETOM Verio, Siemens Healthcare, Germany). Sequence acquisitions included cine and late gadolinium enhancement (LGE) imaging were performed as previously described[7]. Microvascular obstruction (MVO) was defined as hypointense areas within the hyperenhancement region on the LGE images and was manually contoured[7]. We considered an MVO>1.55% of LV mass as prognostically significant based on de Waha et al[5].

Statistical analysis

Continuous variables were expressed as median accompanied by interquartile range. Frequencies were compared using Fisher's exact test. Continuous variables were compared using Mann-Whitney's test or Kruskall Wallis' test, as appropriate. Wilcoxon test were used for paired samples. Correlations between variables were expressed using Spearman rho coefficients. To assess inter-rater reliability, interclass coefficient (ICC) estimates and their 95% confident intervals were calculated based on a mean-rating ($k = 2$), absolute-agreement, 2-way mixed-effects

model. The correlation of the readings of the two readers was also assessed using Spearman's Rho correlation coefficient.

The agreement between IMR_{angio}, $NH\text{-}IMR_{angio}$ and invasive IMR was assessed using Bland-Altman plot. Receiver-operating characteristic (ROC) curve analysis was used to define the diagnostic performance of IMR_{angio} and $NH\text{-}IMR_{angio}$ in detecting CMD. In STEMI, CMD was defined as IMR >40U or MVO>1.55%. In NSTE-ACS and CCS patients, CMD was defined as IMR >25U[40]. Areas under the ROC curves (AUC) were compared using the DeLong method. In STEMI, a hybrid algorithm using both $NH\text{-}IMR_{angio}$ and IMR_{angio} was developed to define the presence of significant CMD (IMR>40 U) in the IRA territory. Lower and upper $NH\text{-}IMR_{angio}$ cut-offs were identified as ≥90% negative predictive value (NPV) and ≥90% positive predictive value (PPV), respectively, for an IMR>40 U. Statistical analysis was performed using SPSS 25.0 (Inc Chicago, Illinois) and MedCalc (Ostend, Belgium). A p value <0.05 was considered statistically significant

RESULTS

Clinical and procedural characteristics

A total of 145 patients were included in the current analysis, including 66 STEMI, 43 NSTEMI and 36 CCS patients. Clinical and procedural characteristics are presented in Table1. Consequently, the agreement between angiography-derived and thermodilution-derived IMR was assessed in a total of 246 measurements (189 coronary vessels) (Figure 2).

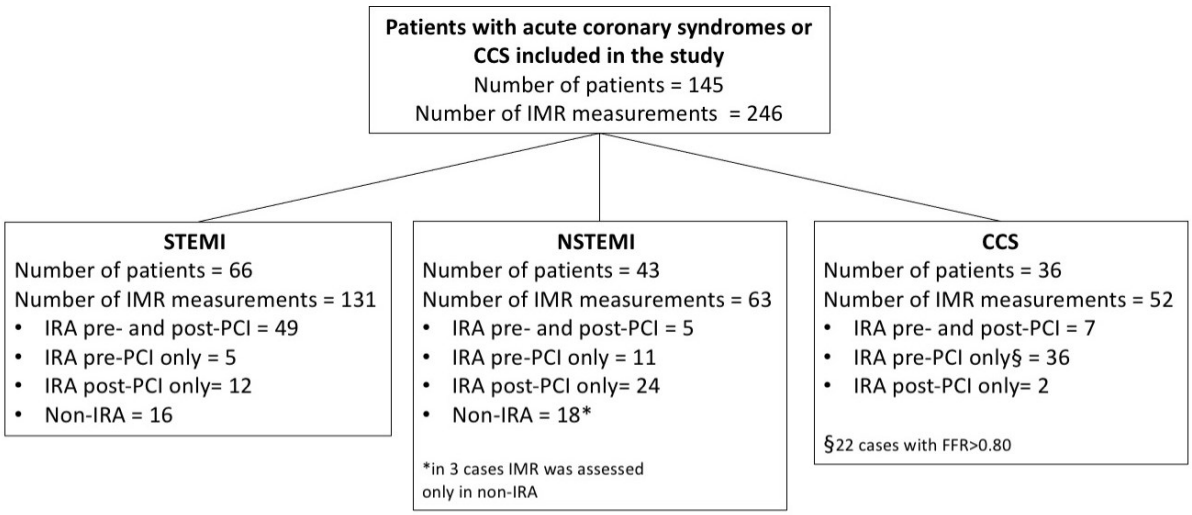


Figure 2. Study Flow Chart.

Coronary microvascular dysfunction across the spectrum of coronary syndromes

Before intervention, IMR was significantly higher in the IRA of STEMI patients (IMR 46.2 [24.6-68.3]) compared with NSTE-ACS (22.3 [18.1-29.0]) and CCS (20.5 [14.1-32.9]; p<0.0001) (Table 1 and Supplementary Figure1), whilst no significant differences in IMR were observed between patients with NSTE-ACS and CCS.

IMR decreased post-PCI in patients with STEMI (IMR 46.2 [24.6-68.3] vs 30.9 [19.2-51.1], p=0.001) but not in patients with NSTE-ACS and CCS (Supplementary Figure1).

Overall, in patients with STEMI, IMR was significantly higher in the IRA compared with the non-IRAs (35.8 [20.2-60.0] vs 18.9 [12.8-26.9], p<0.0001). No difference in IMR was observed between the IRA and non-IRA in NSTE-ACS (22.7[17.2-28.9] vs 18.6[13.3-28.5], p=0.27)(Supplementary Figure2).

In the CCS group 15 coronary vessels in 12 patients presented an abnormal FFR (≤0.80). Within the CCS group a condition of microvascular angina (FFR>0.80 and IMR>25U) was observed in 8 out of 36 patients (22.2%), whist 14 out of 36 (38.9%) did not show either significant epicardial stenosis (FFR>0.80) or presence of

microvascular impairment (IMR<25U). Notably, a substantial agreement was observed between IMR and $IMR_{corrected}$ as shown in the Supplementary Figure 3.

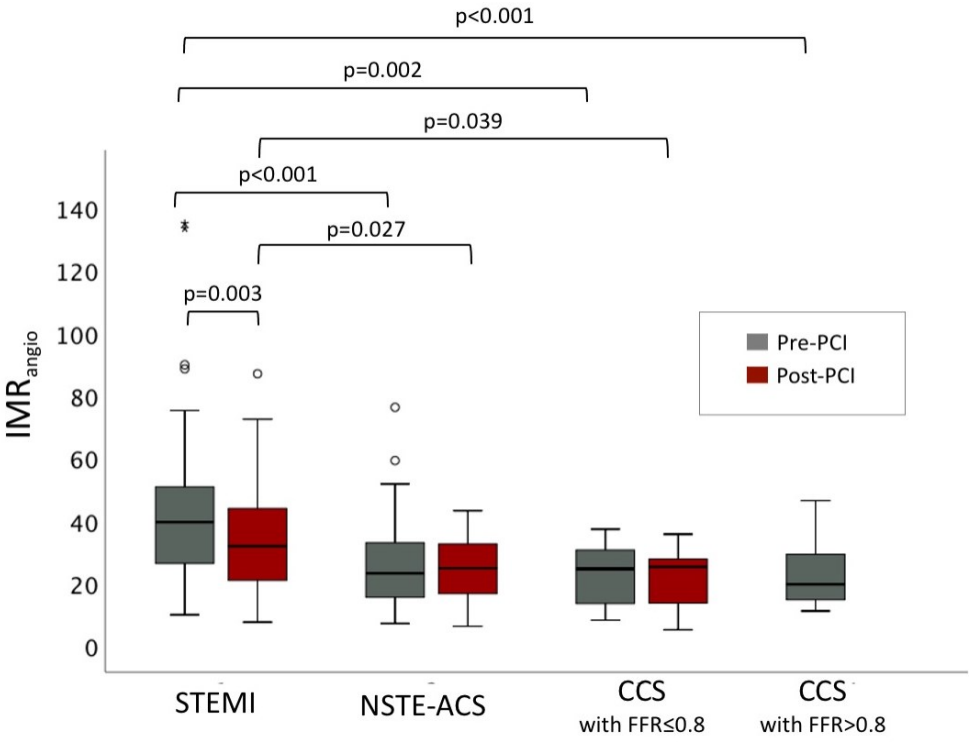


Figure 3. IMR_{angio} across the spectrum of coronary syndromes
Box plots depict IMR_{angio} median values in patients with STEMI, NSTE-ACS and CCS before and after PCI. CCS cases with FFR >0.80 at baseline did not undergo PCI. p-value is provided for statistically significant differences between the subgroups. Other comparisons were not statistically significant.

IMR_{angio} across the spectrum of coronary syndromes

Satisfactory ICC was observed for IMR_{angio} (0.97, 95%CI 0.93-0.99; F=31.8, p<0.0001).

Before intervention IMR_{angio} was significantly higher in the IRA of STEMI patients (39.6 [26.1-50.9]) compared with NSTE-ACS (25.3[16.6-42.3]) and CCS (20.1[14.4-30.6], p<0.0001) (Table 1 and Figure 3). As IMR, IMR_{angio} also decreased significantly post-PCI in STEMI (39.6 [26.1-50.9] vs 31.8 [21.0-45.2], p=0.002) but did not change significantly in NSTE-ACS and CCS (Figure 3).

Notably in the subgroup of CCS patients without obstructed coronary disease (FFR>0.80) IMR_{angio} was significantly higher in patients with microvascular angina

(defined as invasive IMR>25U) compared to those without (31.0[25.3-42-5] vs 16.6[14.1-19.7] p<0.001) (Figure 4).

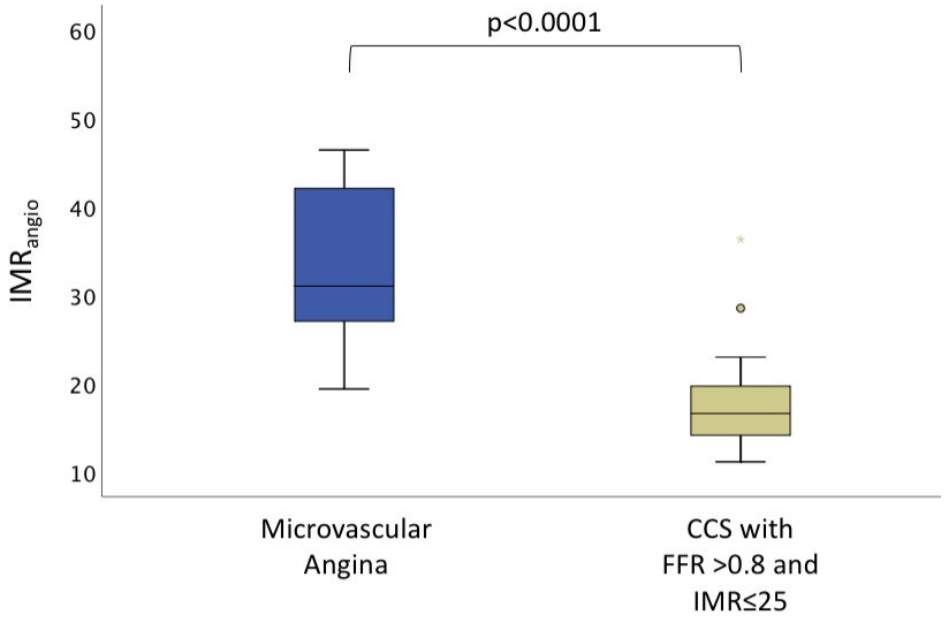


Figure 4. IMR_{angio} in patients with microvascular angina (defined as FFR >0.8 and IMR >25 U) vs patients with unobstructed coronary artery disease (FFR >0.8) but normal microcirculatory function (IMR ≤25 U).

Overall, IMR_{angio} and invasive IMR were significantly correlated (rho=0.78, p<0.0001).

Notably, the correlation was maintained across the whole spectrum of coronary syndromes, both before and after PCI, as well as in the IRA as in the non-IRA for STEMI and NSTE-ACS (Figure5). The Bland Altman analysis showed a significant agreement between IMR and IMR_{angio} especially in cases with IMR below 75 U. Conversely, the absolute numerical values of the two indices are less related in cases of severe microvascular dysfunction (Figure 6). The correlation between IMR_{angio} and IMR in LAD vs non-LAD vessels is presented in the Supplementary Figure 4.

Table 1. Clinical, procedural and haemodynamic data

	STEMI	NSTE-ACS	CCS	p-value
Clinical data				
Age, years	63.5(56.0-71.0)	63.0(56.0-71.2)	67.0(59.0-74.0)	0.244
Sex male, n(%)	56(84.8)	26(60.5)	24(66.6)	0.014
Hypertension, n(%)	33(50.0)	26(60.5)	24(66.6)	0.424
Hypercholesterolaemia, n(%)	24(36.4)	14(32.6)	17(47.2)	0.397
Diabetes, n(%)	9(13.6)	6(13.9)	6(16.7)	0.938
Current smoker, n(%)	34(51.5)	20(46.5)	14(38.9)	0.279
*Target vessel**				
LAD, n(%)	29(43.9)	24(55.8)	27(77.1)	
LCX, n(%)	7(10.6)	8(18.6)	1(2.9)	0.016
RCA, n(%)	28(42.4)	9(20.9)	6(17.1)	
Intermediate, n(%)	2(3.0)	2(4.7)	1(2.9)	
TIMI Flow – pre-PCI				
0	44(66.7)	2(4.6)	0(0.0)	<0.0001
1	6(9.0)	1(2.3)	0(0.0)	
2	10(15.3)	13(30.2)	0(0.0)	
3	6(9.0)	27(62.8)	52(100)	
TIMI Flow – post-PCI				
0	0(0.0)	0(0.0)	0(0.0)	<0.001
1	2(3.0)	0(0.0)	0(0.0)	
2	10(15.2)	3(7.0)	0(0.0)	
3	54(81.8)	40(93.0)	52(100)	
Ischemic time, min	196(127-425)	-	-	-
Coronary physiology data - pre-PCI**				
FFR	0.75(0.61-0.86)	0.85(0.80-0.94)	0.83(0.73-0.90)	0.008
QFR	0.78(0.72-0.84)	0.83(0.78-0.93)	0.87(0.77-0.93)	0.002
CFR	1.30(1.10-1.91)	3.00(1.37-4.46)	2.10(1.58-3.90)	0.001
RRR	1.60(1.33-1.94)	3.00(2.12-4.89)	2.78(1.61-4.28)	<0.0001
IMR	47.5(24.4-68.2)	22.3(17.7-28.9)	20.5(14.7-31.8)	<0.0001
IMRangio	39.6(26.1-50.9)	25.3(16.6-42.3)	20.1(14.4-30.6)	<0.0001
NH-IMRangio	39.9(28.3-60.4)	42.7(25.5-62.8)	36.7(23.5-44.4)	0.110
Coronary physiology data - post-PCI**				
FFR	0.94(0.89-0.98)	0.88(0.84-0.96)	0.87(0.82-0.93)	0.015
QFR	0.96(0.91-0.99)	0.94(0.86-0.97)	0.93(0.88-0.99)	0.182
CFR	1.80(1.41-2.65)	2.59(2.03(3.35)	2.10(1.58-3.47)	0.004

RRR	2.04(1.63-2.81)	2.86(2.00-4.09)	2.41(1.64-3.70)	0.003
IMR	29.7(19.8-49.3)	22.7(15.7-28.4)	15.8(11.9-34.8)	0.035
IMRangio	31.8(21.0-45.2)	24.8(16.5-33.0)	23.9(8.1-27.7)	0.018
NH-IMRangio	45.5(31.7-67.6)	45.1(22.4-58.1)	39.7(23.1-42.6)	0.316

*Target vessel in STEMI and NSTE-ACS corresponds to IRA **complete physiology data is available in Supplementary Table 1

Non-Hyperaemic-IMR$_{angio}$ across the spectrum of coronary syndromes

Satisfactory ICC was observed for NH-IMR$_{angio}$ (0.90, 95%CI 0.64-0.92; F=11.7, p<0.0001).

Before intervention, NH-IMR$_{angio}$ did not differ significantly between STEMI (39.9[28.3-60.4]), NSTE-ACS (42.7[25.5-62.8]) and CCS (36.7[23.5-44.4], p=0.110) (Table 1 and Supplementary Figure 5). However, in STEMI patients NH-IMR$_{angio}$ was significantly higher in the IRA compared with the non-IRA (39.9[28.3-60.4]vs 22.5[21.2-43.3], p=0.031).

Overall, NH-IMR$_{angio}$ showed a significant correlation with IMR in STEMI (rho=0.64, p<0.0001, Figure5), a modest correlation in CCS (rho=0.33, p=0.018) and it was not correlated with IMR in NSTE-ACS (rho=0.23, p=0.121) (Supplementary Figures 6,7).

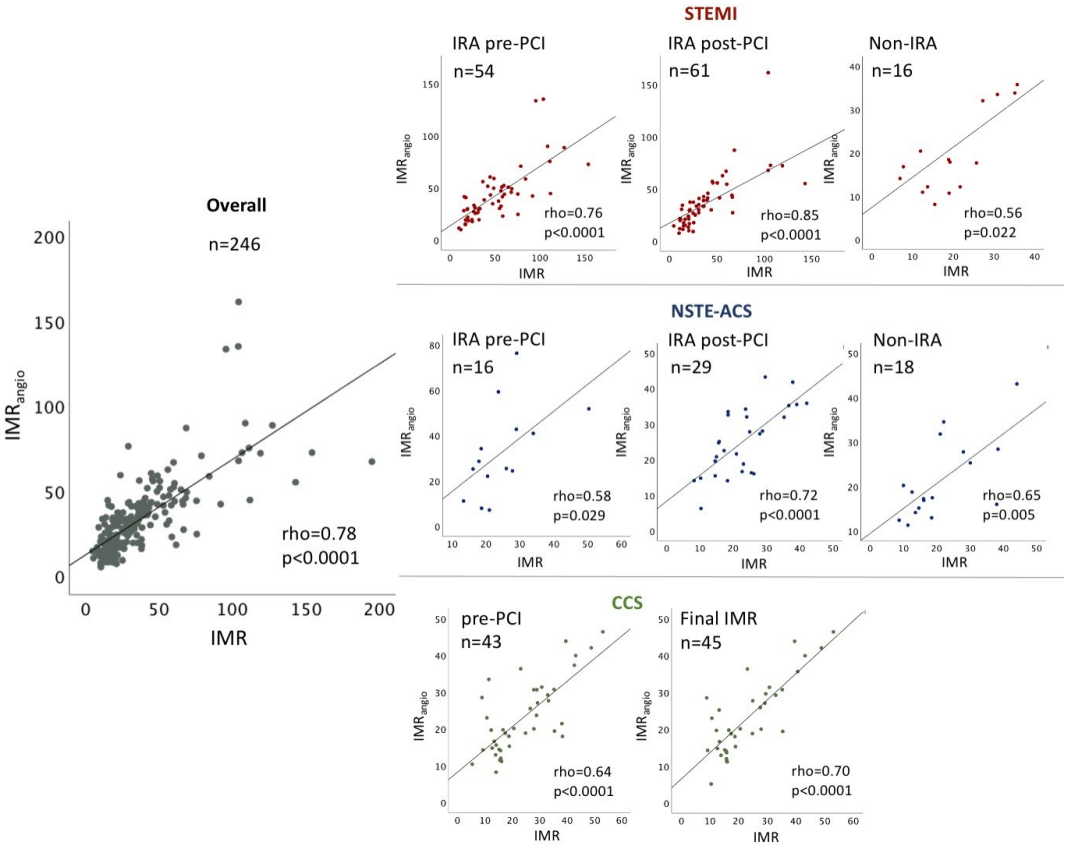


Figure 5. Correlation between IMR_{angio} and IMR

Scatter plots summarise correlations between IMR_{angio} and IMR in patients with STEMI, NSTE-ACS and CCS. Final IMR for CCS include baseline measurements for patients with FFR >0.8 and
post-PCI values for patients with FFR≤0.8 who underwent PCI.

Notably, in the STEMI cohort, the correlation between NH-IMR_{angio} and IMR was maintained when analysis was restricted to the IRA either pre-PCI (rho=0.68, p<0.0001), or post-PCI (rho=0.67, p<0.0001) but not in the non-IRA (rho=0.33, p=0.21).

The Bland Altman analysis confirmed the good agreement between NH-IMRangio and invasive IMR in STEMI, up to severe degree of microvascular dysfunction (Figure 6, Supplementary Figures 6,7). The correlation between NH-IMR_{angio} and IMR in LAD vs non-LAD vessels is presented in the Supplementary Figure 8.

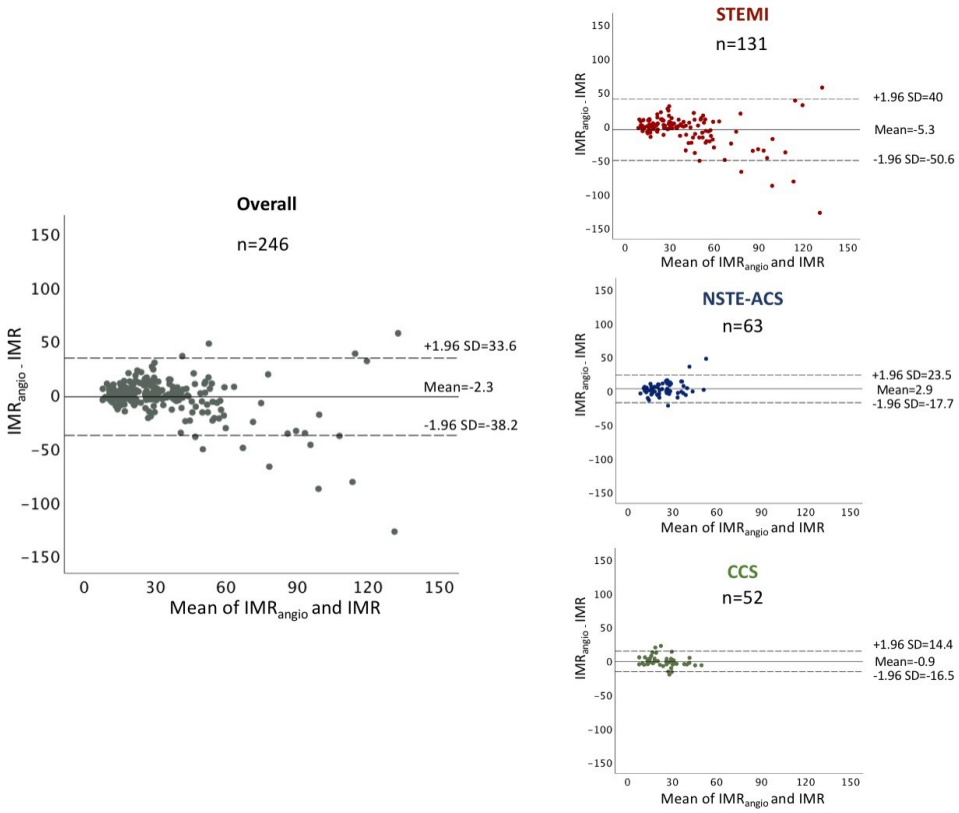


Figure 6. Bland-Altman plots

Bland-Altman plots summarise agreement between IMR_{angio} and IMR in STEMI and NSTEMI/CCS patients.

Microvascular vasodilatory capacity and angiography-derived microcirculatory resistance indices

RRR was significantly lower in patients with STEMI compared with NSTE-ACS and CCS patients, indicating a more severe impairment of microvascular vasodilatory capacity in the STEMI group (Table 1 and Supplementary Figure6). No significant differences in RRR were observed between NSTE-ACS and CCS (Table 1 and Supplementary Figure 9). The median RRR value in the whole cohort was 2.18, with higher proportion of STEMI patients (67.3%) presenting impaired RRR (<2.18) in the IRA, compared with NSTE-ACS (27.9%), CCS (43.7%) and the non-IRA (32.2%, p<0.001).

IMR_{angio} maintained a good correlation with IMR both in the group with low (rho=0.80, p<0.001) as in the group with high RRR (rho=0.64, p<0.001)

(Supplementary Figure 10). Conversely, NH-IMR_{angio} and thermodilution-derived IMR were well related (rho=0.66, p<0.001) in patients with low RRR (<2.18) but less well correlated (rho=0.36, p<0.001) in patients with high RRR (≥2.18) (Supplementary Figure 10).

Diagnostic performance of IMR_{angio} and NH-IMR_{angio}

In patients with STEMI, IMR_{angio} predicted IMR>40 U with an AUC of 0.93 (CI 95%: 0.88-0.98, p< 0.0001) (Figure 7). The best IMR_{angio} cut-off to predict IMR>40 U was 40 (Youden index=0.79). IMR_{angio}>40 U presented a diagnostic accuracy of 88.6%, NPV of 87.9%, PPV of 89.6%, sensitivity of 84.3% and specificity of 92.1%.

A good accuracy of IMR_{angio} was also maintained in NSTE-ACS. IMR_{angio} predicted an invasive-IMR>25 U with an AUC of 0.78 (CI 95%: 0.64-0.93, p<0.0001) (Supplementary Figure 7). The best IMR_{angio} cut-off in NSTE-ACS was 25 and demonstrated a diagnostic accuracy of 73.3%, NPV of 87.1%, PPV of 58.6%, sensitivity of 80.9% and specificity of 69.2%.

In patients with CCS, IMR_{angio} predicted IMR>25 U with an AUC of 0.88 (CI 95%: 0.79-0.97, p<0.0001) (Supplementary Figure 8). The best IMR_{angio} cut-off in CCS was 25 and demonstrated a diagnostic accuracy of 78.4%, NPV of 80.0%, PPV of 76.2%, sensitivity of 72.7% and specificity of 82.8%.

In STEMI patients, NH-IMR_{angio} demonstrated a good diagnostic performance in predicting IMR>40 U in the IRA (AUC=0.82, 95%CI 0.74-0.90, p<0.0001), but was less accurate when compared with IMR_{angio} (p=0.001) (Figure7). The diagnostic performance of NH-IMR_{angio} was however suboptimal in patients with NSTE-ACS (AUC=0.64, 95%CI 0.47-0.81, p=0.11) and CCS (AUC=0.63, 95%CI 0.48-0.79, p=0.10)(Supplementary Figures 7,8).

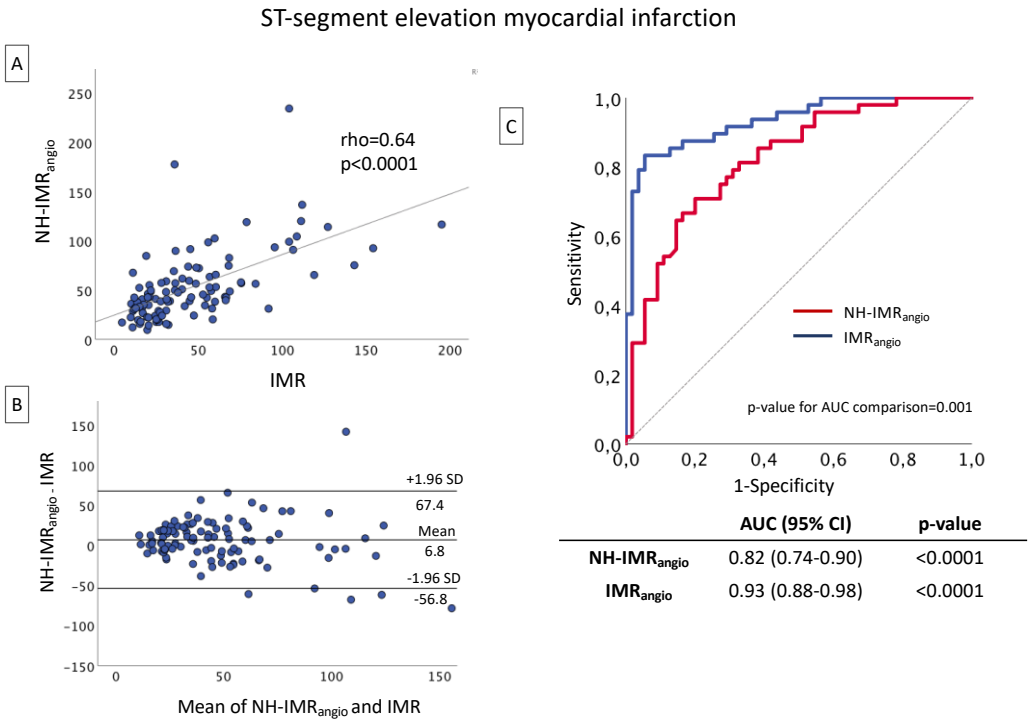


Figure 7. Non-Hyperaemic-IMR$_{angio}$ in the infarct-related-artery of STEMI

Scatter plot (A) and Bland Altman (B) analysis summarise significant correlation and agreement between NH-IMR$_{angio}$ and IMR in patients with STEMI. Panel C shows the ROC curve analysis for IMR$_{angio}$ and NH-IMR$_{angio}$ in predicting a pressure-wire IMR >40 U in the IRA of STEMI.

IMR$_{angio}$, NH-IMR$_{angio}$ and MVO

CMR imaging was performed in 49 STEMI patients (Supplementary Table 1). MVO>1.55% was present in 18 (36.7%) cases. Patients with MVO≥1.55% showed both higher IMR$_{angio}$ (41.0[29.5-64.3] vs 27.4[15.7-38.4], p=0.008) and NH-IMR$_{angio}$ (58.9[42.6-90.8] vs 43.4[30.1-59.1], p=0.026) compared with patients with MVO<1.55% (Supplementary Figure 11). IMR$_{angio}$ (AUC=0.76, 95%CI:0.61-0.91, p=0.007) and NH-IMR$_{angio}$ (AUC=0.71, 95%CI:0.54-0.87, p=0.033) presented fair and comparable diagnostic accuracy in predicting the presence of MVO>1.55% (Supplementary Figure 11).

Hybrid IMR_{angio} algorithm to assess coronary microvascular dysfunction in STEMI infarct-related artery

A cut-off value of <30U of NH-IMR_{angio} presented a NPV=92.3% in excluding an IMR>40 U. Conversely, a NH-IMR_{angio} >90U presented a PPV=93.3% in detecting an IMR>40U.

A hybrid decision-making strategy in which IMR_{angio} was measured only in vessels presenting NH-IMR_{angio} higher than 30U and lower than 90U, yielded an 88.0% agreement with IMR classification, sparing the administration of adenosine in 38.0% of the cases (Figure 8).

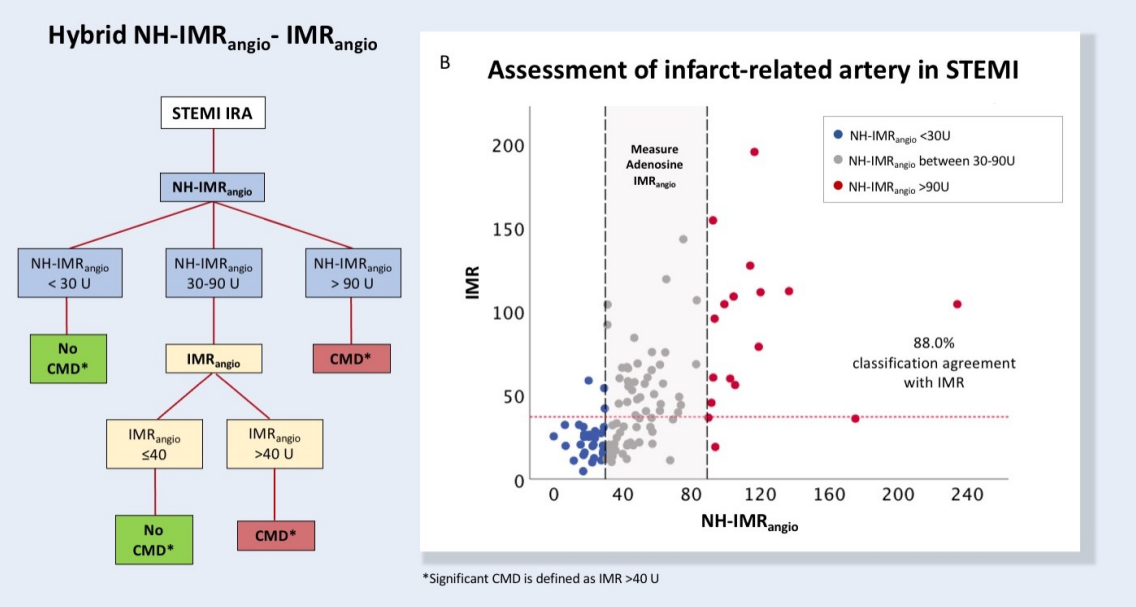


Figure 8. Hybrid IMR_{angio} algorithm in STEMI

Panel A shows the flow-chart of microcirculatory assessment of the IRA in STEMI patients using a "hybrid" NH-IMR_{angio}/ IMR_{angio} decision making strategy. Details of lesion distribution are shown in B. Overall, 38% of the lesions can be assed with high-accuracy by means of NH-IMR_{angio} only. A NH-IMR_{angio} cut-off of 30U presents a NPV of 92% in excluding IMR>40U. Conversely, a NH-IMR_{angio} cut-off of 90U presents a PPV of 93% in detecting IMR>40U. The algorithm offers a diagnostic accuracy in predicting invasive IMR of 88.0%.

DISCUSSION

The main results of our analysis are the following:

1. $IMR_{angio,}$ is a hyperaemic, angiography-based and pressure-wire-free index, with good diagnostic accuracy in defining an abnormal value of invasively measured pressure-wire-derived IMR.
2. The diagnostic accuracy of IMR_{angio} is maintained across the whole spectrum of coronary syndromes,-including STEMI, NTE-ACS and CCS. In STEMI, its diagnostic value is further confirmed by its correlation with MVO on CMR.
3. NH-$IMR_{angio,}$ a non-hyperaemic resting version, maintains a good diagnostic performance in STEMI whilst the same does not hold true in the non-IRA and in NSTE-ACS and CCS. This is likely to be due to the depleted vasodilatory capacity of the coronary microcirculation in STEMI, as reflected by a lower RRR.

Besides the well-established assessment of the epicardial segment of the coronary tree, a comprehensive physiological evaluation of CMD has important prognostic and therapeutic implications[40, 41]. Specifically, in patients with STEMI, the presence of microvascular injury has been associated with an increased risk of adverse outcome[7, 14, 16]. More recently, IMR has also been proposed as an accurate tool to early identify STEMI patients at increased risk of suboptimal reperfusion, who have potential benefit from additional therapies or closer monitoring[38]. Nevertheless, the application of CMD assessment in routine clinical practice remains extremely limited. This has been attributed to the requirement for a pressure-wire assessment and the associated additional procedural time, procedural cost and increased procedural complexity. The need of inducing hyperaemia with adenosine infusion is also a limiting factor.

In order to overcome some of these limitations, we have recently developed and validated IMR_{angio} as an angiography-derived and pressure-wire-free index to assess CMD[42]. However, its validation was limited to a relatively small cohort of STEMI patients. In this study we have extended those observations and shown that IMR_{angio} maintains an excellent diagnostic performance also in patients with NSTE-ACS and CCS compared with pressure-wire-derived IMR.

To the best of our knowledge, this represents one of the few available reports comparing the degree of CMD, measured by pressure-wire derived IMR across the spectrum of coronary syndromes. However, this is the first time it has been done with the newly proposed angiography-derived IMR (IMR_{angio}).

Unsurprisingly, STEMI presentations were characterized by a higher degree of CMD (high IMR) and reduced microvascular vasodilatory capacity (low RRR) compared to NSTE-ACS and CCS.

Notably, IMR, IMR_{angio} and RRR were not significantly different in NSTE-ACS and CCS and they did not differ between the IRA and non-IRA. This is in line with previous observations that microvascular impairment in the non-IRA, when present, is usually not severe and that the observed values of IMR are not significantly different from those measured in patients with CCS[44, 45].

Importantly, IMR_{angio} closely reproduced the measured invasive IMR across the spectrum of coronary syndromes. Notably, on Bland Altman analysis, the agreement between IMR_{angio} and invasive IMR was very close in NSTE-ACS and CCS, whereas it appeared more scattered in STEMI. This different behaviour is clearly due to the inherently higher biological variability of IMR in STEMI, in which the degree of CMD ranges from low to very high. This is in line with our previous observation that the absolute numerical values of IMR and IMR_{angio} are less correlated in cases of extreme (very high IMR) microvascular dysfunction[42]. Consistently, it has been previously shown that the agreement between QFR and FFR is negatively affected by the presence of severe microvascular impairment[46]. Nonetheless, even though the difference between IMR and IMR_{angio} values tends to widen with the severity of microvascular impairment, it remains a clinically meaningful concordance between the two measures using standard conventional thresholds for IMR. In particular, the classification agreement between IMR_{angio} and IMR remains excellent (88.6%) in STEMI, when using the established cut-off of >40 U for both parameters. Similarly, in patients with STEMI patients, the numeric agreement between NH-IMRangio and IMR remains strong up to extreme degrees of microvascular dysfunction, where the correlation between the two indices scatters.

In this study we also tested the accuracy of a non-hyperaemic and adenosine-free version of $IMR_{angio,}$ named $NH\text{-}IMR_{angio}$ against IMR. We observed that it reliably detects abnormal IMR in the IRA in STEMI but it did not do so in the non-IRA and in NSTE-ACS and in CCS. The good correlation of $NH\text{-}IMR_{angio}$ in the IRA in STEMI is dependent on a blunted vasodilatory response of the microcirculation to the hyperaemic agent, as reflected by a low RRR. This important observation is a further reflection of the different functional status of coronary microcirculation across the clinical presentations of STEMI, NSTE-ACS and CCS. When the vasodilatory response to adenosine is blunted, the RRR is exhausted and the difference between basal/non-hyperaemic and hyperaemic resistance is minimal, as observed in the IRA of STEMI[19]. This explains why in this setting, the agreement between a non-hyperaemic index of microvascular resistance ($NH\text{-}IMR_{angio}$) and the invasive (hyperaemic) IMR is maintained. Conversely, when the microvascular vasodilatory capacity is intact and the vasodilatory reserve is preserved, the vascular tone changes significantly after the administration of adenosine. In this case, a non-hyperaemic index of microvascular resistance does not reliably reflect the minimal level of resistance achievable at maximal hyperaemia. This is why in our study, the agreement between $NH\text{-}IMR_{angio}$ and IMR was poor in the non-IRA of STEMI patients, and in NSTE-ACS and CCS, since the corresponding vascular beds were characterised by relatively preserved RRR and IMR.

Interestingly, when assessed against CMR-derived MVO, $NH\text{-}IMR_{angio}$ and IMR_{angio} showed similar correlations. Importantly, similar prediction of MVO is a further proof that $NH\text{-}IMR_{angio}$ and IMR_{angio} could be used, to a certain extent, interchangeably in the IRA of patients with STEMI.

Whether the two angiography-derived indices have similar long-term prognostic value needs to be tested in dedicated studies measuring validated clinical outcomes. Our data suggest that $NH\text{-}IMR_{angio}$ can be a valid and a more practical alternative to assess CMD in the IRA of STEMI undergoing primary PCI. Indeed, when incorporated and combined with IMR_{angio} into a hybrid decision-making algorithm, $NH\text{-}IMR_{angio}$ would allow an adenosine-free microvascular assessment in nearly half of the cases (Figure 8).

Whilst the prognostic value of CMD in STEMI patients is well documented, its prognostic relevance in patients with CCS or with unobstructed coronary disease has only recently been considered[47]. In this setting, a dedicated assessment of the coronary microvascular function in the catheterization laboratory was shown to be effective in reducing symptoms and increasing quality of life and treatment satisfaction[40]. In our study we showed that IMR_{angio} was significantly higher in patients with unobstructed coronary arteries but with high IMR. This means that IMR_{angio} is a potential tool in the assessment of CMD in patients with microvascular angina, in whom the adoption of physiology-based assessment is sometimes perceived as problematic because of the necessity to manipulate with a pressure-wire an unobstructed epicardial coronary artery.

Limitations

The relatively small sample limits the conclusions of our analysis. In particular, the final sample size for each clinical subgroup (STEMI, NSTE-ACS and CCS) has to be acknowledged as a potential limiting factor of our analysis. Secondly, in our study, IMR was used to define CMD with different cut-offs in STEMI and in NSTE-ACS/CCS. The IMR cut-off of 40 is a well-established and validated threshold to define CMD in STEMI[14, 16]. An IMR>25 U has been previously proposed to define an abnormal coronary microcirculatory function in patients with CCS[40]. However, an analogous reference threshold for NSTE-ACS is missing. In our study we applied the same IMR threshold of 25 U used for CCS in NSTE-ACS, and this could explain the lower PPV and NPV observed for IMR_{angio} in NSTE-ACS compared to CCS. In the presence of severe epicardial disease and particularly when FFR is lower than 0.60, IMR tends to overestimate the true microvascular resistance because of the distal vessel underfilling and the collapse of microvessels with consequent falsely elevated microvascular resistance[13, 48]. Moreover, the contribution of collateral flow may cause a falsely increased value of distal coronary pressure measured by the pressurewire[49]. A slight overestimation of the IMR values cannot be excluded by our analysis since the coronary wedge pressure (Pw) was not available in this series. Nevertheless, only 5.7% of the coronary vessels included in the analysis presented a severe epicardial stenosis (FFR <0.60).

Moreover, the corrected IMR obtained applying the Yong formula presented a substantial agreement with the IMR values (Supplementary Figure 3). Therefore, we did not anticipate a significant overestimation of the true IMR in the vast majority of the assessed coronary vessels[3, 49].

In this study the angiographic views for IMR_{angio} analysis were prospectively acquired immediately after invasive IMR measurement. However, in the everyday practice, it may be difficult to be sure of the achievement of the maximal hyperemic status without the use of a pressure-wire. We anticipate that continuous i.v. adenosine infusion for a standardized time > 1 minute should guarantee the achievement of the maximal hyperemic status. This approach needs to be tested in future dedicated studies.

Lastly, other novel angiography-derived indices of microvascular function have been recently developed. In particular, Tebaldi and colleagues proposed an index that included the vessel length and correction for epicardial disease[50]. In this study, IMR_{angio} was not compared with other angiography-derived indices and future dedicated studies are warranted to explore these aspects of angiography-derived CMD assessment.

CONCLUSIONS

IMR_{angio} measured at maximal hyperaemia is a viable and pressure-wire-free alternative to IMR, with the potential of significantly simplifying the assessment of CMD in patients with acute and chronic coronary syndromes. NH-IMR_{angio} represents a reasonable alternative to IMR in the IRA of patients with STEMI, who usually have a blunted response to adenosine, as a consequence of the intra and peri-procedural microvascular injury. Both IMR_{angio} and NH-IMR_{angio} correlated well with MVO on CMR in STEMI patients.

When combined with IMR_{angio} in a hybrid decision-making algorithm, NH-IMR_{angio} can limit the need of inducing hyperaemia in nearly half of the cases, making the assessment of CMD in patients with STEMI even simpler and hence more easily adoptable in future research and clinical practice.

SUPPLEMENTARY MATERIAL

Inclusion criteria

1. Clinical indication for invasive coronary angiography for suspected coronary artery disease

Exclusion criteria

1. Haemodynamic instability
2. Previous history of coronary artery bypass grafting
3. Angiographic evidence of severe left main disease or complex coronary anatomy (such as high tortuosity, very high calcific burden, concomitant presence of chronic total occlusion)
4. Severe chronic kidney disease
5. General contraindications for CMR (specifically for STEMI patients)
6. Suboptimal angiographic imaging quality (not suitable for QFR analysis)

Coronary syndromes definition

STEMI

STEMI was defined as the occurrence of ongoing chest pain for at least 30 minutes associated with ST-segment elevation >2 mm in at least two contiguous leads or new left bundle branch block[1].

NSTE-ACS

NSTE-ACS was defined as the evidence of increased high-sensitivity troponin (above the 99th percentile of the upper reference limit) combined with the occurrence of typical symptoms of myocardial ischemia and/or new significant ST-

T changes (other than ST elevation/new left bundle branch block) on electrocardiogram and/or imaging evidence of new regional wall motion abnormalities[51].

CCS

CCS was defined as story of symptoms compatible with exertional myocardial ischemia and/or non-invasive evidence of coronary artery disease and/or inducible myocardial ischemia[41].

Index of microcirculatory resistance

IMR was measured in a standard fashion using thermodilution technique on the CoroFlow system (Coroventis, Uppsala Sweden).

Maximal hyperaemia was achieved with intravenous infusion of adenosine at a rate of 140 µg/kg/min. Mean transit time was calculated as the average of three transit time measurements during three separate injections of 3ml of room temperature 0.9% saline solution. IMR was then calculated as follows:

$IMR = Pd_{(hyperaemia)} \; x \; tTmean_{(hyperaemia)}$

Where Pd is distal pressure detected by the pressure-wire and tTmean is mean transit time.

Microvascular vasodilatory capacity

Resistive reserve ratio (RRR) was calculated in all patients at the same time-points when IMR was assessed. RRR is a measure of coronary microvascular vasodilatory capacity, describing the ability of the microcirculation to respond to a vasodilatory stimulus (e.g. adenosine infusion)[18]. It is calculated as:

$$RRR = \frac{\mathrm{Pd(resting)} x \; tTmean(resting)}{\mathrm{Pd(hyperemia)} x \; tTmean(hyperemia)}$$

An RRR below the median value was used to define an impaired microvascular vasodilatory capacity.

Quantitative flow ratio measurement

At the same time points when IMR measurement was scheduled, angiographic images at least 25 ° apart were acquired at 15 frame/second with manual injection of contrast dye, both at rest and during maximal hyperaemia, using a monoplane radiographic system (Siemens Healthcare, Germany). In order to avoid any possible confounder of contrast dye injection on IMR assessment, the angiographic views during hyperaemia were acquired after IMR measurement was completed.

QFR was measured also in one of the non-IRA in STEMI and NSTE-ACS cases who had invasive IMR assessment of the non-IRA.

Angiography-derived Index of Microcirculatory Resistance

IMR_{angio} was calculated by starting from the formula for calculation of IMR as previously described[4]:

$IMR = Pd_{(hyperaemia)} \times tTmean_{(hyperaemia)}$

where $Pd_{(hyperaemia)}$ is distal pressure at hyperaemia and $tTmean_{(hyperaemia)}$ is mean transit time at hyperaemia. By multiplying and dividing by hyperaemic aortic pressure ($Pa_{(hyperaemia)}$), the formula becomes:

$$IMR = Pa_{(hyperaemia)} x \frac{Pd_{(hyperaemia)}}{Pa_{(hyperaemia)}} x\ tTmean_{(hyperaemia)}$$

Since QFR is a surrogate of Pd(hyperaemia)/Pa(hyperaemia) ratio, (QFR ~ $\frac{Pd(hyperaemia)}{Pa(hyperaemia)}$), QFR can be used to replace $\frac{Pd(hyperaemia)}{Pa(hyperaemia)}$ in the formula. Similarly, $tTmean_{(hyperaemia)}$ can be expressed as the ratio between the number of frames (Nframes) for contrast dye to travel, during hyperaemia, from the guiding catheter to a distal reference (corresponding to the position of the distal marker of the pressure wire) divided by the frame-acquisition rate (fps).

In this way the formula becomes:

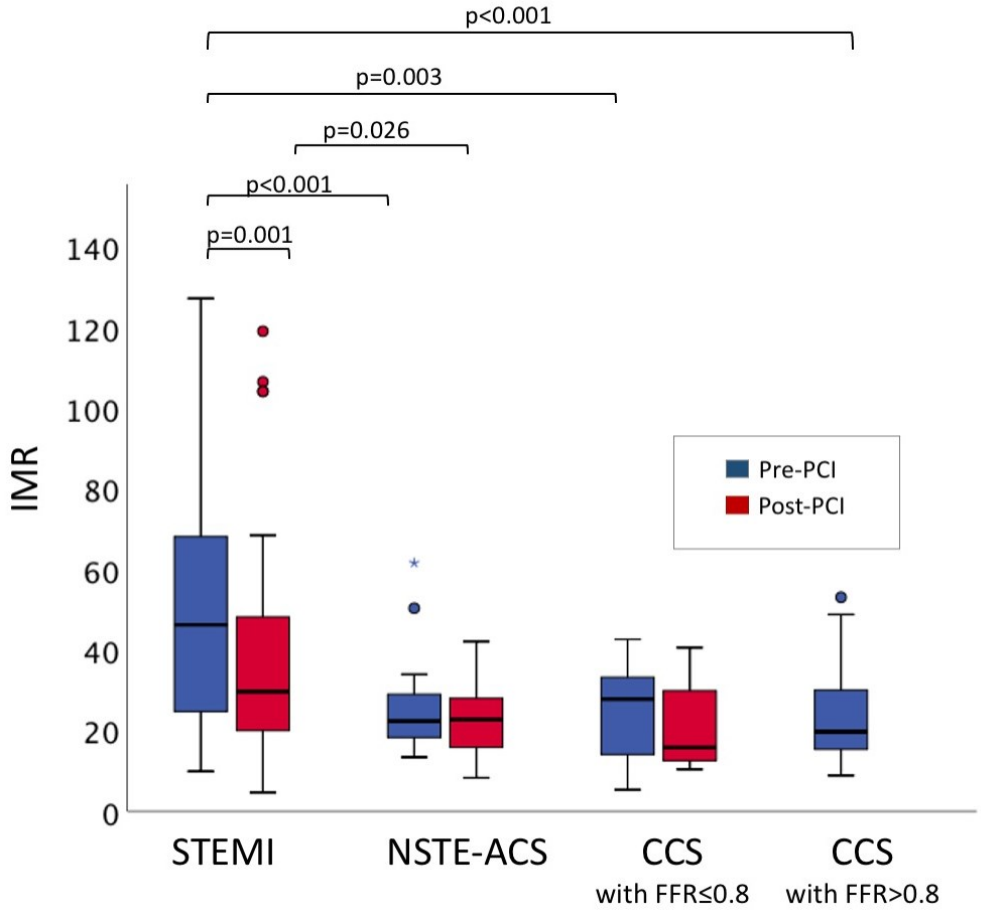
$$IMR_{angio} = Pa_{(hyperaemia)}\ x\ QFR_{(hyperaemia)}\ x\ \frac{Nframes_{(hyperaemia)}}{fps}$$

Supplementary Table 1. Coronary physiology measures across the spectrum of coronary syndromes

	STEMI	NSTEMI	CCS	p-value
Coronary physiology data – pre-PCI				
Resting Pa, mmHg	95(76-108)	89(78-98)	84(74-94)	0.251
Resting Pd, mmHg	77(61-90)	83(70-96)	78(68-86)	0.261
Resting tTmn, sec	1.04(0.71-1.39)	0.86(0.47-1.25)	0.74(0.50-1.22)	0.122
Hyperaemic Pa, mmHg	84(71-103)	85(72-98)	84(72-96)	0.883
Hyperaemic Pd, mmHg	62(47-84)	74(62-83)	70(60-82)	0.151
Hyperaemic tTmn, sec	0.72(0.40-1.17)	0.32(0.23-0.43)	0.34(0.22-0.50)	<0.0001
FFR	0.75(0.61-0.86)	0.85(0.80-0.94)	0.83(0.73-0.90)	0.008
Contrast-QFR	0.78(0.72-0.84)	0.83(0.78-0.93)	0.87(0.77-0.93)	0.002
Adenosine-QFR	0.78(0.67-0.87)	0.84(0.74-0.88)	0.82(0.73-0.93)	0.123
Coronary physiology data - post-PCI				
Pa, mmHg	93(79-105)	85(81-100)	89(74-98)	0.651
Pd, mmHg	90(76-96)	83(77-97)	81(68-98)	0.634
Resting tTmn, sec	0.68(0.45-1.25)	0.59(0.44-1.09)	0.65(0.45-1.38)	0.951
Hyperaemic Pa, mmHg	84(75-94)	82(74-95)	74(44-87)	0.132
Hyperaemic Pd, mmHg	78(68-91)	74(67-86)	76(58-89)	0.297
Hyperaemic tTmn, sec	0.37(0.25-0.65)	0.28(0.21-0.41)	0.30(0.22-0.60)	0.094
FFR	0.94(0.89-0.98)	0.88(0.84-0.96)	0.87(0.82-0.93)	0.015
Contrast-QFR	0.96(0.91-0.99)	0.94(0.86-0.97)	0.93(0.88-0.99)	0.182
Adenosine-QFR	0.95(0.89-0.98)	0.92(0.89-0.96)	0.90(0.85-0.99)	0.301

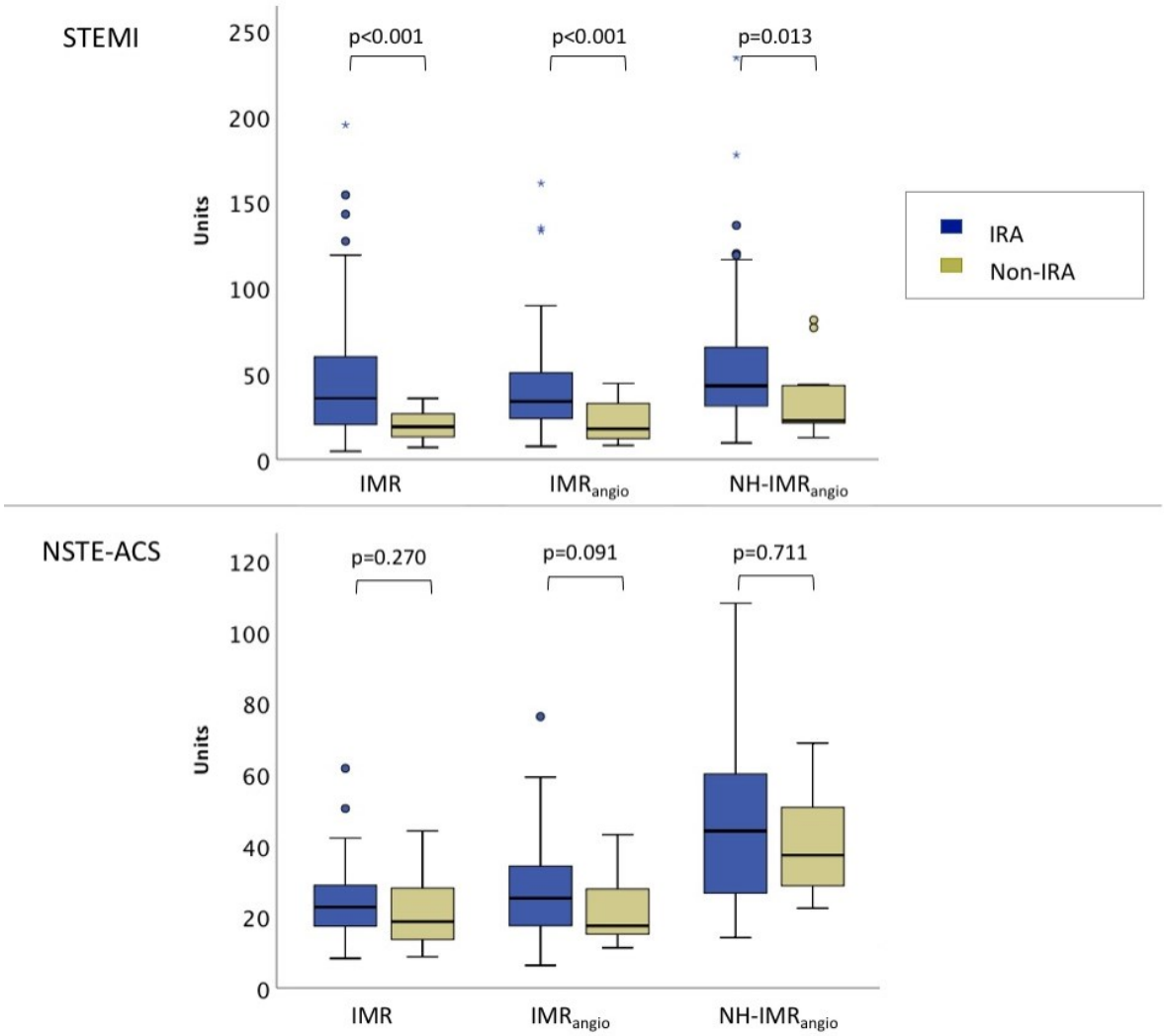
Supplementary Figure 1.

Box plots show IMR median values in patients with STEMI, NSTE-ACS and CCS before and after PCI. CCS cases with FFR >0.80 at baseline did not undergo PCI. p-value is provided for statistically significant differences between the subgroups. Other comparisons were not statistically significant.

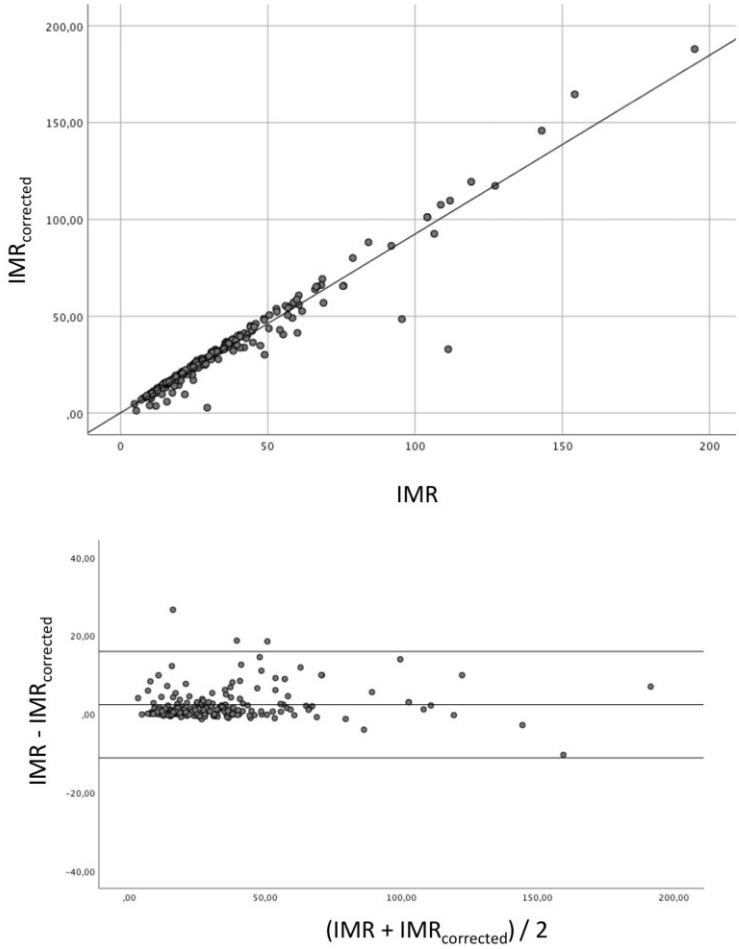


Supplementary Figure 2

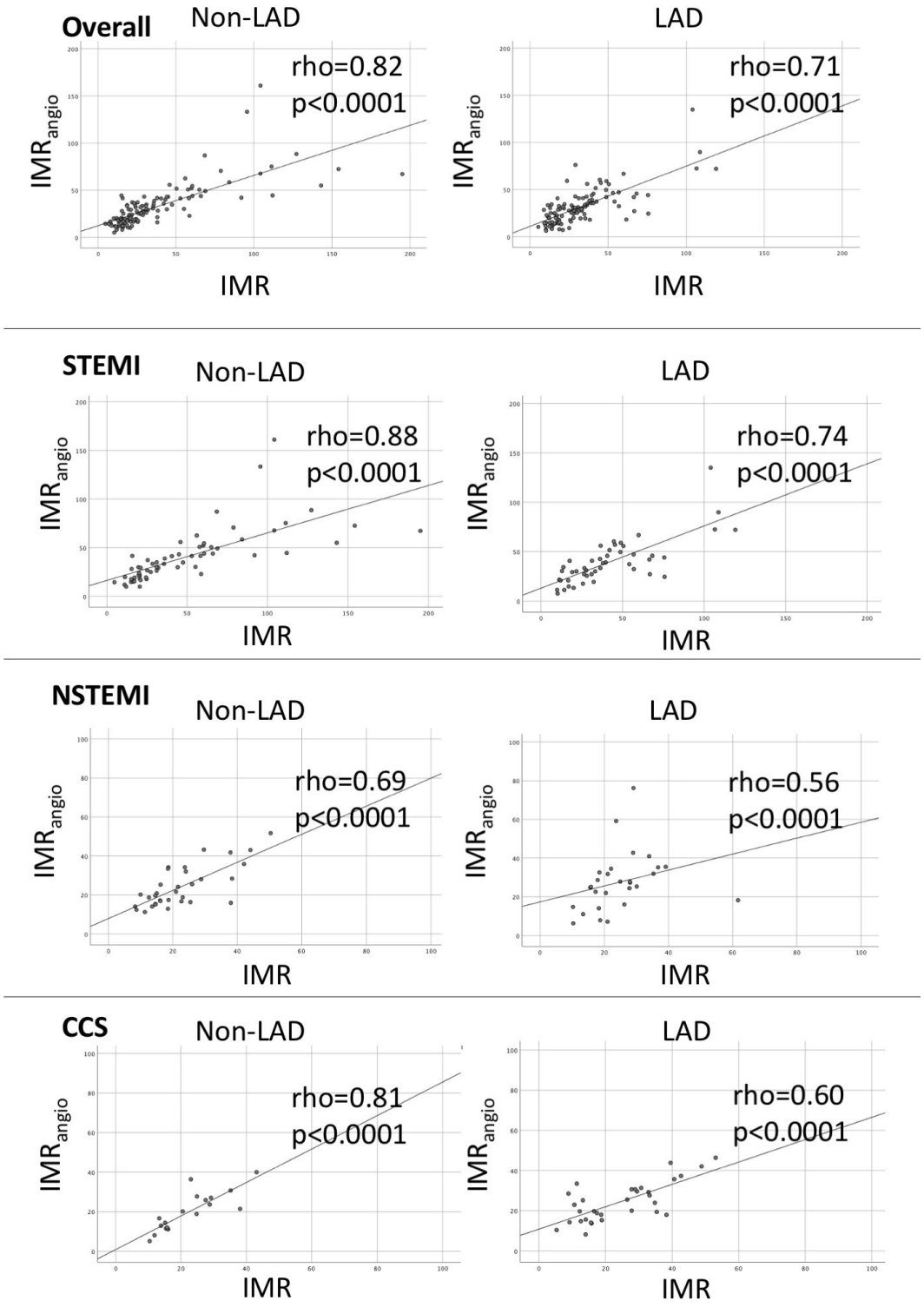
Box plots show IMR, IMR_{angio} and $NH\text{-}IMR_{angio}$ in the infarct-related-artery and non-IRA of patients with STEMI and NSTE-ACS.



Supplementary Figure 3

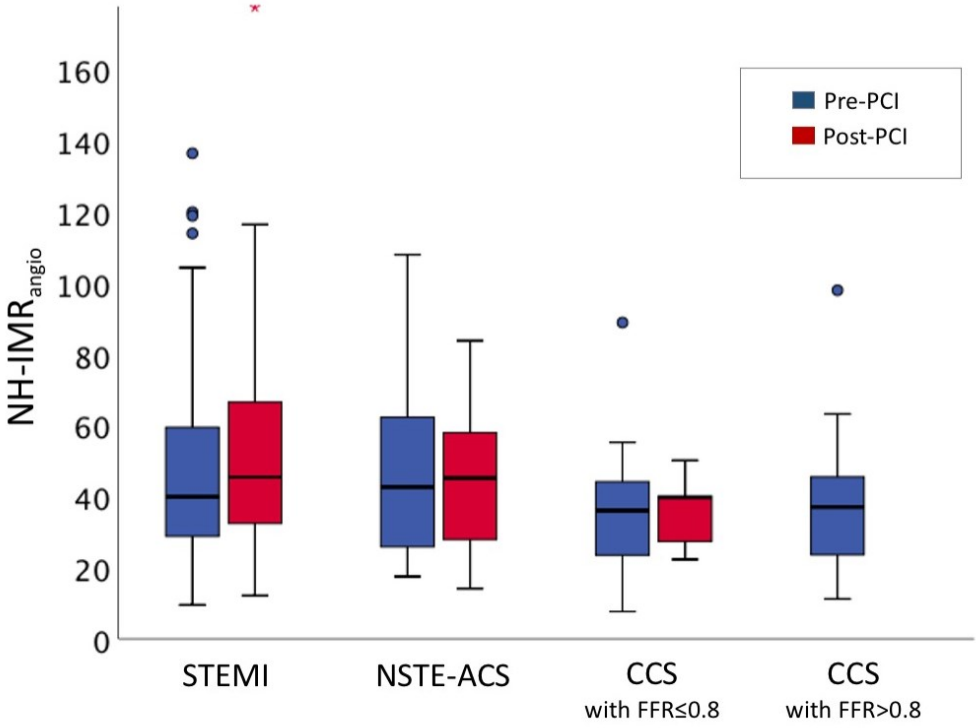


Supplementary Figure 4. Correlation between IMR and IMR_{angio} in LAD vs non-LAD coronary vessels



Supplementary Figure 5

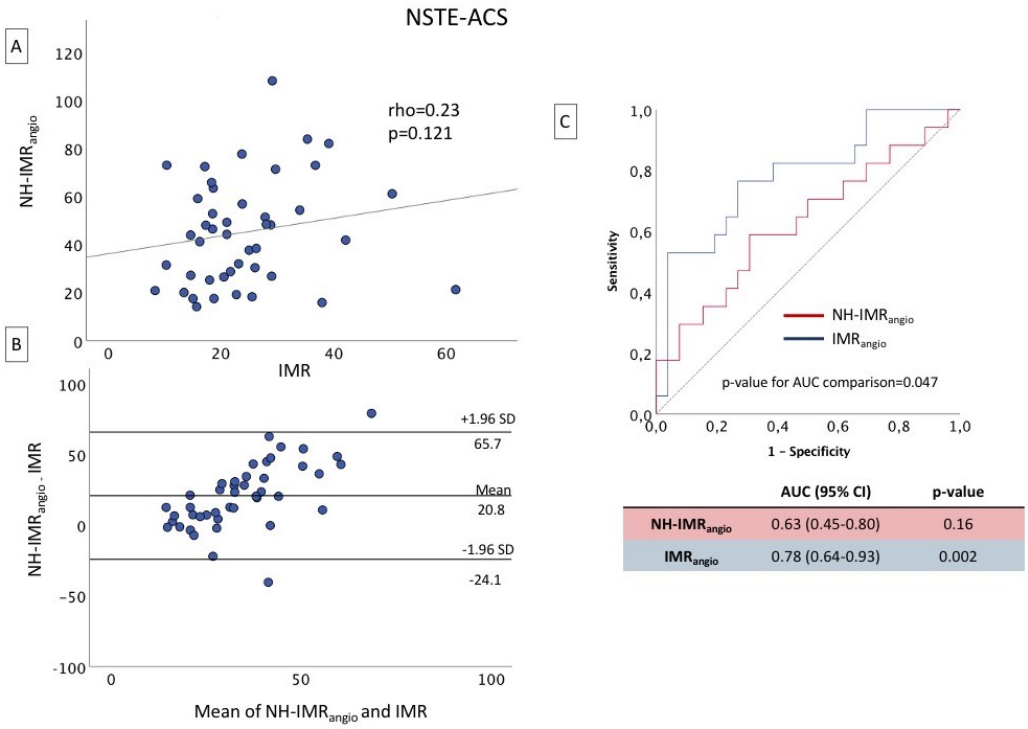
Box plots show NH-IMR$_{angio}$ median values in patients with STEMI, NSTE-ACS and CCS before and after PCI. CCS cases with FFR >0.80 at baseline did not undergo PCI. p-value is provided for statistically significant differences between the subgroups. Other comparisons were not statistically significant.



Supplementary Figure 6

Scatter plot (A) and Bland Altman analysis (B) of NH-IMR_{angio} and IMR in patients with NSTE-ACS.

In panel C is shown the ROC curve analysis of IMR_{angio} and NH-IMR_{angio} in predicting an invasive IMR >25 U.

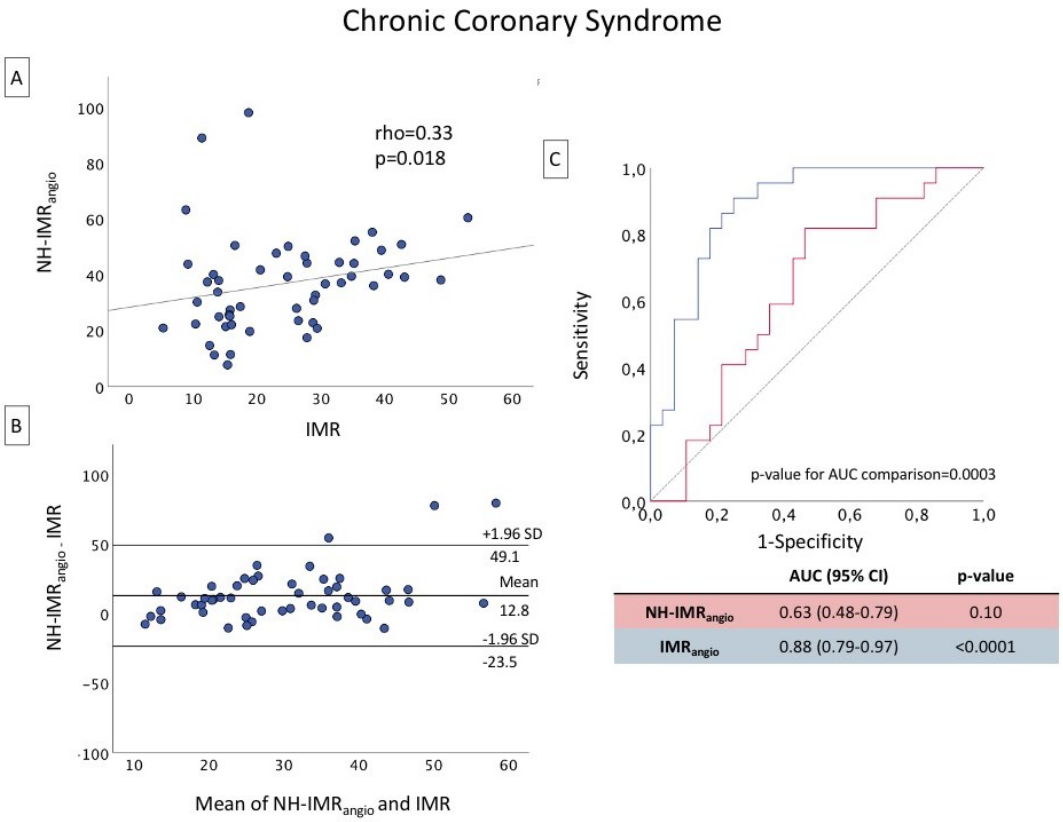


	AUC (95% CI)	p-value
NH-IMR_{angio}	0.63 (0.45-0.80)	0.16
IMR_{angio}	0.78 (0.64-0.93)	0.002

Supplementary Figure 7

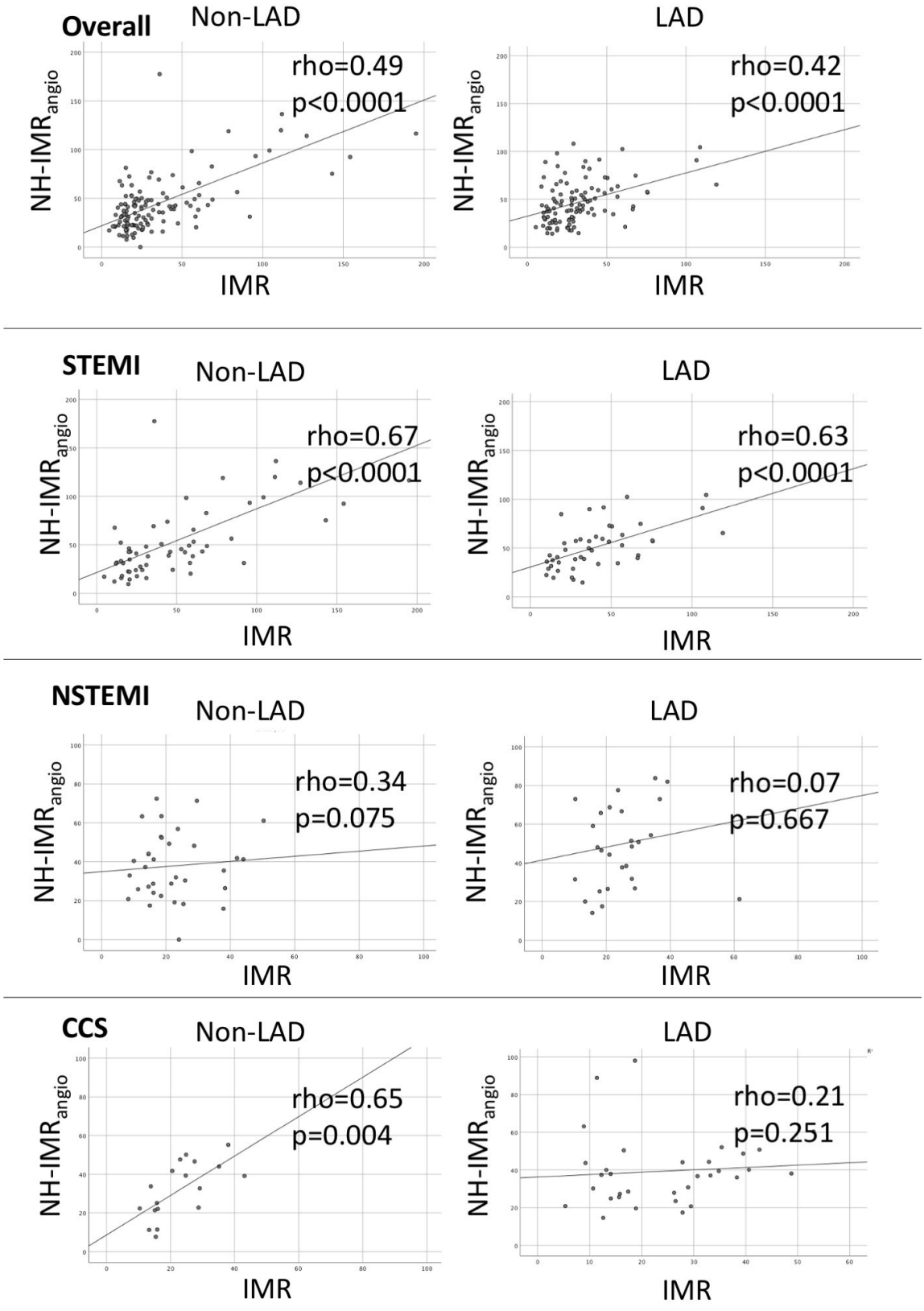
Scatter plot (A) and Bland Altman analysis (B) of NH-IMR_{angio} and IMR in patients with CCS.

In panel C is shown the ROC curve analysis of IMR_{angio} and NH-IMR_{angio} in predicting an invasive IMR >25 U.



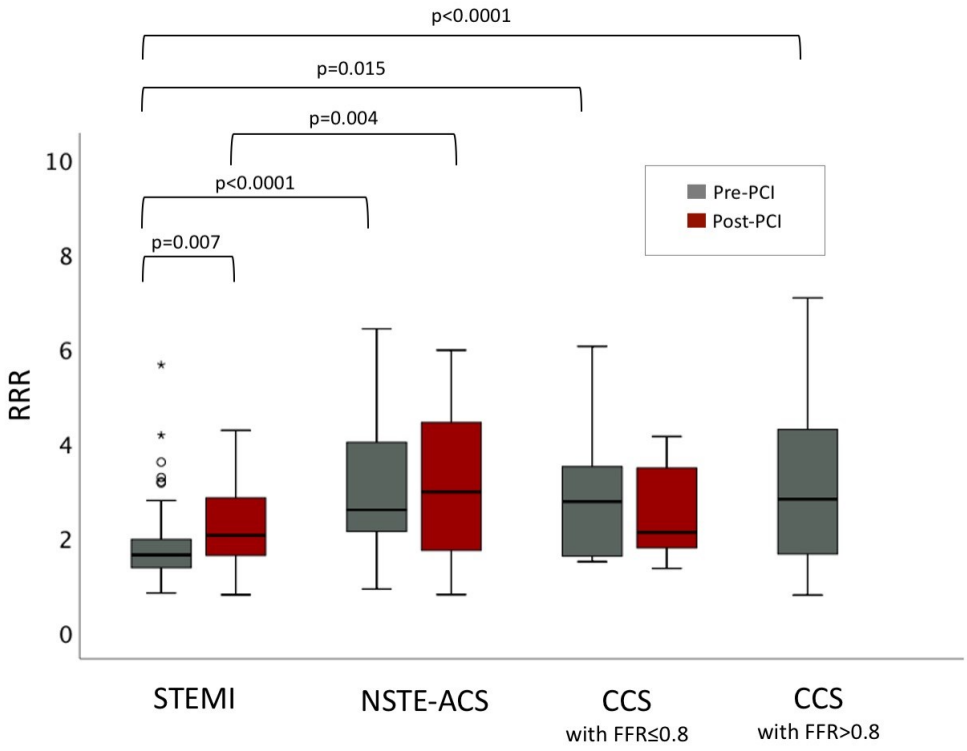
	AUC (95% CI)	p-value
NH-IMR_{angio}	0.63 (0.48-0.79)	0.10
IMR_{angio}	0.88 (0.79-0.97)	<0.0001

Supplementary Figure 8. Correlation between IMR and NH-IMR$_{angio}$ in LAD vs non-LAD coronary vessels.



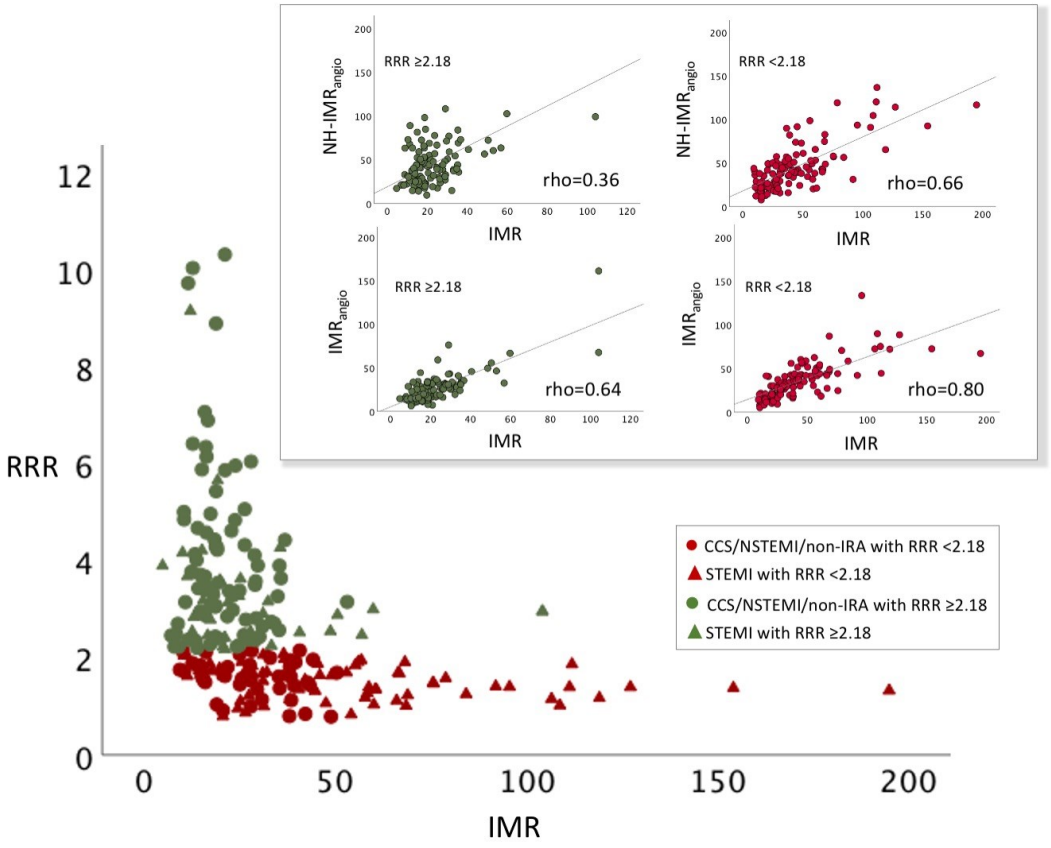
Supplementary Figure 9

Box plots show RRR median values in patients with STEMI, NSTE-ACS and CCS before and after PCI. CCS cases with FFR >0.80 at baseline did not undergo PCI. p-value is provided for statistically significant differences between the subgroups. Other comparisons were not statistically significant.



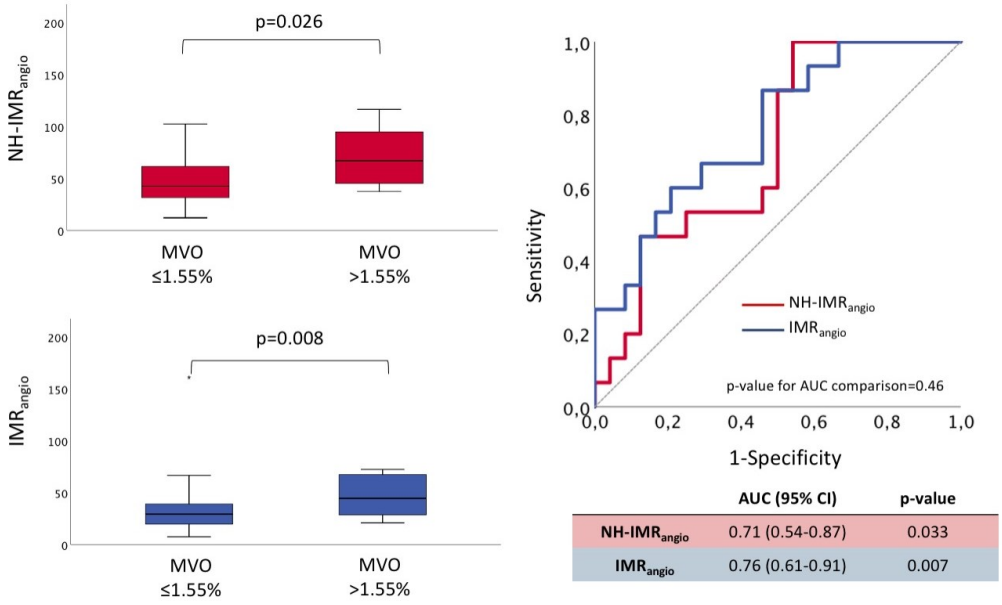
Supplementary Figure 10. Microvascular vasodilatory properties and IMR_{angio}

The relationship between RRR and IMR is shown in the main panel. Coronary lesions have been stratified according to low (<2.18) vs. high (≥2.18) RRR. Notably, the rate of STEMI is significantly higher in the low RRR group (67% vs 33%). The correlation between NH-IMR_{angio} and IMR is preserved in patients in cases with low RRR whereas is poor in cases with high RRR. Conversely, IMR_{angio} showed an excellent correlation with IMR irrespectively of the RRR.



Supplementary Figure 11.

Patients with evidence of CMR-based MVO showed higher IMR_{angio} and NH-IMR_{angio} compared with patients without MVO (A). ROC curve analysis of IMR_{angio} and NH-IMR_{angio} in predicting MVO >1.55% (B).



	AUC (95% CI)	p-value
NH-IMR_{angio}	0.71 (0.54-0.87)	0.033
IMR_{angio}	0.76 (0.61-0.91)	0.007

Supplementary Table 2. CMR characteristics of patients presenting with STEMI

STEMI	n=49
LVEDV(ml)	150 (127-172)
LVESV(ml)	76 (57-98)
LVEF(%)	50 (43-55)
Infarct Size(g)	18 (15-28)
Infarct Size(%)	24.3 (18.0-31.7)
MVO(%)	0.0 (0.0-3.1)
MVO >1.55%	18 (36.7)
LV oedema(%)	41.6(37.2-51.3)
Salvaged Myocardial Area(%)	15.9(10.5-23.5)
Salvaged Myocardial Index(%)	38.7(29.2-49.3)

CHAPTER 5

Long-term prognostic value of angiography-derived index of microcirculatory resistance in patients with STEMI

ABSTRACT

Aims

Microvascular injury evaluation yields prognostically relevant information in ST-segment-elevation myocardial infarction (STEMI) patients. However, widespread adoption of invasive pressure-wire-based evaluation of coronary microvascular function is limited due to cost, technical and procedural complexity. We explored the diagnostic and prognostic potential of non-hyperaemic angiography-derived index of microcirculatory resistance (NH IMR_{angio}) as a pressure-wire and adenosine-free microvascular function evaluation tool.

Methods and Results

A total of 262 STEMI patients were prospectively enrolled in our study. NH IMR_{angio} was retrospectively derived on the infarct related artery (IRA) through a dedicated software-based application of computational fluid dynamics to three-dimensional coronary artery modelling. Invasive pressure-wire-based assessment of the index of microcirculatory resistance (IMR) was performed. Measurements were performed on IRAs at completion of primary percutaneous coronary intervention (pPCI). The combination of all-cause mortality resuscitated cardiac arrest and new heart failure diagnosis was the prespecified primary endpoint.

NH IMR_{angio} showed a good diagnostic performance in identifying microvascular injury AUC0.78(95%CI:0.72-0.84,p<0.0001) with an optimal cut-off at 43U. The primary endpoint occurred in 38(16%) patients at a median follow-up of 4.2(2.0-6.5) years. On survival analysis, NH IMR_{angio}>43U (log-rank test,p<0.001) was equivalent to an IMR>40U (log-rank test,p=0.02) in predicting the primary endpoint (hazard ratio comparison p-value=0.91). NH IMRangio>43U was an independent predictor of the primary endpoint (adjusted HR 2.13,95%CI:1.01-4.48).

Conclusion

NH IMR_{angio} has a reliable diagnostic performance to identify microvascular injury in STEMI patients with a prognostic value equivalent to invasively measured IMR. NH IMR_{angio} can be a feasible alternative to IMR for risk stratification in pPCI treated STEMI patients.

INTRODUCTION

The adoption of primary percutaneous coronary intervention (pPCI) has contributed to improvement of short- and long-term outcome in patients with ST-segment-elevation myocardial infarction (STEMI).[52] However, despite the widespread implementation of guideline based treatment[53], a significant subset of patients still experiences poor outcomes due to the development of heart failure.[54, 55] Severe coronary microvascular injury and subsequent suboptimal reperfusion are key pathological processes underlying post myocardial infarction heart failure development.[56] Microvascular injury in patients admitted with ST-segment-elevation myocardial infarction (STEMI) is biologically[7] and prognostically relevant [57] and cardiac magnetic resonance (CMR) imaging is conventionally accepted as the gold standard for detecting it.[58] However, the relative expense and limited availability of CMR imaging still represents a main barrier to its widespread clinical adoption especially in the emergency pPCI setting.[59] Therefore, to evaluate microvascular injury as early as possible and in the catheterization laboratory, the index of microvascular resistance (IMR), based on the use of a conventional pressure-wire and thermodilution technique, has been proposed and investigated.[60] Our group and others have previously shown the good diagnostic performance of IMR in predicting microvascular injury diagnosed by CMR imaging.[7, 14] Contemporary studies highlight the early- and long-term prognostic implications of an IMR >40U in STEMI patients.[17, 61] Recently, IMR has also been proposed as a tool to triage novel or additional therapies in STEMI, with promising clinical and research implications.[20, 62] Despite its proposed role as a prognostic and theragnostic biomarker [62], the clinical adoption of IMR remains limited. Additional

cost, procedural time, an extra - though small - procedural risk associated with the manipulation of a pressure wire in the infarct related artery (IRA) and patient discomfort due to intravenous adenosine infusion, are amongst some of the barriers to its widespread use. The recent development and application of computational flow dynamics to three-dimensional modelling of the coronary artery represents an excellent opportunity to angiographically derive indices of coronary physiology (such as fractional flow reserve or IMR) avoiding the use of a pressure wire.[63] Our group has recently described a novel, angiography-derived, pressure-wire free index of microcirculatory resistance (IMR_{angio}).[64] This index showed a good diagnostic performance in predicting an invasive IMR >40U in STEMI patients.
The aim of this work was to evaluate the diagnostic and prognostic performance of non-hyperaemic IMR_{angio} (NH IMR_{angio}) in STEMI patients.

METHODS

Patient population

Patients presenting between January 2010 and March 2020 with STEMI at the Oxford Heart Centre were enrolled in the prospective OxAMI (Oxford Acute Myocardial Infarction) cohort study. STEMI was diagnosed in the presence of chest pain lasting for at least 30 minutes accompanied by ST segment elevation (>2 mm) in at least 2 anatomically contiguous leads. The current study is based on prospectively enrolled participants who had pressure-wire based IMR measurements. Pressure-wire-based coronary physiology was not performed if any of the following exclusion criteria were met: i) haemodynamic instability, ii) history of coronary artery bypass grafting, iii) severe chronic renal failure, iv) angiographic evidence of severe left main disease or complex coronary anatomy (tortuous IRA, presence of a chronic total occlusion), and v) pPCI performed with plain old balloon angioplasty. This retrospective analysis includes all participants on whom both invasive IMR measurement and angiography-derived coronary physiology assessment was feasible. The patient flow diagram is reported in Supplementary figure S1.
pPCI was performed in standard fashion with the use of adjunctive therapies (mechanical thrombectomy, glycoprotein IIb/IIIa) and choice of stenting technique

at the operator's discretion. The Thrombolysis in Myocardial Infarction (TIMI) trial grading system was used as a semiquantitative angiographic tool to assess coronary flow before and at completion of pPCI on the IRA.[65] ST-segment resolution was calculated using surface electrocardiography acquired before and at 90 minutes after pPCI as described previously.[66] We defined an ST-segment resolution ≥70% as complete. The OxAMI study design has been previously described in detail.[3] The study protocol was approved by the local ethics committee (10/H0408/24) and conducted in accordance with the Declaration of Helsinki.

Non-hyperaemic IMR_{angio} measurement

NH IMR_{angio} was measured on the IRA, using two dedicated coronary angiographic projections acquired at the end of the pPCI procedure. The methodology employed and its reproducibility have been published previously.[64] In brief, three-dimensional quantitative coronary angiography and quantitative flow ratio (QFR) analyses were performed using QAngio® XA 3D software (Medis, Leiden, the Netherlands). NH IMR_{angio} was computed using the following formula:

$$NH\ IMR_{angio} = Pa(resting)\ x\ QFR\ x\ \frac{Nframes}{fps}$$

where *Pa* was the post PCI mean aortic pressure at resting conditions, *Nframes* was the number of angiographic frames from contrast dye to travel from the guiding catheter to a distal reference (placed at the distal third of the IRA) in resting conditions and fps was the frame-acquisition rate.

All analyses were performed at the OxACT corelab (University of Oxford, Oxford Heart Centre, John Radcliffe Hospital, Oxford, UK) by independent operators blinded to physiology and clinical outcome data. Disagreement was resolved by consensus.

Invasive measurement of coronary physiology indices

Invasive assessment of coronary physiology indices on the IRA was performed with pressure-wire technology (Abbott, Santa Clara, California, US or Certus, St. Jude Medial, St. Paul, Minnesota, US) and a thermodilution technique at the end of the pPCI as previously reported.[3] Briefly, measurements were taken after intracoronary injection of 250μg of isosorbide dinitrate. Transit time was calculated as the average of transit time measurements during three separate injections of 3 ml of

0.9% room temperature saline at resting and hyperaemic conditions. Hyperaemia was induced by intravenous adenosine infusion at a rate of 140μg/kg/min. IMR was defined as the product of mean distal coronary pressure and mean transit time during hyperaemia.[14] Coronary flow reserve (CFR) was expressed as the ratio of resting to hyperaemic mean transit times.[14] Resistive reserve ratio (RRR) was defined as the ratio of resting to hyperaemic coronary microcirculatory resistance.[67] Based on established literature, IMR and CFR were dichotomized using thresholds of 40U and 2.0, respectively.[14]

Clinical Follow-up

The primary clinical outcome of the study was the hierarchical composite endpoint of all-cause mortality, resuscitated cardiac arrest and new heart failure diagnosis. The secondary clinical outcome of the study was the hierarchical composite endpoint of cardiac mortality, resuscitated cardiac arrest and new heart failure diagnosis. Heart failure was defined as the development of new heart failure symptomatology and/or prescription of diuretics in conjunction with supporting new non-invasive imaging findings of left ventricular systolic dysfunction (left ventricular ejection fraction (LVEF) <50%) and/or raised brain natriuretic peptide. Follow-up was performed through electronic case record review, clinic visit and telephone contact.

Statistical Analysis

We tested the normality assumption of continuous variables with statistical (Shapiro-Wilk test) and graphical (histogram) means. We expressed continuous variables as mean ± standard deviation or median (25^{th} to 75^{th} percentile) as appropriate and categorical variables as numbers (percentage). Between-group comparisons for categorical variables were performed using Pearson's χ^2 or Fisher's exact test as appropriate, while continuous variables were compared using Student's t-test, Mann-Whitney U test or Kruskal-Wallis test. Correlations between variables were expressed using Spearman rho coefficients. We used receiver operator characteristic curve analysis to explore the diagnostic utility of NH IMR_{angio} to predict an IMR >40U. We then selected the optimal NH IMR_{angio} cut-off value to predict an IMR >40U by identifying the value that maximised Youden's *J* statistic.

We subsequently performed survival analyses for the primary and secondary endpoints stratified according to i) NH IMR_{angio} and ii) IMR (both expressed as dichotomous variables) using Kaplan Meier and Cox proportional hazard regression modelling methods. In a sensitivity analysis we also added CFR (as a dichotomous variable) to the survival analyses for the primary and secondary endpoints. We compared the hazard ratios of dichotomised NH IMR_{angio}, IMR and CFR with paired Student's t-tests.[68] To evaluate the prognostic utility of NH IMR_{angio} (as a dichotomous variable) for our primary endpoint we constructed multivariate Cox regression models adjusted for clinical, procedural, angiographic and echocardiographic variables. Missingness for ST segment resolution was addressed by creating a third group for the variable (yes/no/unknown) and median imputation for discharge echocardiography LVEF%. Proportional hazard assumptions were graphically and statistically assessed. Explanatory variables with a p-value of <0.1 at univariate analysis were entered in the model using a conditional backward stepwise method. We measured the goodness of fit using concordance (C-statistic) and deviance statistics. By adding NH IMR_{angio} in a baseline multivariate model, we assessed for improvement in model performance by performing a likelihood ratio test.

Statistical analyses were performed with SPSS version 26 (IBM Inc. New York USA) and R studio version 1.3 (*survival*, *survminer*, *forestplot* and *survcomp* packages). All tests were two-sided and □ was set at 0.05.

RESULTS

Study population

A total of 262 patients with both post pPCI invasive IMR and NH IMR_{angio} assessment were included in the current analysis. Complete follow-up data was available for 241 participants (92%) with a median follow-up of 4.2 (2.0-6.5) years. Baseline clinical, procedural and imaging characteristics are reported in Table 1. Data about post pPCI NH IMR_{angio} and invasive indices of coronary physiology indices are presented in Table 2. The median IMR and NH IMR_{angio} values at the end of the procedure were 33 (20 - 55) and 43 (30 - 59) units respectively.

Table 1. Clinical, Procedural & Echocardiographic Characteristics

Total Number	**262**
Clinical	
Age, years	62 ± 11
Male gender, n (%)	215 (82)
Hypertension, n (%)	119 (46)
Hypercholesterolemia, n (%)	101 (39)
Diabetes, n (%)	41 (16)
Smoker, n (%)	110 (42)
Previous cardiology history, n (%)*	37 (14)
Family history of IHD, n (%)	101 (39)
Procedural	
Ischemic time, minutes	180 (122, 317)
Target vessel	
LAD, n(%)	119 (45)
LCX, n(%)	25 (10)
RCA, n(%)	109 (42)
Other, n(%)	9 (3)
TIMI Flow – pre-PCI	
0	197 (75)
1	22 (8)
2	30 (12)
3	13 (5)
TIMI Flow – post-PCI	
0	0 (0)
1	3 (1)

2	33 (13)
3	226 (86)
Complete ST segment resolution, n (%)*	151 (73)
Discharge Echocardiography LVEF, %*	50 (45, 56)

IHD: ischaemic heart disease; LAD: left anterior descending artery; LCx: left circumflex artery; LVEF: left ventricle ejection fraction; PCI: percutaneous coronary intervention; RCA: right coronary artery; TIMI: the Thrombolysis in Myocardial Infarction; *Degree of missingness: Previous cardiological history = 0.4%, Complete ST segment resolution = 21%, Discharge LVEF % Echocardiography = 2%.

Table 2. Post PCI pressure-wire- and angiography-derived coronary physiology indices

Total Number	**262**
Pressure-wire-derived	
Resting Pa, mmHg	92 ± 18
Resting transit time, s*	0.69 (0.48, 1.13)
Hyperaemic Pa, mmHg*	83 ± 16
Hyperaemic Pd, mmHg	76 (67, 87)
Hyperaemic transit time, s	0.43 (0.28, 0.78)
FFR*	0.94 (0.90, 0.98)
IMR	33 (20, 55)
CFR*	1.5 (1.1, 2)
RRR*	1.7 (1.3, 2.3)
Angiography-derived	
Fixed flow QFR*	0.95 (0.90, 0.98)
Contrast QFR	0.96 (0.90, 0.99)
NH IMR_{angio}	43 (30, 59)

CFR: coronary flow reserve; FFR: fractional flow reserve; IMR: index of microvascular resistance; NH IMR_{angio}: non-hyperaemic IMR_{angio}; Pa: aortic pressure; Pd: distal pressure; PCI: percutaneous coronary intervention; QFR: quantitative flow ratio; RRR: resistive reserve ratio; *Degree of missingness: resting transit time = 1.1%, hyperaemic Pa= 0.4%, FFR = 0.4%, CFR = 1.1%, RRR= 1.1%; Fixed flow QFR = 0.8%.

Diagnostic performance of NH IMRangio

NH IMR_{angio} was significantly correlated with IMR (rho=0.50, p<0.0001) and predicted an IMR >40U with an AUC of 0.78 (95% CI: 0.72-0.84, p<0.0001) (Supplementary figure 2). The optimal NH IMR_{angio} cut-off to predict an IMR >40U was 43U (Sensitivity: 77%, Specificity: 67%) coinciding with the median NH IMR_{angio} value of 43U observed in the overall study cohort. Patients with a high NH IMR_{angio} (>43U) were characterised by longer ischaemic times, worse rates of post pPCI TIMI flow grade, lower occurrence of complete ST-segment resolution, and lower left ventricular ejection fraction at discharge than patients with a low NH IMR_{angio} (Table S1). A high NH IMR_{angio} (>43U) was associated with a significantly higher degree of invasively assessed microvascular dysfunction (expressed by either IMR, CFR or RRR) than a low NH IMR_{angio} (Table S2).

Prognostic value of NH IMR_{angio}

At 7 years of follow-up, the primary and secondary endpoints occurred in 38 (16%) and 30 (12%) participants respectively. All-cause death occurred in 13 (5%) participants (4 cardiac deaths), two (1%) had a resuscitated cardiac arrest and 28 (12%) had a new diagnosis of heart failure. Cox regression analyses showed that a post pPCI NH IMR_{angio} >43U was significantly associated with a higher risk of both the primary and secondary endpoints, HR 3.43 (95%CI: 1.67-7.07, p=0.001) and HR 3.32 (95%CI: 1.48-7.47, p=0.004) respectively. A post pPCI IMR >40U was also significantly associated with a higher risk of the primary and secondary endpoints HR 2.07 (95% CI: 1.09-3.92, p<0.03) and HR 2.17 (95% CI 1.06-4.48, p<0.04). The comparison of the hazard ratio estimates of NH IMR_{angio} >43U and IMR >40U did not yield a statistically significant difference for either endpoint (p=0.91, and p=0.85). In an exploratory analysis, a CFR ≤2.0 was significantly associated with a higher risk of the primary and secondary endpoints HR 3.82 (95% CI 1.17-12.43, p<0.03) and 4.56 (95% CI 1.08-19.18, p<0.04). Pairwise comparisons CFR ≤2.0 with IMR >40U and NH IMRangio >43U hazard ratios were not statistically significant. The Kaplan Meier curves displaying the relationship between survival-free from the primary and secondary endpoints for high versus low NH IMR_{angio} and IMR are shown in Figures 1 and 2 respectively.

The profiles of the survival curves are similar. Kaplan Meier survival curves displaying the relationship between survival free from the primary and secondary endpoints for high and low NH IMR_{angio}, IMR and CFR are displayed in Supplementary figures 3 and 4.

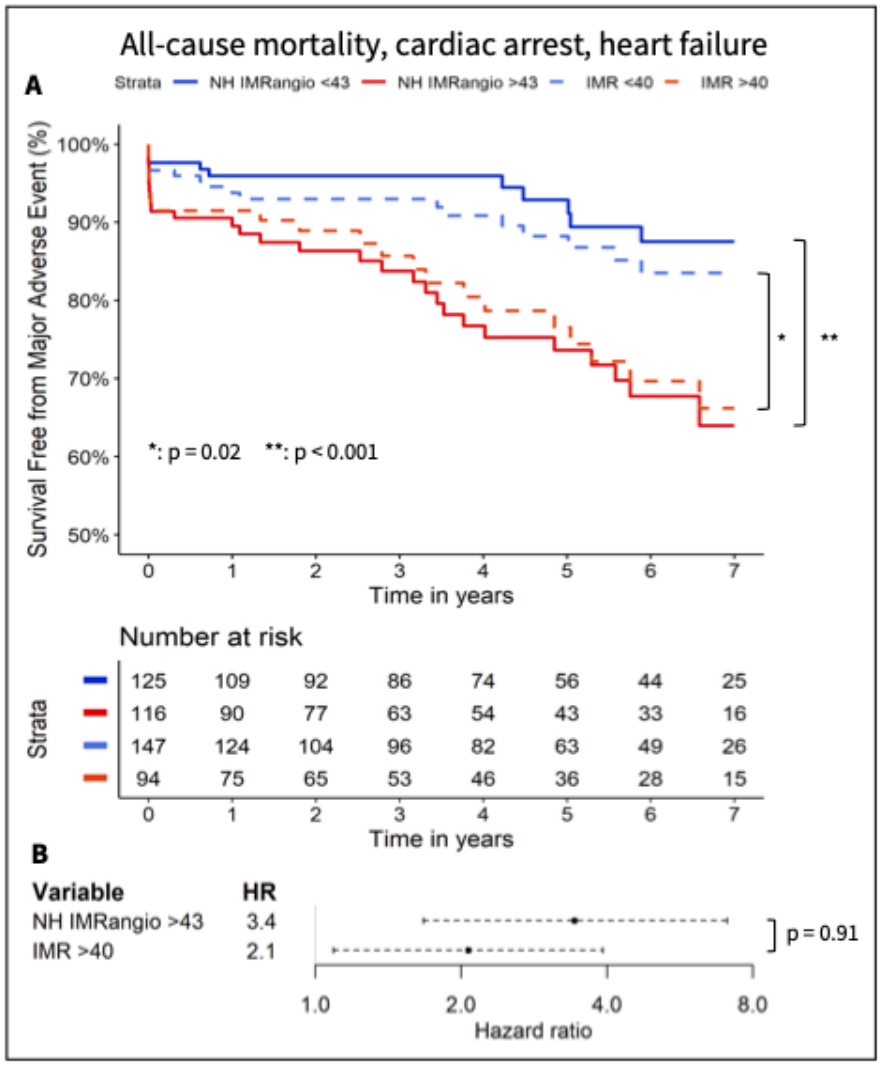


Figure 1: Kaplan Meier curves of freedom from all-cause mortality, resuscitated cardiac arrest, new heart failure diagnosis with high vs low i) NH IMR_{angio} and ii) IMR **(A)**. Forrest plot displaying the hazard ratio of high i) NH IMR_{angio} and ii) IMR **(B)**. HR: hazard ratio; IMR: index of microcirculatory resistance; NH IMR_{angio}: non-hyperaemic $IMR_{angio.}$

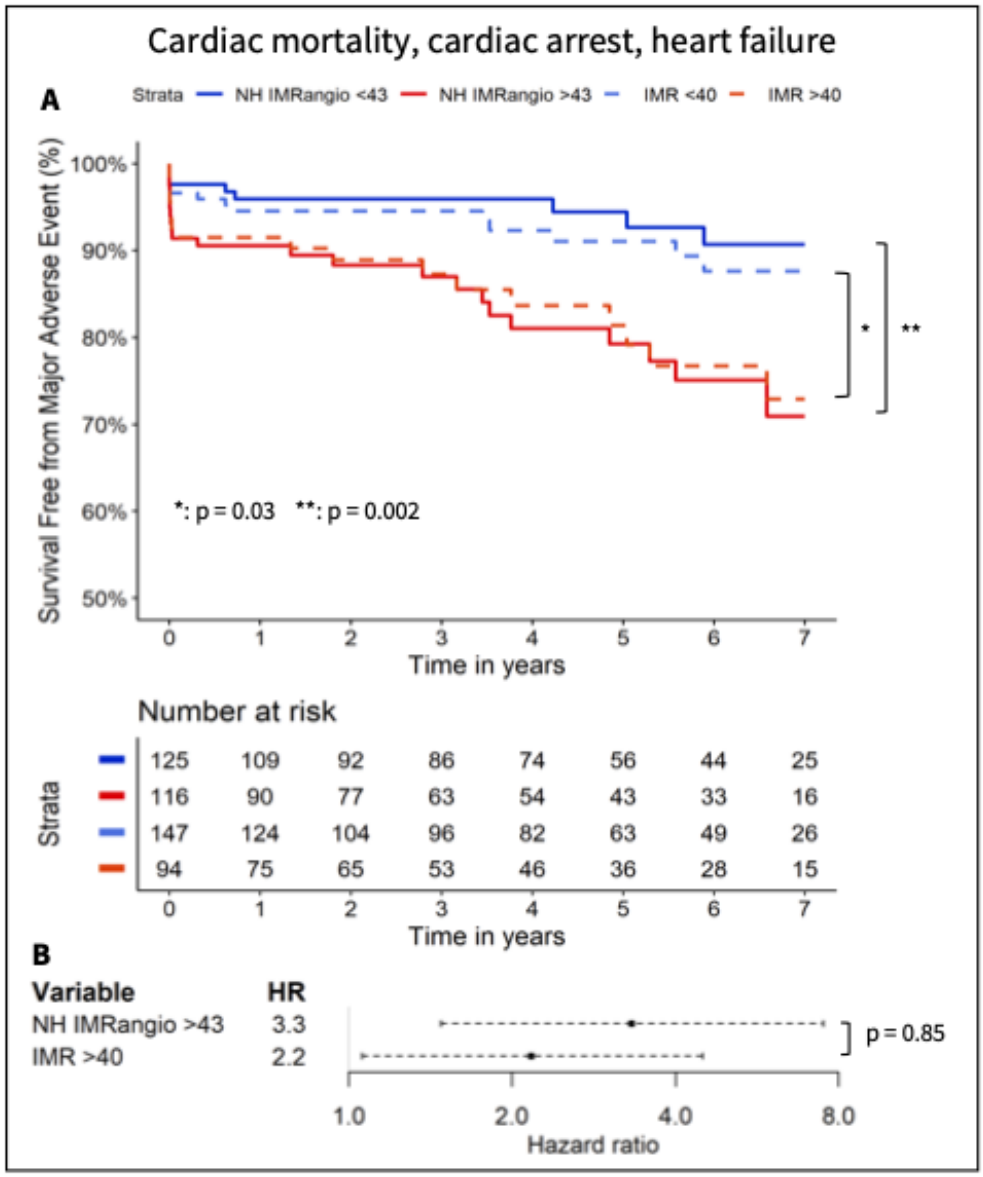


Figure 2: Kaplan Meier curves of freedom from cardiac mortality, resuscitated cardiac arrest, new heart failure diagnosis with high vs low i) NH IMR_{angio} and ii) IMR **(A)**. Forrest plot displaying the hazard ratio of high i) NH IMR_{angio} and ii) IMR **(B)**. HR: hazard ratio; IMR: index of microcirculatory resistance; NH IMR_{angio}: non-hyperaemic IMR_{angio}.

Kaplan Meier landmark curves displaying the relationship between high versus low NH IMR_{angio} and survival-free from the primary and secondary endpoints after 30 days are shown in Figure 3.

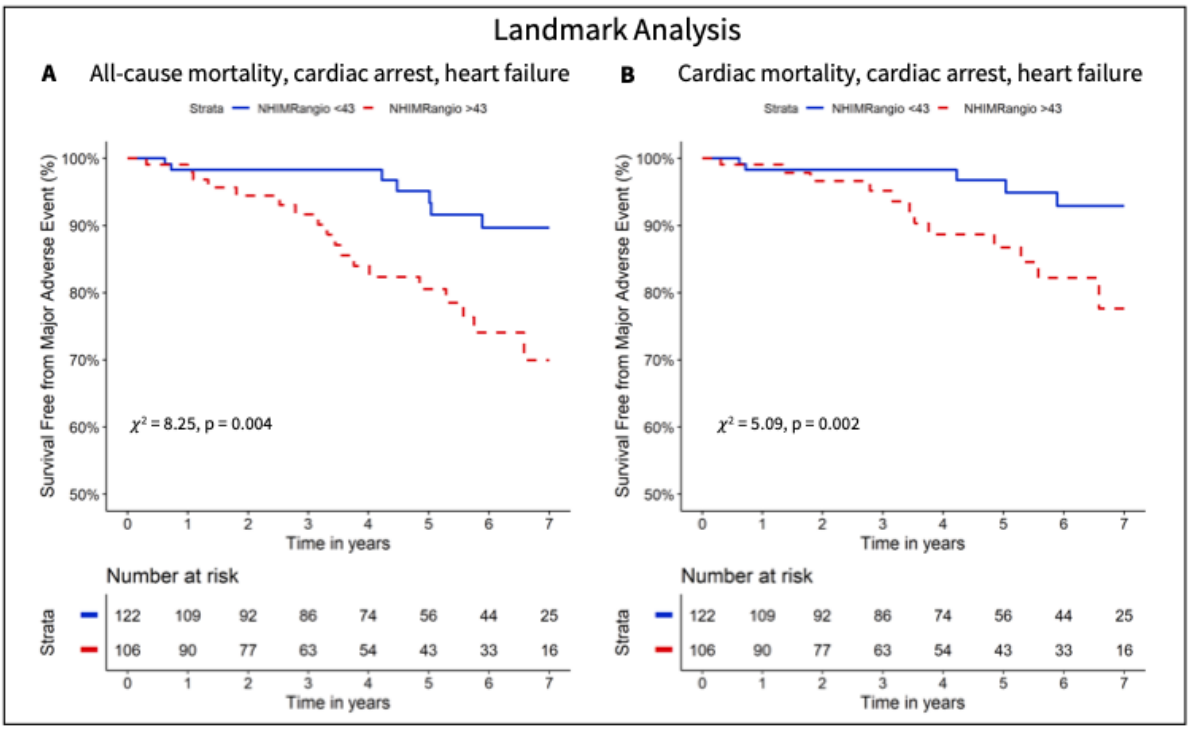


Figure 3: Landmark analysis (30 days onward) Kaplan Meier curves of freedom from all-cause mortality, resuscitated cardiac arrest, new heart failure diagnosis **(A)** and cardiac mortality, resuscitated cardiac arrest and new heart failure diagnosis **(B)** stratified according to high versus low NH IMR_{angio}. *NH IMR_{angio}: non-hyperaemic IMR_{angio}.*

To further evaluate the prognostic utility of NH IMR_{angio} >43U a multivariate Cox regression analysis was performed. The univariate and multivariate predictors of the primary endpoint are listed in Table 3. NH IMR_{angio} >43U was an independent predictor of the primary endpoint, adjusted HR 2.13 (95% CI: 1.01-4.48, p<0.05) in a model with age, ischaemic time, and discharge LVEF%. Kaplan Meier curves displaying the relationship between adjusted survival free of a major adverse event and high versus low NH IMR_{angio} are shown in Figure 4. The addition of NH IMR_{angio} >43U as a variable to a cox regression model including age, ischaemic

time and discharge LVEF% yielded a good model (C-statistic 0.82, χ^2: 67) with a significant improvement in predictive performance (χ^2 difference: 4.30, p=0.04).

Table 3. **Univariate and Multivariate Predictors of All-cause mortality, cardiac arrest, heart failure (p ≤0.1)**

Univariate Predictors	**Hazard Ratio**	**95% CI**	**p-value**
Age (per 1 year increase)	1.07	1.03-1.10	<0.001
Male gender	2.01	1.00-4.07	0.05
LAD as IRA	2.07	1.09-4.00	0.03
Ischaemic time (per 1min delay)	1.00	1.00-1.00	0.04
Discharge Echocardiography LVEF (per % increase)*	0.91	0.89-0.94	<0.0001
NH IMRangio >43U	3.43	1.67-7.07	0.001
Multivariate predictors			
Age (per 1 year increase)	1.07	1.03-1.11	<0.001
Ischaemic time (per min)	1.00	1.00-1.00	0.034
Discharge Echocardiography LVEF (per % increase)	0.92	0.90-0.95	<0.0001
NH IMRangio >43U	2.13	(1.01-4.48)	0.047

CI: confidence interval; IMR: index of microvascular resistance; IRA: infarct related artery; LAD: left anterior descending artery; LVEF: left ventricle ejection fraction; NH IMR_{angio}: non-hyperaemic IMR_{angio}; *Missingness for discharge echocardiography LVEF% was addressed by median imputation.

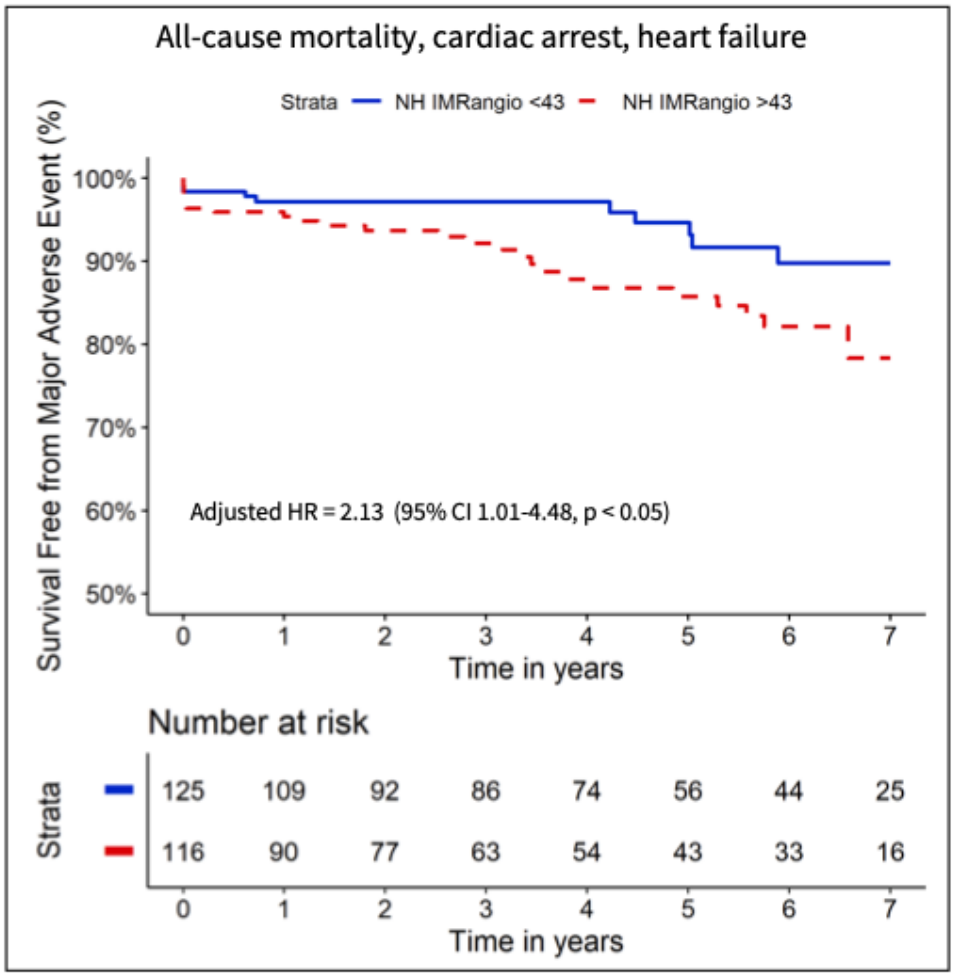


Figure 4: Kaplan Meier curves of freedom from all-cause mortality, resuscitated cardiac arrest, new heart failure diagnosis with high vs low NH IMR_{angio} adjusted for age, ischaemic time, LVEF%.
aHR: adjusted hazard ratio; CI: confidence interval; LVEF%: left ventricle ejection fraction; NH IMR_{angio}: non-hyperaemic $IMR_{angio.}$

DISCUSSION

To our knowledge, this is the first study to assess the diagnostic and prognostic value of a dedicated pressure-wire and adenosine-free index (NH IMR_{angio}) for the assessment of coronary microvascular dysfunction/injury in STEMI patients. Microvascular dysfunction/injury in STEMI is prognostically important [57, 61] due to the resulting poor structural and functional myocardial recovery.[7, 69] Although CMR remains the gold standard investigation for microvascular injury characterisation[58], the associated cost and limited availability – particularly in the emergency care setting – pose some technical and logistic constraints in its routine adoption as an early tool for risk stratification and guidance of additional/alternative therapeutic strategies. .[59] For this reason, biomarkers derived from invasive assessment coronary microvascular function at the time of pPCI (e.g. IMR) represent a reasonable and reliable alternative with a demonstrated complementary value providing a grading of the severity of microvascular obstruction detected by CMR imaging.[7, 60] The advantage of being immediately measurable in the catheterization laboratory has facilitated the evaluation of IMR as a theragnostic and prognostic biomarker[62]. However, its clinical adoption remains limited due to a number of factors including additional cost and procedural time. These constraints can be overcome with angiography-derived pressure wire-free indices of coronary physiology indices. Our recent validation of the angiography derived index of microcirculatory resistance (IMR_{angio}) against pressure-wire-based IMR represented the first study in this field.[64]

Diagnostic performance of NH IMR_{angio}

In the current analysis, we have expanded our previous results by showing that the non-hyperaemic version of IMR_{angio} – NH IMR_{angio} - reliably predicts microvascular injury as defined by an IMR >40U in the IRA of STEMI patients. Notably, the optimal NH IMR_{angio} cut-off of >43U derived from the ROC analysis coincides with the median NH IMRangio value in our cohort. Using a non-hyperaemic index to evaluate microvascular injury in the IRA of STEMI patients is still reliable, as the vasodilatory response to adenosine in the IRA has been shown to be blunted. [14, 19] In our cohort, the median value of RRR - a dedicated index to express the vasodilatory capacity of coronary microcirculation - was 1.7. This value is

suggestive of a depressed coronary microvascular vasodilatory capacity in this cohort of STEMI patients, a finding consistent with previous reports.[19, 67] This depressed vasodilatory capacity can explain why, particularly in STEMI patients, a non-hyperaemic index such as NH IMR_{angio} retains a fair degree of diagnostic accuracy in identifying microvascular injury.

Prognostic value of NH IMR_{angio}

The main finding of the current study is the observation that an NH IMR_{angio} >43U, measured in the IRA of STEMI patients at the end of pPCI, is equivalent to an IMR >40U in predicting long-term adverse events. The survival curves of patients stratified according to low or high values of IMR or NH IMR_{angio} present a similar profile, while the stratification is prognostically significant for both indices. The hazard ratios of a high IMR (>40U) or NH IMR_{angio} (>43U) are not significantly different, further supporting the prognostic equivalence of the two indices. The prognostic equivalence is also maintained when analysing a stricter cardiac endpoint excluding non-cardiac mortality. These findings are consistent with the results and effect estimates reported in the seminal work by Fearon and colleagues on the prognostic role of invasive and pressure-wire based IMR in STEMI patients.[61] Kaplan-Meier survival curves separate early on, suggesting a prognostic role of NH IMR_{angio} for early cardiac complications; a finding corroborated by previous IMR based work.[17] Since this early separation could influence our analysis, we conducted a landmark analysis from 30 day onwards. An NH IMR_{angio} >43U retained its significance in this landmark analysis suggesting that the long-term prognostic performance is not only driven by early events. This can be explained by the significant contribution of new heart failure diagnoses to our combined endpoints. In our previous work, we have already shown that hyperaemic IMR_{angio} is significantly elevated in patients with clinically significant microvascular obstruction assessed by cardiac magnetic resonance.[64] This provides a further biologically plausible explanation for the prognostic significance of NH IMR_{angio} we report herein [64].

Finally, this study proves that an NH IMR_{angio} >43U is an independent predictor of adverse events, with an associated two-fold increased risk of a poor clinical outcome at 7 years follow-up. Our findings resonate with previously published

findings of the independent prognostic value of an IMR> 40U in predicting long-term outcomes.[14, 61] Specifically adding NH IMR_{angio} into a model with other clinically relevant and universally available variables, incrementally improved the predictive performance of the model itself, supporting independent and incremental prognostic significance of NH IMR_{angio} as a novel tool for risk stratification.

Limitations

Despite it being the first study to evaluate a pressure-wire and adenosine-free method to assess coronary microvascular function early on in the catheterization laboratory, the relatively small sample size and single-centre nature ought to be acknowledged as limitations. For this reason, further testing in larger and external cohorts is needed to further corroborate our findings and to increase the precision of the reported effect estimates. We recognise that our cohort study might have been subject to selection bias due to the exclusive inclusion of patients in whom invasive coronary physiology measurements were performed. This might have led to the unintentional inclusion of a relatively intermediate-low risk cohort of STEMI patients, as reflected by the relative low rate of adverse events at follow up. Despite this limitation, NH IMR_{angio} retained its diagnostic and prognostic accuracy and it is possible that its performance could improve in a larger "all comers" cohort. Similarly, on a practical level it must also be considered that the real unmet need is to improve risk-stratification in patients at intermediate risk of adverse events. Patients presenting with high-risk features (multiple comorbidities, haemodynamic instability, complex coronary anatomy) have already "declared" their risk category, whilst it is the majority of "intermediate risk" patients (like the ones included in our analysis) that would benefit the most from personalised and stratified medicine approaches based on theragnostic indices.[70]

Finally, from a translation to clinical practice perspective, we acknowledge that a limitation of this work is represented by the off-line evaluation of NH IMR_{angio}.. Even though our tool should be formally evaluated in a "real-time" setting, there is little doubt about its suitability as a real-time catheterisation laboratory tool. A large body of evidence suggests that real-time measurement of QFR is not only feasible but significantly quicker than pressure-wire based coronary physiology

evaluation.[71, 72]. We are anticipating that these results are likely to be extended to NH IMR_{angio} real time evaluation, as the time-limiting factor in computation of NH IMR_{angio} is indeed the QFR measurement.

CONCLUSIONS

Non-hyperaemic Angiography-derived IMR_{angio} has a reliable diagnostic performance to identify microvascular injury in STEMI patients with a prognostic value equivalent to invasively measured IMR. NH IMR_{angio} can be a feasible alternative to IMR for risk stratification in pPCI treated STEMI patients.

SUPPLEMENTARY MATERIAL

Supplementary Figure 1

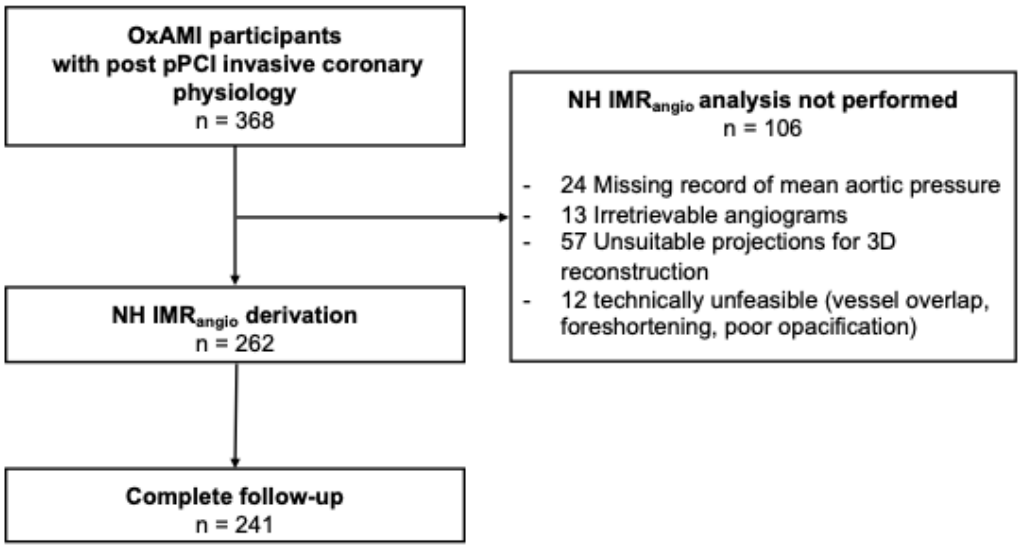


Figure S1: Patient flow diagram
NH IMR_{angio}: non-hyperaemic $IMR_{angio.}$; pPCI: primary percutaneous coronary intervention; 3D: three dimensional.

Supplementary Figure 2

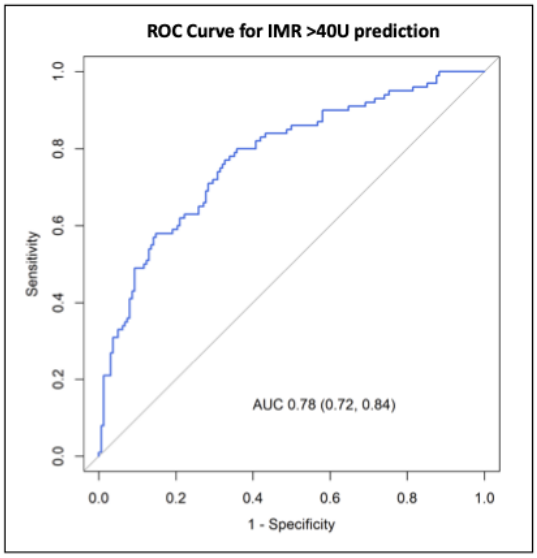


Figure S2: Diagnostic utility of NH IMR_{angio} in identifying an IMR >40U.
AUC: area under the curve; CMD: coronary microvascular dysfunction; IMR: index of microcirculatory resistance; NH IMR_{angio}: non-hyperaemic IMR_{angio}; ROC: receiver operating characteristic.

Supplementary Figure 3

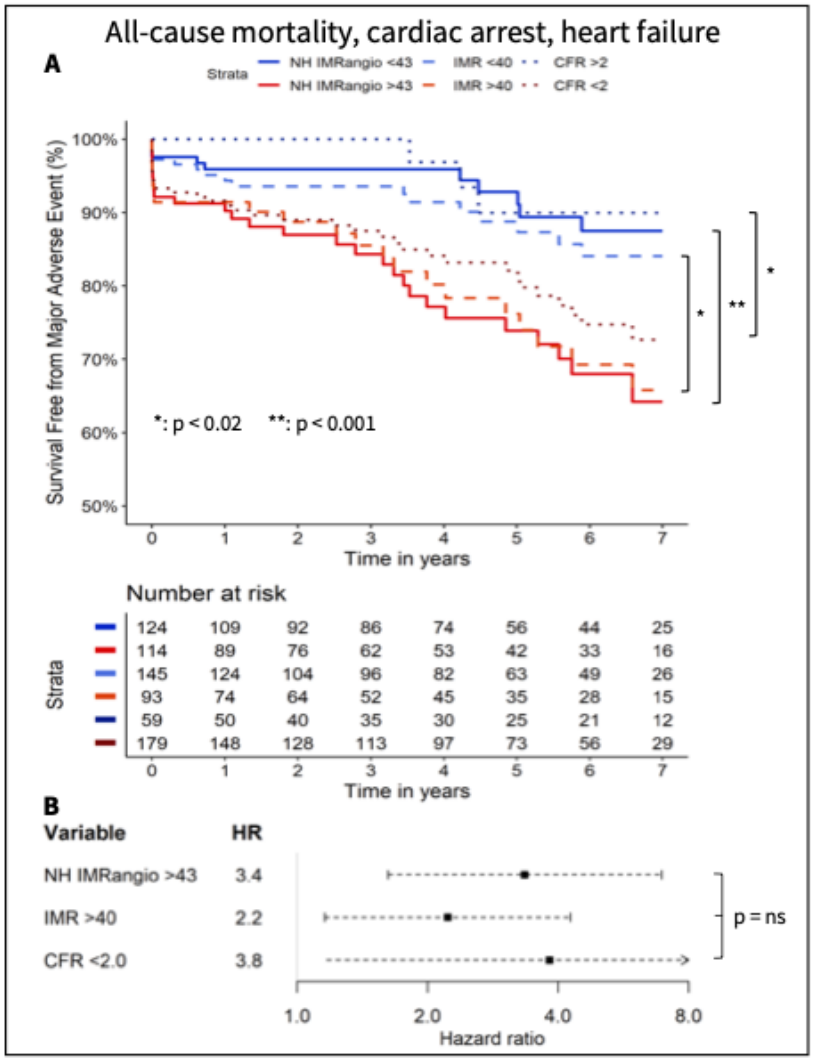


Figure S3: Kaplan Meier Curves of freedom from all-cause mortality, resuscitated cardiac arrest, new heart failure diagnosis with high vs low i) NH IMR_{angio} and ii) IMR **(A)**. Forrest plot displaying the hazard ratio of high i) NH IMR_{angio} and ii) IMR **(B)**. CFR: coronary flow reserve; HR: hazard ratio; IMR: index of microcirculatory resistance; NH IMR_{angio}: non-hyperaemic $IMR_{angio;}$ ns= not significant.

Supplementary Figure 4

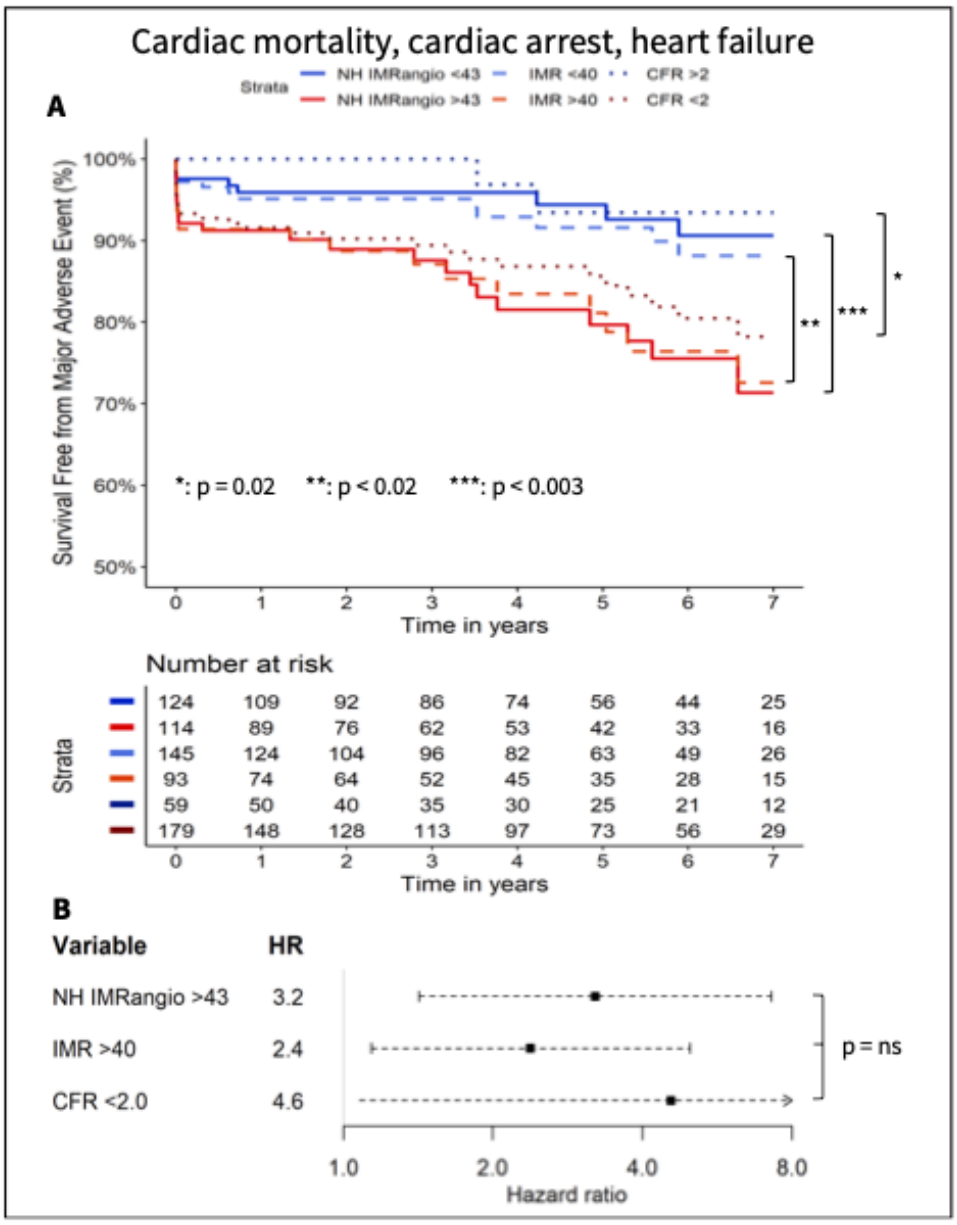


Figure S4: Kaplan Meier curves of freedom from cardiac mortality, resuscitated cardiac arrest, new heart failure diagnosis with high vs low i) NH IMR_{angio}, ii) IMR and iii) CFR **(A)**. Forrest plot displaying the hazard ratio of high i) NH IMR_{angio}, ii) IMR, and iii) CFR **(B)**. CFR: coronary flow reserve; HR: hazard ratio; IMR: index of microcirculatory resistance; NH IMR_{angio}: non-hyperaemic $IMR_{angio;}$ ns= not significant.

Table S1. Clinical, Procedural & Echocardiographic Characteristics according to low versus high NH IMR_{angio}

	NH IMR_{angio} ≤43	**NH IMR_{angio} >43**	**p value**
Total Number	**136**	**126**	
Age, years	60 ± 11	64 ± 11	**<0.01**
Male gender, n (%)	118 (87)	97 (77)	**0.04**
Hypertension, n (%)	56 (42)	63 (50)	0.17
Hypercholesterolemia, n (%)	50 (37)	51 (41)	0.54
Diabetes, n (%)	23 (17)	18 (14)	0.56
Smoker, n (%)	66 (49)	44 (35)	**0.03**
Previous cardiology history, n (%)*	17 (13)	20 (16)	0.45
Family history of IHD, n (%)	55 (40)	46 (37)	0.51
Target vessel			
LAD, n(%)	55 (41)	64 (51)	
LCX, n(%)	12 (9)	13 (10)	0.37
RCA, n(%)	63 (46)	46 (37)	
Other, n(%)	6 (4)	3 (2)	
TIMI Flow – pre-PCI			
0	103 (76)	94 (75)	
1	11 (8)	11 (9)	0.84
2	14 (10)	16 (13)	
3	8 (6)	5 (4)	
TIMI Flow – post-PCI			
0	0 (0)	0 (0)	
1	0 (0)	3 (2)	**<0.001**
2	8 (6)	25 (20)	
3	128 (94)	98 (78)	
Ischemic time, minutes	158 (113, 293)	201 (137, 337)	**0.02**
Complete ST segment resolution, n(%)*	87 (82%)	64 (64%)	**0.003**
Discharge echocardiography LVEF*, %	53 (47, 56)	49 (43, 55)	**0.03**

IHD: ischaemic heart disease; LAD: left anterior descending artery; LCx: left circumflex artery; LVEF: left ventricle ejection fraction; MVO: microvascular obstruction; NH IMR_{angio}: non-hyperaemic IMR_{angio}; PCI: percutaneous coronary intervention; RCA: right coronary artery; TIMI: the Thrombolysis in Myocardial Infarction. *Degree of missingness: Previous cardiological history = 0.4%, Complete ST segment resolution = 21%, Discharge LVEF % Echocardiography = 2%

Table S2. Post PCI invasive and angiography-derived coronary physiology indices according to low versus high NH IMR_{angio}.

	NH IMR_{angio} ≤43	**NH IMR_{angio} >43**	**p-value**
Total Number	**136**	**126**	
Pressure-wire-derived			
Resting Pa, mmHg	88 ± 16	97 ± 18	**<0.001**
Resting transit time, s*	0.59 (0.38, 0.83)	0.88 (0.59, 1.40)	**<0.001**
Hyperaemic Pa, mmHg*	80 (70, 89)	85 (74, 95)	**<0.001**
Hyperaemic Pd, mmHg	72 ± 14	82 ± 16	**<0.001**
Hyperaemic transit time, s	0.33 (0.24, 0.55)	0.59 (0.36, 1.03)	**<0.001**
FFR*	0.92 (0.88, 0.97)	0.95 (0.90, 0.99)	**0.002**
IMR	25 (17, 35)	46 (29, 85)	**<0.001**
CFR*	1.6 (1.2, 2.1)	1.4 (1.1, 1.9)	**0.044**
RRR*	1.8 (1.3, 2.5)	1.6 (1.2, 2.2)	**0.022**
Angiography-derived			
Fixed flow QFR*	0.95 (0.90, 0.98)	0.96 (0.90, 0.99)	0.651
Contrast QFR	0.95 (0.89, 0.98)	0.97 (0.91, 0.99)	**0.039**
NH IMR_{angio}	31 (22, 37)	60 (50, 77)	**<0.001**

CFR: coronary flow reserve; FFR: fractional flow reserve; IMR: index of microvascular resistance; NH IMR_{angio}: non-hyperaemic IMR_{angio}; Pa: aortic pressure; Pd: distal pressure; PCI: percutaneous coronary intervention; QFR: quantitative flow ratio; RRR: resistive reserve ratio; *Degree of missingness: resting transit time = 1.1%, hyperaemic Pa= 0.4%, FFR = 0.4%, CFR = 1.1%, RRR= 1.1%; Fixed flow QFR = 0.8%.

CONCLUSIONS

The results reported in the current thesis contribute to improve the knowledge on coronary physiology in patients with STEMI and to develop alternative methods to assess the severity of CMD in patients undergoing PPCI.

The main conclusions that can be drawn by this work are the following:

1. Post-ischaemic severe CMD assessed either by invasive IMR or by CMR is associated with adverse clinical outcome at long-term follow up after STEMI. In particular, IMR>40 U or the presence of MVO was associated with more than 4-fold increase in the risk of mortality, heart failure or cardiac arrest. Notably, patients with both high IMR and MVO represent a subgroup at high risk of adverse events during the first year of follow up after STEMI.
2. Pressure-bounded CFR is a novel index derived using standard pressure-wire technology. Pb-CFR is associated with the presence of CMD in STEMI. However, the diagnostic performance of pb-CFR is inferior to invasive IMR.
3. Based on 3-D reconstruction of the coronary artery and computational fluid dynamics, we developed an angiography-derived IMR. IMR_{angio} demonstrated an excellent diagnostic performance compared with invasive IMR and with CMR-based MVO in patients with STEMI.
4. IMR_{angio} is well correlated with invasive IMR across the spectrum of acute and chronic coronary syndromes. In patients with STEMI, non-hyperemic IMR_{angio} (NH-IMR_{angio}) demonstrated good accuracy in detecting CMD and can be used as an alternative to IMR.
5. NH-IMR_{angio} demonstrated a good prognostic value in a retrospective analysis of the OxAMI study. In particular, NH-IMR_{angio} >43 U was associated with the composite adverse outcome, including mortality, heart failure and cardiac arrest, at long-term after STEMI.

CLINICAL AND RESEARCH IMPLICATIONS

This PhD thesis has important clinical ramifications for the contemporary management of STEMI patients. By overcoming the technical and logistic limitations associated with pressure-wire based assessment of IMR, the novel IMR_{angio} can facilitate a wider adoption of coronary microvascular assessment in STEMI patients, particularly in cost-constrained healthcare settings. This could translate into an improvement in risk stratification and the implementation of stratified medicine approaches for the deployment of novel or adjunct therapeutics and the selection of dedicated clinical pathways in STEMI care.

Beyond its proposed clinical role, IMR_{angio} may have important implications for research. IMR_{angio} could indeed facilitate recruitment into clinical trials of patients selected according to their risk of coronary microvascular injury/dysfunction or act as a new and easily available surrogate outcome measure for microvascular injury/dysfunction.

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