# CORONARY MICROCIRCULATION AND PERIPHERAL ENDOTHELIAL FUNCTION EVALUATION AFTER ACUTE ST ELEVATION MYOCARDIAL INFARCTION TREATED WITH PRIMARY ANGIOPLASTY

**SÉRGIO BRAVO CORDEIRO BAPTISTA**

**Thesis for the PhD degree in Medicine**
**Subspecialty of Clinical Investigation**
**NOVA Medical School, Lisbon**

**September 2016**

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**SÉRGIO BRAVO CORDEIRO BAPTISTA**

**Tese para a obtenção do grau de Doutor em Medicina**
**na especialidade de Investigação Clínica**
**na Faculdade de Ciências Médicas de Lisboa**

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**Tese para a obtenção do Grau de Doutor em Medicina na especialidade de Investigação Clínica**

**Setembro de 2016**

**Para a Teresa,**
**e para a Mariana, a Sofia e o Miguel**

***Not everything that can be counted counts.***
***Not everything that counts can be counted.***

*William Bruce Cameron, 1963*

# CONTENTS

## Contributions and responsibilities

The candidate was responsible for the design, implementation (including logistics and financial), patient inclusion, data registry and statistical analysis of the study, as well as for the full writing of this thesis.

All patients included were admitted to Hospital Prof. Doutor Fernando da Fonseca´s Cardiology Department (Director until 2013: Prof. Doutor Victor Gil; since 2013: Dr. Carlos Morais).

The acute invasive procedure (IMR measurement) was performed either by the candidate, or by one of the Interventional Cardiologists of the Interventional Cardiology Unit of Hospital Prof. Doutor Fernando da Fonseca (Coordinator: Dr. Pedro Farto e Abreu).

The EndoPAT evaluations were performed in the Endothelial Function Laboratory of Hospital Prof. Doutor Fernando da Fonseca (Director: Prof. Doutor Victor Gil), either by the candidate, Dra. Mariana Faustino or one of the trained technicians.

The Echocardiographic evaluations were performed in the Echocardiography Unit of Hospital Prof. Doutor Fernando da Fonseca (Coordinator: Dr. António Freitas), by adequately trained physicians. Their analysis was performed by Dr. António Freitas and Dra. Mariana Faustino.

The ECGs were performed by trained cardiopulmonary technicians.

All laboratory evaluations were performed by the Clinical Pathology Department of Hospital Prof. Doutor Fernando da Fonseca (Director: Dra. Luísa Sancho).

The cardiac magnetic resonance exams were performed in Hospital dos Lusíadas, Lisbon (Cardiovascular Department, coordinated by Prof. Doutor Victor Gil and Imaging Department, coordinated by Drª Graça Correia), by Dr. João Abecasis, who was also responsible for their interpretation.

# LIST OF PUBLICATIONS

## Papers in Periodicals with Referees

This thesis resulted so far in the following publications:

- Baptista SB, Faustino M, Simões J, Nédio M, Monteiro C, Lourenço E, Leal P, Farto e Abreu P, Gil V. **Endothelial dysfunction evaluated by peripheral arterial tonometry is related with peak TnI values in patients with ST elevation myocardial infarction treated with primary angioplasty**. *Microvasc Res*. 2016 May;105:34-9. doi: 10.1016/j.mvr.2015.12.010. Epub 2015 Dec 22.

- Faustino M, Baptista SB, Freitas A, Monteiro C, Leal P, Nédio M, Antunes C, Abreu PF, Gil V, Morais C. **The Index of Microcirculatory Resistance as a Predictor of Echocardiographic Left Ventricular Performance Recovery in Patients With ST-Elevation Acute Myocardial Infarction Undergoing Successful Primary Angioplasty**. *J Interv Cardiol.* 2016 Apr;29(2):137-45. doi: 10.1111/joic.12278. Epub 2016 Mar 1.

- Baptista SB, Faustino M, Brizida L, Loureiro J, Augusto J, Abecasis J, Monteiro C, Leal P, Nédio M, Farto e Abreu P, Gil V, Morais C. **Early peripheral endothelial dysfunction predicts myocardial infarct extension and microvascular obstruction in patients with ST elevation myocardial infarction.** *Submitted*

A forth manuscript is currently being prepared:

- Baptista SB, Faustino M, Loureiro J, Brízida L, Freitas A, Augusto A, Abecasis J, Monteiro C, Leal P, Nédio M, Antunes C, Farto e Abreu P, Gil V, Morais C. **The index of microvascular resistance as a surrogate for myocardial infarct extension, microvascular obstruction and left ventricular remodelling in patients with ST elevation myocardial infarction treated by primary angioplasty.**

## Papers in Conference Proceedings

This thesis resulted in the following publications as abstracts in conference proceedings:

- Baptista SB, Faustino M, Gil V. **Severity Of Coronary Artery Disease And Endothelial Dysfunction Evaluated By Peripheral Arterial Tonometry.** *Journal of the American College of Cardiology* 2013;62 (18_S1):B183-B183

- Baptista SB, Faustino M, Abreu PF, Gil V. **Impact of endothelial dysfunction evaluated by peripheral arterial tonometry in the extension of ST elevation myocardial infarction treated with primary angioplasty.** *Journal of the American College of Cardiology* 2013;62 (18_S1), B183-B183

- Baptista SB., Faustino M., Loureiro J., Brizida L., Alves P., Nedio M., Monteiro C., Lourenço E., Leal P., Farto e Abreu P., Gil V., Morais C. **Index of microcirculatory resistance in patients with STEMI treated with primary angioplasty: relation with other indicators of microvascular reperfusion, infarct size and regional systolic function.** *Eurointervention* 2015; Abstracts EuroPCR 2015; Euro15A-MA006.

- Baptista SB., Faustino M., Loureiro J., Brizida L., Alves P., Nedio M., Monteiro C., Lourenço E., Leal P., Farto E Abreu P., Gil V., Morais C. **Predictors of microcirculatory dysfunction in STEMI patients treated with primary angioplasty.** *Eurointervention* 2015; Abstracts EuroPCR 2015; Euro15A-MA009

- Faustino M, Baptista SB, Freitas A, Monteiro C, Leal P, Nédio M, Antunes C, Farto e Abreu P, Gil V, Morais C. **Coronary index of microcirculatory resistance and echocardiographic parameters evolution in patients with ST-elevation acute myocardial infarction treated with primary angioplasty.** *European Heart Journal* 2015;36 (suppl 1):240

- Faustino M, Baptista SB, Freitas A, Augusto JA, Leal P, Nédio M, Antunes C, Abreu PF, Gil V, Morais C. **Global longitudinal strain and coronary microcirculation status in prediction of left ventricle functional recovery after ST elevation myocardium infarction.** *Eur Heart J Cardiovasc Imaging* 2015;16(suppl 2):S156-S182.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ΔP | Pressure gradient |
| 2D | Two-dimensional |
| ACE | Angiotensin-converting-enzyme |
| ACEi | Angiotensin-converting-enzyme inhibitors |
| ACS | Acute coronary syndromes |
| ADMA | Asymmetrical dimethylarginine |
| ARB | Angiotensin II receptor blockers |
| AUC | Area under the curve |
| BMI | Body mass index |
| BNP | B-type natriuretic peptide |
| CABG | Coronary artery bypass graft |
| CAD | Coronary artery disease |
| CAMs | Cellular adhesion molecules |
| ceCMR | Contrast enhanced cardiac magnetic resonance |
| CFR | Coronary flow reserve |
| CI | Confidence interval |
| CK | Creatine kinase |
| CKD | Chronic kidney disease |
| CK-Mb | Mb fraction of creatine kinase |
| CT | Cardiac tomography |
| cTFC | Corrected TIMI frame count |
| CV | Cardiovascular |
| DBP | Diastolic blood pressure |
| DICOM | Digital Imaging and Communications in Medicine |
| ECG | Electrocardiogram |
| Echo | Echocardiogram |
| EDHF | Endothelium-dependent hyperpolarizing factor |
| EECP | Enhanced external counterpulsation |
| FMD | Flow-mediated dilation |
| FRS | Framingham Risk Score |
| GLS | Global longitudinal strain |
| HbA1c | Glycosylated haemoglobin |
| HF | Heart failure |
| hMR | Hyperaemic microvascular resistance index |
| HR | Hazard ratio |
| hs-CRP | High sensitive C-reactive protein |
| IMR | Index of microcirculatory resistance |
| $IMR_{true}$ | Index of microcirculatory resistance corrected for collateral flow |
| IQR | Interquartile range |
| IS | Infarct size |
| ISR | In-stent restenosis |

| | |
|---|---|
| IVUS | Intravascular ultrasound |
| L_RHI | Logarithmic transformation of reactive hyperaemia index |
| LA | Left atrium |
| LAD | Left anterior descending artery |
| LCx | Left circumflex artery |
| LDF | Laser Doppler flowmetry |
| LDL-C | Low-density lipoprotein cholesterol |
| L-FMC | Low flow mediated constriction |
| Lp-PLA2 | Lipoprotein-associated phospholipase A2 |
| LV | Left ventricle |
| LVEdV | Left ventricular end diastolic volume |
| LVEF | Left ventricular ejection fraction |
| LVEsV | Left ventricular end systolic volume |
| MACE | Major adverse cardiac events |
| MBG | Myocardial blush grade |
| MCE | Myocardial contrast echocardiography |
| MI | Myocardial infarction |
| MVO | Microvascular obstruction |
| NO | Nitric oxide |
| NST-ACS | Non-ST elevation acute coronary syndrome |
| NSTEMI | Non-ST elevation myocardial infarction |
| NT-pro-BNP | N-terminal pro b-type natriuretic peptide |
| NYHA | New York Heart Association classification for heart failures symptoms |
| OR | Odds ratio |
| OxLDL | Oxidized low-density lipoprotein |
| Pa | Aortic pressure |
| PAI-1 | Plasminogen activator inhibitor-1 |
| PAT | Peripheral arterial tonometry |
| PCI | Percutaneous coronary intervention |
| Pd | Distal pressure |
| PET | Positron emission tomography |
| PGI2 | Prostaglandin I2 (Prostacyclin) |
| P-PCI | Primary (urgent) percutaneous coronary intervention |
| PzF | Zero-flow pressure |
| Q | Flow |
| R | Resistance |
| RCA | Right coronary artery |
| RHI | Reactive hyperaemia index |
| ROC | Receiver operating characteristic |
| SBP | Systolic blood pressure |
| SK | Streptokinase |
| SMC | Smooth muscle cells |
| SPECT | Single-photon emission computed tomography |
| STEMI | ST elevation myocardial infarction |

| | |
|---|---|
| SYNTAXsc | Synergy Between PCI With Taxus and Cardiac Surgery Score |
| Tmn | Mean transit time |
| TMPG | TIMI myocardial perfusion grade |
| TnI | Troponin I |
| $TnI_{AUC}$ | Area under the curve of troponin |
| $TnI_{peak}$ | Peak value of troponin |
| t-PA | Tissue plasminogen activator |
| VOP | Venous occlusion plethysmography |
| WMSI | Wall motion score index |

## LIST OF STUDY ACRONYMS

| | |
|---|---|
| APPROACH | Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease |
| ARTS | Arterial Revascularization Therapies Study |
| BARI | Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy |
| PROSPECT | Providing Regional Observations to Study Predictors of Events in the Coronary Tree |
| SYNTAX | Synergy Between PCI With Taxus and Cardiac Surgery |
| TIMI | Thrombolysis In Myocardial Infarction |

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# ABSTRACT

**Introduction:** Despite achieving normal epicardial coronary artery flow after primary percutaneous coronary intervention (P-PCI), a significant proportion of patients with acute ST elevation myocardial infarction has a poorer outcome because of microvascular coronary damage and/or dysfunction. Endothelial dysfunction may play a role in this microvascular coronary damage after STEMI, and its evaluation by peripheral arterial tonometry may be useful to predict the extent of microvascular coronary damage and the extent of myocardial infarction.

**Objectives**: To evaluate the relation of early peripheral endothelial dysfunction, as measured by the reactive hyperemia index (RHI, obtained by peripheral arterial tonometry) and the index of microcirculatory resistance (IMR) immediately after P-PCI and to access the relation between RHI and IMR values and: 1) the extent of myocardial infarct evaluated by contrast enhanced cardiac magnetic resonance (ceCMR) and troponin release; 2) the extent of microvascular obstruction (MVO), evaluated by ceCMR and by other available indirect indicators; 3) late (3 months) left ventricular remodelling, measured by echocardiography.

**Methods:** Observational, prospective cohort study. Patients with a first STEMI successfully treated with P-PCI, hemodynamically stable and without contra-indications for adenosine administration were included. After successful P-PCI, IMR was determined, using a pressure-wire. RHI was evaluated acutely and after 24 hours, using EndoPAT; endothelial dysfunction was defined as RHI<1.67, and RHI was also analysed by tertiles. Corrected TIMI frame count (cTFC) and TIMI myocardial perfusion grade (TMBG) were evaluated at the end of the procedure. Blood tests for cardiac biomarkers were collected on admission and on scheduled intervals during the first 48 hours. ECGs were recorded before and immediately after P-PCI and at 90 and 180 minutes, for ST resolution evaluation. Left ventricular global and regional function were evaluated by echocardiography at baseline and at 3 months. ceCMR was performed on the 7-8$^{th}$ day post-MI.

**Results**: 60 patients were included (48 males, mean age 59.6±12.7 years). In the first acute RHI values were higher than expected (mean 2.15±0.58) suggesting important technical pitfalls; no relation was found between this acute RHI and any of the infarct extent or microvascular obstruction indicators. Mean RHI values measured at 24 hours were 1.87±0.60. Patients with an RHI<1.67 on this second evaluation tended to have higher IMR (median 40.5 IQR 54.4 vs. median 22.0 IQR 26.0, p=0.09), worse ST resolution, worse angiographic (cTFC and TMPG) results and had more MVO in the ceCMR (54.1% vs. 11.1%, p=0.03). They also had significantly larger infarcts as evaluated by peal TnI (p=0.024) and AUC TnI (p=0.012) and a tendency to have larger infarcts in the ceCMR. Left ventricular ejection fraction (LVEF) was lower and wall motion score index (WMSI) was higher in the first Echocardiogram in these patients. IMR median values were 24 (IQR 33). IMR strongly correlated with MVO on the ceCMR (r=0.91, p<0.0001; ROC curve 0.723, CI95% 0.500-0.896, p=0.018). Patients with IMR>24 had significantly worse ST resolution and angiographic indicators of microvascular dysfunction. IMR also correlated with infarct mass (r=0.70, p<0.001) and salvage mass (r=0.35, p=0.014) in the ceCMR. Patients with IMR>24 had significantly higher peak (p=0.013) and AUC (p=0.003) TnI. LVEF improved significantly only in patients with IMR<24 (p=0.01). IMR independent predictors were age, glucose and HbA1c.

**Conclusions**: RHI measured in the acute phase of STEMI after P-PCI seems to be unfeasible. RHI measured 24h after the P-PCI is feasible and predicts infarct size and MVO, confirming endothelial dysfunction as an important mechanism in microvascular dysfunction in STEM patients. IMR is strongly correlated with MVO and predicts both infarct size and LV remodelling.

# RESUMO

**Introdução:** Apesar da normalização do fluxo coronário epicárdico após intervenção coronária percutânea primária (ICP-P), uma proporção significativa dos doentes com enfarte agudo do miocárdio com elevação do segmento ST (EAMcST) têm piores resultados clínicos devido ao desenvolvimento de lesão ou disfunção microvascular coronária. A disfunção endotelial provavelmente desempenha um papel nesta lesão microvascular coronária e a sua avaliação por tonometria arterial periférica poderá ser útil para prever a extensão da lesão microvascular e a extensão do enfarte.

**Objectivos**: Avaliar a relação da disfunção endotelial periférica precoce, avaliada pelo índice de hiperémia reactiva (IHR, obtido por tonometria arterial periférica) com o índice de resistência da microcirculação (IRM), medido imediatamente após a ICP-P e estimar a relação entre o IHR e o IRM e, 1) a extensão do enfarte, avaliada por ressonância magnética cardíaca com contraste (RMCc) e pela curva de libertação de Troponina I; 2) a extensão da obstrução microvascular (OMV), avaliada por RMCc e por outros indicadores indirectos; 3) a remodelagem ventricular esquerda tardia (aos 3 meses), avaliada por ecocardiografia.

**Métodos**. Estudo observacional, prospectivo, de coorte. Foram incluídos doentes com um primeiro EAMcST, tratados com sucesso por ICP-P, hemodinamicamente estáveis e sem contra-indicações para administração de adenosina. Depois da ICP-P, o IRM foi medido usando um fio de pressão. O IHR foi avaliado na fase aguda e novamente 24 horas depois da ICP-P. A disfunção endotelial foi definida como um IHR<1,67 e o IHR foi também analisado por tercis. Os indicadores angiográficos de reperfusão (contagem corrigida de *frames* e grau de perfusão miocárdica TIMI) foram avaliados no final da ICP-P. Foram colhidas análises na admissão e em horários definidos nas primeiras 48 horas para avaliação da Troponina I. Antes, imediatamente após e 90 e 180 minutos depois da ICP-P foram registados electrocardiogramas, para avaliação da resolução das alterações do segmento ST. A função ventricular esquerda global e segmentar foi avaliada por ecocardiografia após a ICP-P e aos 3 meses. A RCMc foi efectuada ao 7-8º dia após o EAMcST.

**Resultados**: Foram incluídos 60 doentes (48 homens, idade media 59,6±12,7 anos). Na primeira avaliação, os valores de IHR foram muito superiores ao esperado (média 2,15±0,58), provavelmente por erros técnicos incontornáveis, não se relacionando com nenhum dos indicadores de extensão do enfarte ou de OMV. Na segunda avaliação, às 24h, os valores médios de IRH foram 1,87±0,60. Os doentes com IRH <1,67 tiveram tendencialmente valores mais elevados de IRM (mediana 40,5 IIQ 54,4 vs. mediana 22,0 IIQ 26,0, p=0,09), pior resolução do segmento ST, piores resultados nos indicadores angiográficos de OMV e maior probabilidade de ter OMV na RMNc (54,1% vs. 11,1%, p=0,03). Também tiveram enfartes de maior dimensão na avaliação pela TnI I máxima (p=0,004) e pela área sob a curva de TnI (p= 0,012). A fracção de ejecção do ventrículo esquerdo (FEVE) foi menor e o score de motilidade segmentar (SMS) maior nestes doentes. A mediana do IRM foi 24 (IIQ 33). O IRM correlacionou-se fortemente com a OMV avaliada na RMNc (r=0.91, p<0.001; curva ROC 0,723, IC95% 0,500-0,896, p=0,018). Nos doentes com IRM >24, a resolução do ST foi significativamente menor e os indicadores angiográficos de reperfusão foram significativamente piores. O IRM também se correlacionou com a massa de enfarte (r=0,70, p<0,001) e a massa de miocárdio salvo (r=0,35, p=0,014) na RMCc. Os doentes com IRM>24 tiveram valores significativamente mais elevados de TnI máxima (p=0,013) e ASC de TnI (p=0,003). A FEVE melhorou de forma significativa apenas nos doentes com IMR<24 (p=0,01). Os preditores independentes do IRH foram a idade, a glicemia na admissão e a HbA1c na admissão.

**Conclusões**: Não parece ser possível avaliar de forma fidedigna o IHR na fase aguda do EAMcST após ICP-P. O IHR medido 24h após a ICP-P é mensurável de forma adequada e prevê a dimensão do enfarte e da OMV, confirmando a disfunção endotelial como um mecanismo importante na disfunção microvascular em doentes com EAMcST. O IRM correlaciona-se fortemente com a OMV e permite prever a dimensão do enfarte e o risco de remodelagem ventricular esquerda.

# INTRODUCTION

# 1. The scope of the problem: microcirculatory dysfunction in patients with acute STEMI

The interventional cardiologists that perform primary percutaneous coronary interventions have a common insecurity when they face a patient with an occluded coronary artery in the setting of an acute ST elevation myocardial infarction: *what if opening the artery is not enough*?

This anguish can take several forms: either more immediate – like the no-reflow phenomenon, the inability to improve patient's pain or the unexpected absence of ST resolution – or later – like unexpected severe left ventricular remodelling or left ventricular dysfunction in the follow-up, even after a successful and timely procedure. All these questions and fears seem to have a common ground, one we need to understand better in order to further improve STEMI patients outcomes: the coronary microcirculation.

The diagnosis and treatment of acute ST elevation myocardial infarction (STEMI) has undoubtedly been the subject of intense investigation over the last decades. As a result, prompt implementation of both mechanical (primary percutaneous coronary intervention) and pharmacological (adjuvant anti-platelets and anticoagulants) approaches to reopen the occluded coronary artery is nowadays universally accepted as the treatment of choice to improve survival in STEMI patients.[1,2]

Notwithstanding all the improvements we have seen in primary PCI programs, with dramatic reductions in the time between symptom onset and the intervention, and despite a normal epicardial coronary artery flow is almost always achieved in a timely fashion after primary percutaneous coronary intervention (P-PCI), a significant proportion of patients (from 20% to 60%) has a poor outcome because of microvascular coronary damage.[3,4] In fact, microvascular perfusion is often impaired after P-PCI, and reperfusion of the epicardial coronary artery will not always guarantee reperfusion at the myocardial tissue level[5].

The extent of this microvascular coronary dysfunction has been shown to be an important and independent contributor to subsequent changes in left ventricular geometry and performance.[6,7] Patients with impaired microvascular perfusion have larger infarcts, as evaluated by CK and troponin release, less electrocardiographic ST elevation resolution, larger long-term left ventricular wall motion abnormalities and lower left ventricular ejection fraction, and larger necrotic areas as evaluated by cardiac magnetic resonance. As a consequence of these, coronary microvascular dysfunction is associated with higher event rates, risk of progression to heart failure and mortality.

The precise mechanisms underlying coronary microcirculation dysfunction before and after the restoration of epicardial blood flow are largely unknown and likely to be multifactorial. Understanding what happens in the microcirculation in the setting of a ST elevation myocardial infarction is thus very relevant. Most research done on this matter, however, has focused on the changes occurring on microcirculation *after* the STEMI and very little is known about the effect of *pre-existent* microvascular coronary dysfunction.

The recent development of invasive techniques for the precise evaluation of the coronary microcirculation (such as the resistance – IMR) and of non-invasive methods for the evaluation of endothelial dysfunction (such as digital peripheral arterial tonometry - PAT) opens a new window of opportunity for the understanding of the pathophysiological processes occurring in coronary microvasculature in patients with STEMI.

I have been deeply involved in the area of functional evaluation of coronary disease in the past few years, both locally[8–13]and at a national[14] and international levels[15–17] and I am currently involved in several trials evaluating new non-hyperaemic technologies for physiological assessment of intermediate lesions and/or new clinical indications. Therefore, both I and all the Interventional Cardiology Department in which I work are very familiar with the use of pressure-wire technology, both for clinical and investigational purposes.

On the other hand, I am also quite familiar with the PAT technology, following the creation of an Endothelial Function Laboratory by Prof. Victor Gil. This laboratory has conducted several studies, not only in coronary artery disease,[18–21], but also in other clinical conditions, like rheumatologic diseases[22], obesity[23–25] or erectile dysfunction[26], in which I had the opportunity to collaborate.

Therefore, using these new invasive (IMR) and non-invasive (EndoPAT®) techniques with the intention to improve our understanding of the coronary microcirculation dysfunction in STEMI patients was a natural step.

In the following pages, the evidence that supports this study will be presented, organized in six sections:

- **Section 2** is a short description of the normal coronary microcirculation.
- **Section 3** presents the changes thought to occur in acute STEMI and the prevailing theories on the role of microcirculation, with particular focus on the endothelial function and on the gaps in knowledge that led to the current study.
- **Section 4** outlines the tools available for evaluating coronary microvascular circulation.
- **Section 5** focus on the index of microcirculatory resistance the clinical evidence currently available.
- **Section 6** is dedicated to describing the tools available for endothelial function evaluation.
- **Section 7** further details non-invasive peripheral arterial tonometry (EndoPAT).

Finally, a short summary of the fundaments of this thesis are presented in **Section 8**.

# 2. The normal coronary microcirculation

## 2.1. Anatomy and function of the coronary arterial system

The coronary arterial system can be subdivided into three functional compartments: conductive vessels, pre-arteriolar vessels and arterioles.[27–29] The coronary blood flow is driven by the pressure difference between the aortic sinus and the coronary sinus (or the right atrium pressure) across these three compartments:

- **Conductive vessels** (corresponding to epicardial arteries) are the first compartment and in the absence of obstructive stenosis, they offer very little resistance to coronary blood flow (even at maximum hyperemia), serving mainly as conductance vessels. Approximately 60% of their wall thickness consists of the muscular media, which can respond to changes in aortic pressure and modulates coronary tone in response to flow-mediated endothelium-dependent vasodilators, circulating vasoactive substances and neural stimuli.
- The intermediate compartment is represented by **pre-arterioles**, which are resistive vessels connecting the conductive arteries to the arterioles. They are epicardial (extra-myocardial) vessels and have a diameter in the range of 200 to 500 µm. These vessels react to changes in shear stress and intravascular pressure to preserve adequate perfusion pressure in the distal arteriolar bed, being responsible for approximately 25% of the total coronary vascular resistance.
- The distal compartment consists of **arterioles**. They are smaller than 200 µm in diameter and are the main regulatory component of the coronary circulation, representing approximately 55% of the total coronary vascular resistance. Arterioles are usually subdivided in two categories, according to their diameter and the mechanism(s) that regulate their tone[30]: endothelium-dependent vasoreactivity prevails in the larger arterioles (100–200 µm) and translates flow-related stimuli into vasomotor responses, i.e. vasodilation with increase in flow and vice versa. Medium-sized microvessels (40–100 µm in diameter) react predominantly to intraluminal pressure changes sensed by stretch receptors located in vascular smooth muscle cells (myogenic control), i.e. they constrict when the intraluminal pressure increases and, conversely, dilate when the pressure decreases. Finally, the tone of the smaller arterioles (vessels less than 40 mm in diameter) is modulated by the metabolic activity of the myocardium.

The arterioles are responsible for the process of coronary autoregulation[31,32]: coronary flow is regulated independently of the arterial perfusion pressure despite large variations in this pressure. Vasodilatation of the smaller arterioles is induced by increased metabolic activity, which leads to pressure reduction in the medium-sized microvessels and myogenic dilation, which, in turn, increases flow upstream resulting in endothelium-dependent vasodilation (Figure 1).

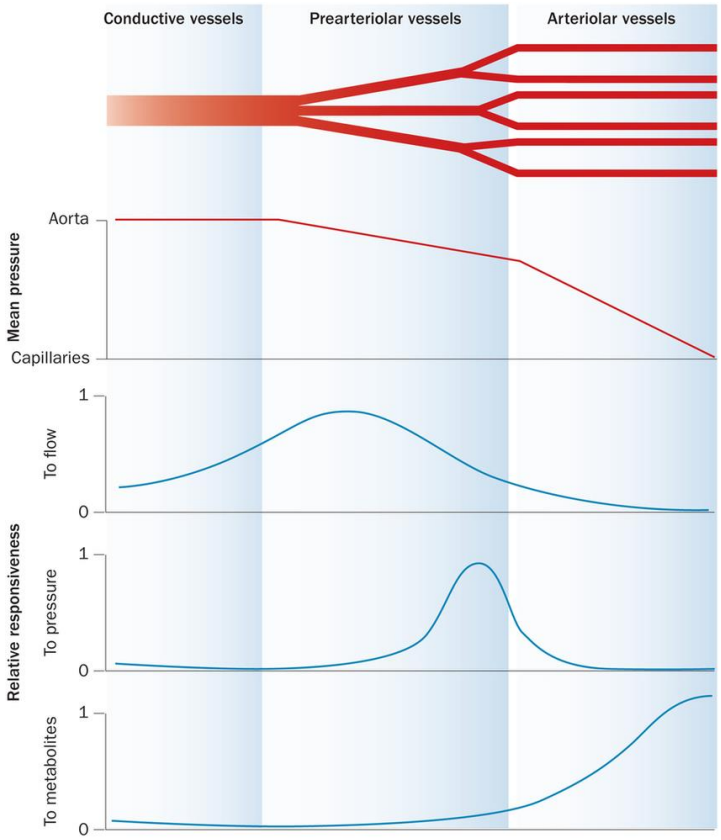



**Figure 1 – Coronary arterial circulation**

Conductive vessels and proximal prearterioles are most responsive to flow-dependent dilation. Distal prearterioles are most responsive to changes in intravascular pressure (and are mainly responsible for autoregulation of coronary blood flow), whereas arterioles are most responsive to changes in the myocardial concentration of metabolites, and are mainly responsible for the metabolic regulation of coronary blood flow (Adapted from Camici et al[33])

These mechanisms effectively and efficiently allow the microcirculation to regulate myocardial perfusion both at rest and at different levels of myocardial metabolic demand.[30] When pressure falls to the lower limit of autoregulation, coronary resistance arteries are maximally vasodilated by intrinsic stimuli, and flow becomes pressure-dependent, resulting in the onset of subendocardial ischemia. Resting coronary blood flow under normal hemodynamic conditions averages 0.7 to 1.0 mL/min/g and can increase four to fivefold during vasodilation.[34]

## 2.2. Regulation of coronary vascular tone

Coronary blood flow is adjusted to the metabolic needs of the myocardium by at least three essential regulators of coronary tone:

- The **metabolic vasodilatory system**, through the production of adenosine and simultaneous opening of the ATP-dependent $K^+$ channels within the myocardial cells (both potent vasodilators) in the presence of an increase in the oxygen consumption and metabolic demand[35];
- The **neurogenic control system,** through adrenergic activation of α-receptors, resulting in arteriolar vasoconstriction. Vasoconstriction mediated by α1-receptor acts mainly on the larger coronary arteries, whereas both α1- and α2-receptor activity is involved in

regulating the degree of vasoconstriction of the smaller resistance vessels.[29] These influences are opposed by the vasodilatory effect of vascular β-receptor stimulation and metabolic mechanisms. Importantly, cholinergic stimulation, normally vasodilatory because it releases nitric oxide, becomes vasoconstrictive when the endothelium is damaged (see below).

- The **vascular endothelium**, that acts both through vasodilation and vasoconstriction, playing a central role in the regulation of vascular tone, since it closely interacts with the two other systems involved:
    - Endothelial-dependent vasodilation is mediated by nitric oxide (which is a very short-lived vasodilating factor) and by the endothelium-dependent hyperpolarizing factor (EDHF), both secreted by healthy endothelial cells in response to i) vascular shear forces associated with increased coronary flow[36]; ii) adenosine (acting on endothelial ATP-dependent $K^+$ channels[37]); iii) or other agonists (e.g. released from platelets or cardiac nerves). Prostacyclin, or prostaglandin I2 (PGI2), is also produced in the coronary endothelium of collateral vessels and causes tonic vasodilation.
    - Endothelial-dependent vasoconstriction is mediated by endothelin-1 which activates protein kinase C in vascular smooth muscle to produce coronary constriction and competes with endothelium-derived relaxing factors. This effect is enhanced in diseased atherosclerotic arteries with extensively damaged endothelium.[38]

## 2.3. The vascular endothelium

The vascular endothelium is a monolayer of cells covering the internal lumen of all blood vessels, separating the blood from the vascular wall and organ tissues. The vascular endothelium has different functions[39]:

- It plays a crucial role in vascular tone and blood flow regulation (as described in the previous section);
- It is an anti-coagulant surface, with an active role on platelet adhesion and aggregation, and on thrombosis.[40] Under physiological conditions, the endothelium prevents thrombus formation through a number of mechanisms: thrombomodulin, protein S, heparin sulphate, and tissue factor pathway inhibitor are all endothelium-derived inhibitors of coagulation, whereas PGI2, NO, and surface-bound CD39 inhibit platelet aggregation.[41]
- It regulates vascular permeability to plasma constituents between blood and tissues[42];
- It contributes to vascular homeostasis and repair.[43]

Additionally, the endothelium also actively produces proinflammatory and anti-inflammatory molecules, and is the actual target for circulating inflammatory mediators that are synthesized by other cell types, including platelets, leukocytes, hepatocytes, and adipocytes.[41]

# 3. Coronary microcirculation dysfunction

## 3.1. Classification and mechanisms of coronary microcirculation dysfunction

Coronary microvascular dysfunction has been widely studied in the last two decades, particularly in patients chest pain despite having normal coronary arteriograms (i.e. microvascular angina).

In an attempt to summarize the mechanisms involved and its clinical translation, Camici and Crea proposed[44] (and recently reviewed[45]) a classification for the different clinical types of coronary microvascular dysfunction (Table 1). Of the four types of microvascular dysfunction proposed, Types 3 (obstructive coronary artery disease) and 4 (iatrogenic) are the ones involved in patients with acute ST elevation myocardial infarction.

The authors also put forward the main pathogenic mechanisms more likely to be involved in these types of coronary microvascular dysfunction (luminal obstruction by thrombotic debris, endothelial dysfunction, smooth muscular cell dysfunction and autonomic dysfunction).

**Table 1 – Classification of coronary microvascular dysfunction**

| | Clinical Setting | Main pathogenic mechanisms |
|---|---|---|
| **Type 1**: In the absence of myocardial diseases and obstructive CAD | Risk factors<br>Microvascular angina | Endothelial dysfunction<br>SMC dysfunction<br>Vascular remodelling |
| **Type 2**: In myocardial diseases | Hypertrophic cardiomyopathy<br>Dilated cardiomyopathy<br>Anderson-Fabry's disease<br>Amyloidosis<br>Myocarditis<br>Aortic stenosis | Vascular remodelling<br>SMC dysfunction<br>Extramural compression<br>Luminal obstruction |
| **Type 3**: In obstructive CAD | Stable angina<br>Acute coronary syndrome | Endothelial dysfunction<br>SMC dysfunction<br>Luminal obstruction |
| **Type 4**: Iatrogenic | PCI<br>Coronary artery grafting | Luminal obstruction<br>Autonomic dysfunction |

Adapted from Camici and Crea.[45] CAD: coronary artery disease; SMC: smooth muscle cells; PCI: percutaneous coronary intervention

However, the exact role of each of the abovementioned mechanisms is difficult to identify, particularly in pathogenic processes. In fact, a substantial number of hypotheses for the pathophysiology of coronary microvascular dysfunction has been proposed in the literature[44,46] including:

- Structural changes:
    - Luminal obstruction, caused by microembolization in acute coronary syndromes or after recanalization.

  - Vascular wall infiltration (e.g. in infiltrative diseases, like Anderson-Fabry cardiomyopathy)
  - Altered microvascular remodelling, through sclerosis of small arteries and arterioles with perivascular fibrosis, swollen endothelial nuclei in capillaries, and irregular lumina of small arteries.[47]
  - Vascular rarefaction and perivascular fibrosis (e.g. in aortic stenosis and arterial hypertension).
- Functional changes:
  - Endothelial dysfunction, including the release of substances with opposing effects (such as endothelin, thromboxane $A_2$, prostaglandin $H_2$, and superoxide) and resulting in a shift from a net dilator response to a net constrictor response to a variety of stimuli. Endothelial dysfunction also involves a switch from a quiescent to an activated state promoting inflammatory responses, chemokine and adhesion molecule expression, and subsequent interaction with platelets and leukocytes.[48]
  - Smooth muscle dysfunction, as showed by a reduced coronary blood flow response to endothelium-independent vasodilators (e.g. adenosine, dipyridamole, papaverine) in patients with microvascular angina.[49]
  - Microvascular spasm and sympathetic dysfunction, mediated via both α1 and α2-adrenoceptors in epicardial conduit arteries and microvessels[50,51], e.g. after coronary revascularization.
- Extravascular factors
  - Extramural compression (aortic stenosis, hypertrophic cardiomyopathy, arterial hypertension)
  - Reduction in diastolic perfusion time (aortic stenosis).

However, these mechanisms could not be critically tested, establishing clear and accepted pathophysiological concepts. In fact, there is no adequate information on the relevance of individual mechanisms in general, let alone in individual patients. As such, the stratification of individual patients and the rational development of targeted strategies is underdeveloped, as recently acknowledged in the 2015 William Harvey Lecture on Basic Science at the European Society of Cardiology Congress in London[46], dedicated to coronary microvascular dysfunction.

## 3.2. Coronary microcirculation dysfunction in STEMI

Despite the fact that primary PCI is highly successful in restoring epicardial coronary blood flow, when performed in a timely fashion, reperfusion at the myocardial level is not accomplished in a significant proportion of patients (from 20 to 60%, depending on the technique used for its evaluation[5]). The negative prognostic implications (both on the risk of left ventricle remodelling and on the risk of hard endpoints, including death) associated with coronary microvascular damage have been repeatedly confirmed, whatever non-invasive or invasive indicator of microvascular dysfunction/obstruction is used (Table 2).

**Table 2 – Main studies showing the prognostic impact of coronary microcirculation dysfunction on adverse remodelling and mortality after primary PCI**

| Author, Year of publication | Indicator of microvascular dysfunction/obstruction | Number of Patients | Risk measure |
|---|---|---|---|
| **Risk of adverse remodelling** | | | |
| Bax et al, 2004[52] | Doppler-wire CFR | 73 | OR 0.28 (0.14-0.41) |
| Araszkiewicz et al, 2006[53] | TIMI myocardial perfusion grade | 145 | OR 3.15, 95% CI 1.35-7.31 |
| Galiuto et al, 2008[54] | Myocardial contrast Echo | 110 | OR 12.7, 95% CI 2.65-61.2 |
| Lombardo et al, 2012[55] | ceCMR | 36 | OR 3.1 95% CI 1.45-6.64 |
| Faustino et al, 2016[56] | Index of microcirculatory resistance | 40 | HR 0.562, P<0.002 |
| **Risk of death** | | | |
| Van´t Hoof. 1998[57] | TIMI myocardial perfusion grade | 777 | OR 2.6 95% IC 1.2-5.4 |
| Morishima et al, 2000[58] | TIMI flow 0 (no reflow) | 120 | OR 5.3, 95% CI 1.90-15 |
| Yamamuro et al, 2002[59] | Coronary flow velocity | 169 | HR 12.8, p<0.001 |
| Henriques et al, 2003[60] | TIMI myocardial perfusion grade | 924 | OR 4.7, 95% CI 2.3-9.5 |
| Bolognese et al, 2004[7] | Myocardial contrast Echo | 124 | OR 0.26, 95% CI 0.09-0.72 |
| Sorajja et al, 2005[4] | ECG | 456 | OR 7.1, 95% CI 1.52-33.3 |
| Ndrepepa et al, 2010[61] | TIMI flow | 1406 | OR 1.66, 95% CI 1.17-2.36 |
| De Waha et al, 2010[62] | ceCMR | 438 | OR 5.12 95% CI 1.09-24.06 |
| Van de Hoef et al, 2013[63] | Doppler-wire CFR | 100 | OR 4.09 95% CI 1.18-14.17 |
| Fearon et al, 2013[64] | Index of microvascular resistance | 253 | OR 3.95 95% CI 1.26-15.00 |

CFR: coronary flow reserve; ceCMR: contrast-enhanced cardiac magnetic resonance;

In spite of this, if evidence is weak and mainly theoretical in stable disease, the exact role and mechanisms of coronary microvascular dysfunction is even less clear in patients with acute coronary syndromes, and particularly in patients with acute ST elevation myocardial infarction.

Traditionally, coronary microvascular dysfunction in this setting is seen *as a consequence* of the primary epicardial event and/or of the coronary reperfusion, either pharmacological (thrombolysis) or mechanical (primary PCI). However, an alternative explanation is that either *pre-existing* or *simultaneous* coronary microvascular dysfunction may have by itself pathophysiological importance and contribute to the extension of the myocardial infarction, left ventricular remodelling and future events.[65]

### 3.2.1. The "classical theory" – coronary microvascular dysfunction as a secondary phenomenon

The classical theory, which assumes that coronary microvascular dysfunction is a consequence of myocardial infarction, has been the prevailing one in past decades. It is based on the hypothesis that ST elevation myocardial infarction is primarily a large epicardial vessel event, as a consequence of a plaque rupture, leading to thrombus formation and subsequent complete occlusion of the vessel.[66]

This hypothesis was widely accepted, since it offered an explanation for the surprising observation that frequently STEMI patients did not have severe stenosis associated with the

acute infarction.[67] According to this view, the coronary microcirculation is just an "innocent bystander" in the acute phase of the acute STEMI, becoming dysfunctional as a result of the occlusive event.[65]

Several mechanisms were hypothesized for this secondary impairment of the coronary microcirculation in STEMI patients (in line with the classification described in Section 3.1 of this Introduction) and three are generally accepted as the most relevant: mechanical obstruction, endothelial dysfunction and reperfusion injury.

1. **Mechanical obstruction**, by distal embolization of atherothrombotic debris, is the mechanism that seems more intuitive – frequently it is even visualized during primary PCI. It was initially proposed based on animal studies with microspheres, injected in the coronaries and resulting in myocardial infarction.[68] Clinically, this phenomenon was confirmed in autopsy studies in patients with unstable angina (in which intermittent fragmentation of thrombi, with peripheral embolization causing microembolic occlusion of small intramyocardial arteries associated with microinfarcts, was documented in 73% of cases[69]) and in patients who died after balloon angioplasty or thrombolysis (microemboli were observed in 81% of patients, and were associated with infarct extension, new myocardial infarction or new ECG abnormalities[70]). Iatrogenic embolization during primary PCI was also the hypothesis for the observed impairment in coronary microcirculation (assessed by correct TIMI frame count) in a study performed in STEMI patients evaluated by IVUS both before and after angioplasty with stent. In this study, the investigators showed that the decrease in plaque volume (assessed by IVUS) was significantly larger in patients with inadequate flow than in those with better reflow.[71] More recently, in patients with STEMI treated by primary PCI, it was showed that distal embolization, confirmed visually by the operator, was associated with larger infarct size and more frequent transmural necrosis, evaluated by contrast enhanced CMR. These patients also had more often microvascular obstruction, as evaluated by first-pass enhancement ceCMR.[72]

   However, despite all this evidence and the easy-to-understand logic behind it, the clinical results with protection, thrombectomy and/or aspiration devices have been disappointing, as showed both by recent meta-analyses of randomized trials[73,74] and by large registries.[75] Currently, these devices are not recommended as routine treatment in patients with acute ST elevation myocardial infarction.[1,2,76,77] Still, it must be acknowledge that the fact that these devices failed does not mean that distal embolization did not occur: it may be that a significant amount of distal embolization occured before any type of medical or procedural intervention, yielding the myocardial microcirculation dysfunction and thereby limiting the therapeutic potential of these procedures.[65]

2. **Endothelial dysfunction**, as described above, seems to be involved in microvascular dysfunction through several pathways in acute coronary patients. Thromboembolic distal embolization by itself results in release of vasoactive factors, but the coronary plaques also have the same potential, even without promoting distal mechanical obstruction. In fact, vasoactive factors, like endothelin-1[78–80] and tissue factor[81], both potent vasoconstrictors, are increasingly expressed in active coronary lesions and in an

experimental model in rat it was shown that rupture of atherosclerotic lesions induced rapid and marked increases in distal vascular resistance, without significant distal embolization.[82] Accordingly, increased microcirculatory resistance has been demonstrated in patients with unstable angina[83] and after balloon angioplasty of thrombotic lesions.[84] Additionally, oxidative stress and ischemia *per se* may reduce the bioavailability of nitric oxide, further contributing to microvascular dysfunction.[65]

3. **Reperfusion injury** is a controversial entity[65,85], mainly because the experimental data that support its concept failed to prove in the clinical setting. Basically, it translates the pathological deleterious events that may occur as a result of the restoration of coronary flow, including changes in myocardial contractile performance (myocardial stunning), myocyte viability (infarction extension), arrhythmogenic threshold (reperfusion arrhythmias) and, again, endothelial function (vascular stunning).[65,86] A number of pathophysiologic mechanisms have been postulated, including histological evidence of platelet and leukocyte accumulation and activation in the myocardial microcirculation, leading to thrombosis, vasoconstriction and release of free oxygen radicals, proteases, and pro-inflammatory mediators; as a consequence, there may be a reduction in nitric oxide bioavailability and activation of endothelin and of the local renin-angiotensin system. Additionally, complement activation leads to the release of histamine and to an increase in cell permeability, resulting in endothelial cell and myocyte swelling, interstitial oedema, and further stimulation of leukocyte adherence.[65]
   However, this evidence was obtained in experimental models, and the pharmacological interventions developed based on these results (including calcium channel blockers, glycoprotein IIb/IIIa inhibitors, nicorandil and anti-neutrophils antibodies) all failed to improve clinical results in patients.[5,65,87] The only possible exception is adenosine, which showed promising results in some trials, despite failing in others.[88,89]

The main limitation of this view of coronary microvascular dysfunction as a consequence of the myocardial infarction relates to the fact that the studies that support it were not designed to evaluate if the abnormalities were already present *before* or *during* the myocardial infarction, as opposed to *after* the event. Additionally, the plaque rupture theory has been challenged in recent years, with the finding that plaque rupture is much more common than previously thought and that most plaque ruptures remain clinically silent. The best evidence on this comes from the PROSPECT trial, in which 697 patients with acute coronary syndromes underwent three-vessel coronary angiography and intravascular ultrasonographic imaging (IVUS) after percutaneous coronary intervention. Subsequent major adverse cardiovascular events, over a median follow-up time of 3.4 years, were found to be equally attributable to recurrence at the site of culprit lesions and to nonculprit lesions.[90] Additionally, in a substudy of PROSPECT including only nonculprit ruptured plaques (seen in 14% of patients with ACS), these were not associated with adverse outcomes, as compared to patients without ruptured plaques.[91]

### 3.2.2. Primary coronary microvascular dysfunction in STEMI

While systemic factors such as thrombotic-fibrinolytic balance and local factors such as collateral blood flow have to be taken into consideration, it is reasonable to propose the myocardial microcirculation as a potential modulating factor in patients with ST elevation myocardial infarction, assuming the presence not only of the vulnerable plaque but also of the vulnerable myocardium and its microcirculation[65].

If this is the case, we would expect coronary microvascular dysfunction to be present not only in the culprit artery, but also in other arteries not involved in the infarction. This was exactly what Uren and colleagues found in 13 patients with STEMI in which they performed positron emission tomography in the first week: coronary flow reserve was found to be decreased not only in the culprit, but also in non-culprit arteries.[92] More recently, the same result was found in a larger cohort (n=100) of STEMI patients, in which coronary flow reserve was measured both in the culprit artery and in one reference vessel not related with the infarction, using a Doppler wire. The authors found that a reduced CFR in the non-culprit artery was related with worse outcomes after 10 years of follow-up, with a significant increase in total mortality (hazard ratio, 4.09; 95% confidence interval, 1.18–14.17; P=0.03).[63] Similar results were also found in several studies in patients with angiographically normal or minimally diseased coronary arteries, in which lower CFR values (measured with a Doppler wire) were related to an increase in long-term events.[93,94]

The presence of endothelial coronary dysfunction (as measured by the flow response to intracoronary infusion of acetylcholine) was also a predictor of events in patients without evidence of epicardial coronary lesions.[95] Taken together, this evidence clearly suggest that the presence of myocardial microcirculatory dysfunction is a strong predictor of clinical outcome, including future acute coronary events (particularly myocardial infarction), even in the absence of hemodynamically significant epicardial disease. In other words, this data argue *against* microvascular coronary dysfunction as *just* a secondary phenomenon, suggesting that it may also play a role *before* and *during* events.

Further data suggesting that there may be a coronary microvascular "*milieu*" that exposes patients to a higher risk of events comes from the studies linking inflammation and coronary artery disease. In fact, widespread activation of neutrophils across the coronary vascular bed has been reported in patients with unstable angina, regardless of the location of the culprit stenosis.[96] Additionally, C-reactive protein serum concentration has been identified as an independent predictor of a blunted coronary blood flow response to adenosine (endothelium-independent) and substance P (endothelium-dependent), in patients undergoing elective PCI[97]. Similarly, in patients with normal coronary angiograms, a significant inverse correlation was noted between C-reactive protein concentrations and myocardial blood flow responses to cold pressor testing by 13 N-ammonia and PET imaging[98].

However, the strongest data in favour of the primary coronary microvascular dysfunction hypothesis in STEMI patients comes from pharmacological studies, particularly with statins,

known to improve microvascular function.[99,100] In fact, several trials showed that previous treatment with statins:

- Reduces the incidence and extent of myocardial injury in patients referred for elective PCI[101];
- Significantly reduces (by 74%) the incidence of no-reflow in STEMI patients treated with primary PCI[102];
- Reduces (by 20%) the risk of developing ST-segment elevation or evolving to full acute myocardial infarction in ACS patients[103];
- Improves clinical outcomes in patients with ACS, an effect that is completely abrogated if statins are prematurely discontinued after the onset of symptoms[104].

Taken together, this data suggest that a pre-existing transient or permanent microcirculation dysfunction may contribute to the development and prognosis of ACS, via reduction of coronary blood flow, leading to an alteration of shear stress and thereby aggravation of endothelial function on epicardial level as well as aggravation of thrombus formation.[65] In this view, microvascular impairment and the consequent reduction of flow would be the primary factor and intracoronary thrombi would then develop *after* the onset of myocardial ischemia, as defended by William Roberts in his famous editorial published in 1974 in Circulation[105], in which he stated that "*coronary thrombosis is a consequence rather than the precipitating cause of acute myocardial infarction*".

In summary, as Amir Lerman elegantly stated in his landmark paper "Microcirculatory dysfunction in ST-elevation myocardial infarction: cause, consequence, or both?", while, with the available evidence, we cannot confirm which of these two theories is more correct, and assuming that both may be right to some extent, "*if indeed microcirculatory dysfunction is ever demonstrated to be one of the major contributors to the evolution and not just the consequence of an acute myocardial infarction, this could substantially alter future research directions and approaches to therapy*".[65]

## 3.3. Endothelial function: a core player throughout the *continuum* of coronary artery disease

Whatever theory concerning microvascular coronary dysfunction in patients with ST elevation myocardial infarction is correct (cause, consequence or both), endothelial function seems to be always at the core of the proposed mechanisms.

Indeed, as described in the previous sections, endothelial function plays a central role in microcirculation tone and blood flow regulation, thrombosis and coagulation, inflammation and vascular permeability, haemostasis and repair. Thus, it is not surprising to see current evidence suggesting that endothelial dysfunction occurs early in the process of atherogenesis and contributes to the *formation*, *progression*, and *complications* of the atherosclerotic plaque[106]:

- **In the early stage of atherosclerosis**, several studies have shown that patients with cardiovascular risk factors but no clinical evidence of atherosclerosis have endothelial

dysfunction[107–109] and that this endothelial dysfunction is an independent predictor of future cardiovascular events.[110,111] In fact, endothelial dysfunction has been reported in relation with most risk factors for atherosclerosis, including hypertension[112], diabetes[113], hyperlipidaemia[114], and ageing.[115,116] However, the presence of endothelial dysfunction appears to have an incremental prognostic value, after control for these classical risk factors[117–120], which may be explained by the fact that it reflects the overall burden of risk, including other so far unknown factors[121].

Conversely, as endothelial function is associated with risk factors, the absence of endothelial dysfunction appears to predict a particularly favourable state.[122]

These observations strongly suggest that endothelial dysfunction is a common mechanistic link between risk factors and the development of atherosclerosis.[123] Interestingly, endothelial dysfunction itself can cause myocardial ischemia, even in the absence of relevant coronary stenosis.[124]

- In patients with established **stable coronary artery disease**, endothelial dysfunction, either measured in the coronary arteries by acetylcholine infusion[125], or evaluated peripherally[126], was associated with a worse prognosis.

  Even in patients with mild or non-obstructive coronary artery disease, the presence of endothelial dysfunction was independently associated with adverse cardiovascular events, in several studies performed with different methods.[95,127–129]

- Finally, in patients with **acute coronary syndromes**, endothelial function, measured in the peripheral circulation, has been shown to be an independent predictor of events[130], and subsequent normalization of endothelial function in these patients predicts a lower risk.[131,132] Similarly, in patients with ST elevation myocardial infarction treated with primary angioplasty, peripheral endothelial dysfunction was associated with larger infarctions as measured by troponin peak levels[18] and endothelial function improvement six months after the event also correlated with lower end-diastolic left ventricular volumes.[133]

Importantly, in most studies performed in all phases of the atherosclerotic process (risk factors -> stable disease -> acute coronary syndromes), endothelial function was evaluated peripherally by non-invasive techniques (see Section 6), as a surrogate for coronary endothelial dysfunction. In fact, endothelial dysfunction appears to be a *systemic* vascular process that not only mediates the development of the atherosclerotic plaque but may also modulate its clinical course.

## 4. Methods for the evaluation of microcirculation in patients with acute ST elevation myocardial infarction

Currently, no technique allows direct visualization of the coronary microcirculation *in vivo* in humans. However, since coronary microcirculation dysfunction may result from functional and not necessarily structural abnormalities (or represents a combination of both mechanisms), even if there was a technique that could clearly visualize the anatomy of the coronary microcirculation

in humans *in vivo*, it would still be an incomplete evaluation. Therefore, microcirculatory function is indirectly assessed using several invasive and noninvasive techniques that enable the measurement of parameters that (under normal circumstances) are strongly dependent on the functional integrity of the coronary microcirculation.

Consistent with its primary hemodynamic function, functional techniques for the assessment of the coronary microvasculature rely on the measurement of coronary blood flow, which changes mainly as a result of alterations in vascular tone. Although there are several imaging techniques that allow estimating coronary blood flow, at present, the most definite evaluation of the coronary microcirculation remains invasive.

In the following pages, the most used invasive and non-invasive techniques for the evaluation of microvascular coronary circulation in patients with acute ST elevation myocardial infarction are described in short. The index of microcirculatory resistance, used in this study, will be detailed in Section 5.

## 4.1. Non-invasive methods

### 4.1.1. ECG ST resolution

ECG ST-segment resolution is a simple, cheap and validated tool to evaluate acutely microvascular perfusion[134] and has been used for several years in fibrinolysis and primary angioplasty trials.[3,4]

After primary PCI, incomplete ST resolution has been related to coronary microvascular obstruction and worse clinical outcome.[135] Different methods have focused on the assessment of multiple leads or single leads showing maximum ST elevation at baseline and a consensus is still lacking about which leads to analyse, the optimal timing of electrocardiogram analysis, and whether standard ECG or continuous ECG monitoring is preferable.

Assessment of single lead ST resolution showing maximum ST elevation at baseline seems to be as accurate as the sum of ST resolution measurements.[136,137]

Additionally, residual ST-segment elevation was found to be an independent marker of coronary microvascular obstruction[138] and a predictor of events after primary PCI.[139]

### 4.1.2. Imaging techniques

**Positron emission tomography (PET)** is a well-established non-invasive technique for the assessment of coronary blood flow[140,141], as it allows the determination of absolute regional myocardial blood flow at rest and in response to various stimuli. Myocardial blood flow measurement using PET is achieved by continuous monitoring of the radioactivity emitted by an intravenously administered tracer, in the circulation and the myocardium. The kinetics of radiotracer uptake in the myocardium are derived from time-activity curves in the left ventricular cavity and the myocardium; fitting these time-activity curves with an operational equation provides accurate estimates of myocardial blood flow. Importantly, however, it may lack sensitivity and specificity for the diagnosis of coronary vasomotor dysfunction and, in general, is unable to differentiate between epicardial and microvascular abnormalities.[142]

**Myocardial contrast echocardiography (MCE)** uses ultrasound to visualize contrast microbubbles with a rheology similar to that of red blood cells that freely flow within patent microcirculation while lack of intra-myocardial contrast opacification is due to microvascular obstruction that predicts functional recovery after STEMI.[54] It has proven to be a useful tool for identifying patients with the no-reflow phenomenon after interventional or thrombolytic treatment for STEMI.[54,143–145] However, it has several limitations: moderate spatial resolution, operator dependency, and incomplete left ventricular coverage with suboptimal visualization of the lateral wall, and semi-quantitative assessment of coronary microvascular obstruction.

**Contrast enhanced cardiac magnetic resonance (ceCMR)** allows multislice imaging with high tissue contrast and high spatial resolution, enabling accurate quantification of coronary microvascular dysfunction and obstruction, and infarct size. Coronary microvascular dysfunction and obstruction appear as an absence of gadolinium enhancement during first pass and lack of gadolinium enhancement within a necrotic region (late gadolinium hyper-enhancement). Coronary microvascular obstruction evaluated by ceCMR correlates with MCE, and other angiographic and invasive indexes[146] and is an independent predictor of adverse clinical outcome, alone or adjusted by other factors, such as infarct size and left ventricular ejection fraction.[62,147–150]

Other imaging modalities, including CT-derived coronary flow reserve and hybrid positron emission tomography-computed tomography are still mainly investigational and have not been widely used in STEMI patients.

Finally, periungueal capillaroscopy is a simple and reliable non-invasive technique allowing evaluation of cutaneous microcirculation. However, it has been used mainly in patients with Raynaud's phenomenon and in patients with connective tissue diseases, and there is no experience with this technique in patients with coronary artery disease. [151]

## 4.2. Invasive methods

### 4.2.1. Angiographic indexes

Invasive TIMI flow, TIMI frame count and TIMI myocardial perfusion grade have all been proposed in the evaluation of microcirculation after acute ST elevation myocardial infarction.

- The **thrombolysis in myocardial infarction (TIMI)** score grading system describes the rate of blood flow in the epicardial vessels, ranging between no flow at all (Grade 0) to a normal flow rate (Grade 3)[152,153] (for more details, see Population and Methods, Section 3.4.1 - TIMI flow grade, page 89). TIMI flow <3 is a marker of both coronary microvascular obstruction and of larger infarct size and has been shown to affect prognosis both at short and long-term follow-up.[58] However, the value of this index is limited, since coronary microvascular obstruction may occur in nearly 35% of patients with TIMI 3 flow.[146]

- The **corrected TIMI frame count (cTFC)** index corresponds to the number of frames required for contrast medium to reach a standardized distal landmark (see Population and Methods, Section 3.4.2 - Corrected TIMI frame count, page 89). It further stratifies the prognosis of patients with TIMI flow 3 and correlates with invasive assessment of coronary flow reserve.[154]

- The **TIMI myocardial perfusion grade (TMPG)** and the **myocardial blush grade (MBG)**, assess the intensity of the radio-opacity of myocardial tissue after an epicardial coronary injection of contrast medium (MBG), as well as its wash-out rate (TMPG). An intense myocardial blush and fast wash-out of contrast medium indicate optimal microvascular reperfusion.[60,155] Both are scored on a scale of 0–3, the latter indicating optimal perfusion (see Population and Methods, Section 3.4.3 - TIMI myocardial perfusion grade (TMPG), page 90). A MPG grade 0–1 can be observed in up to 50% of patients with TIMI flow grade 3.[57]

Using both TIMI flow and TIMI myocardial perfusion grade[156], coronary microvascular obstruction can be defined as a TIMI flow grade <3 *or* a TIMI flow grade 3 with a TMPG/MPG 0 to 1.

### 4.2.2. Doppler wire-derived coronary blood flow reserve

Coronary flow reserve (CFR) represents the extent to which the coronary circulation can increase myocardial blood flow in response to exercise or a hyperaemic stimulus. It is calculated by dividing hyperaemic flow by resting flow. Accordingly, the calculation of CFR

assumes that maximal vasodilatation is achieved by abolishing coronary vasomotor tone, by intravenous administration of endothelium-independent vasodilators (mostly adenosine). CFR evaluates simultaneously the epicardial and microcirculatory compartments of the coronary tree, although, theoretically, in the absence of epicardial vessel disease CFR reflects microvascular function.[157]

Invasive evaluation of CFR was first performed with a Doppler guidewire, positioned into the distal part of the coronary artery. With the sensor at the tip of this wire, coronary flow velocity at rest and during hyperaemia can be measured, and the ratio of maximum to baseline coronary flow velocity can be calculated.[158]

However, this technique has several problems, including frequent guidewire positional changes (motion of the patient, or breathing), which disturbs the Doppler signal and limit the feasibility of the procedure to less than 70% of all arteries.[159] Other technical pitfalls that can hamper signal acquisition include obstruction of the guiding catheter, inaccurate calibration, turbulent flow, and signal loss.[160]

Still, in patients with ST elevation myocardial infarction, CRF evaluated with a Doppler wire was shown as a good prognostic marker for LV function recovery after anterior myocardial infarction treated with primary PCI.[52,161] More recently, the prognostic value of microvascular function as determined using Doppler wire for predicting long-term cardiac mortality was assessed in both infarct-related and reference coronary arteries in patients immediately after primary PCI for ST-segment elevation myocardial infarction. During follow-up (median 11 years), a CFR <2.1 in a reference vessel was associated with a fourfold increased risk of long-term cardiac mortality, whereas a target-vessel CFR <1.5 was associated with an increase in short-term (but not long-term) risk of cardiac mortality.[63]

### 4.2.3. Pressure wire-derived coronary blood flow reserve

In an attempt to overcome the limitations of the Doppler wire, thermodilution-derived CFR was introduced. For this technique, a pressure wire is used to measure temperatures, and CFR is calculated using the principle of thermodilution: by giving short manual injections of 3 cc saline at room temperature into the coronary artery, thermodilution curves are generated and mean transit times at hyperemia and baseline can be calculated. Because coronary flow is inversely proportional to the mean transit time of a bolus of cold saline needed to travel down the coronary artery, CFR can be easily calculated using the ratio of mean transit times.

With this technique, successful measurement of CFR can be performed in 95% of patients.[159] CRF measurements performed this way correlated well with standard CFR, both in experimental model and in humans.[158,162]

However, CFR (measured both by Doppler-wire or pressure-wire) varies with age and sex in healthy humans[163,164], making it impossible to define a clear cut-off value below which microvascular function could be deemed abnormal. Additionally, since coronary blood flow

under resting conditions is dependent on determinants of myocardial oxygen demand (namely heart rate, contractility and ventricular load) and CFR being the ratio of peak hyperaemic-to-resting flow, it is therefore affected by these same determinants, a fact that can also affect the reproducibility of the ratio.[165]

CFR it is also significantly influenced by epicardial vessel disease and thus does not distinguish epicardial from microcirculatory disease.[158] For all these reasons, CFR alone is not a good tool for evaluating the microcirculation in ST elevation myocardial infarction patients.

### 4.2.4. Index of microcirculatory resistance

To overcome the limitations of CFR and allow for the isolated evaluation of the microcirculatory coronary compartment, a new index, the index of microcirculatory resistance (IMR) was developed.[166] The IMR is based on the assumption that microvascular resistance (that provides an independent assessment of microcirculatory function) can be calculated by dividing the distal coronary pressure by absolute coronary flow. According to the methodology previously described for CFR, IMR can be calculated as the distal coronary pressure divided by the inverse of the hyperaemic mean transit time (a correlate to absolute flow), measured simultaneously with the coronary pressure wire.

The fundaments, methodology and clinical evidence for this technique will be further developed in Section 5.

### 4.2.5. Doppler and Pressure combined indexes – hyperaemic microvascular resistance index (hMR) and zero-flow pressure (Pzf)

Recently, a single dual sensor wire (Doppler and pressure) was made available (Combo Wire, Volcano Therapeutics), allowing for the simultaneous measurement of phasic distal pressure and flow velocity. With this wire and dedicated software, and using measurements performed at baseline and at hyperaemia induced by adenosine, both hMR (defined as the ratio of average coronary distal pressure and average instantaneous peak velocity during hyperaemia) and Pzf (which is the distal coronary pressure when theoretically the flow in a coronary artery would cease; since it is not possible to measure this directly, as *in vivo* coronary flow does not cease under normal circumstances, Pzf is extrapolated from pressure-velocity loops[167]) can be measured.

The reproducibility of repeated hyperaemic resistance parameters derived from distal pressure and velocity measurements was confirmed in patients with stable angina.[168] In patients with acute STEMI, a few studies were performed, with promising results: In 2003, Shimada and colleagues[169] studied Pzf using a Doppler wire and assessing arterial pressure from the guiding catheter in patients undergoing primary PCI for anterior STEMI, and showed

that Pzf correlated with viability evaluated by PET. In 2007, it was confirmed that Pzf values are increased after myocardial infarction.[170] More recently hMR, Pzf, and Doppler coronary flow reserve were measured with the combo Doppler-pressure wire in patients after anterior STEMI. All measures correlated with peak creatinine kinase-myocardial and CMR measures of infarct size, and Pzf was found to be higher in those with >75% infarct transmurality.[171] In 2015, hMR and Pzf were found to be related to microvascular injury (assessed by ceCMR) and myocardial perfusion (evaluated with PET).[172] In the same year, another study compared Pzf, hMR and IMR, and suggested that the former was a better predictor of the extent of myocardial infarction (assessed with ceCMR) than the latter two.[173] There is only one study reporting clinical outcomes in STEMI patients treated with primary PCI, and it also confirmed hMR as a strong predictor of a combined endpoint of death and hospital admission for heart failure.[174] However, unlike other wire-based techniques, hMR and Pzf have not been validated in animal or human models. Furthermore, there are concerns that hMR measurement may not be accurate in patients with severe epicardial stenosis, since it does not account for collateral flow (and hence may lead to an overestimation of true microvascular resistance in the presence of a severe stenosis).

# 5. The index of microcirculatory resistance (IMR)

## 5.1. Definition and evaluation of IMR

The IMR is a measurement of the minimum achievable microcirculatory resistance in a target coronary artery territory and thus it provides a quantitative assessment of the microvascular integrity. Unlike CFR, which provides a combined assessment of both the epicardial and the microvascular beds, IMR enables a specific quantitative assessment of the status of the microvascular coronary circulation (Figure 2).

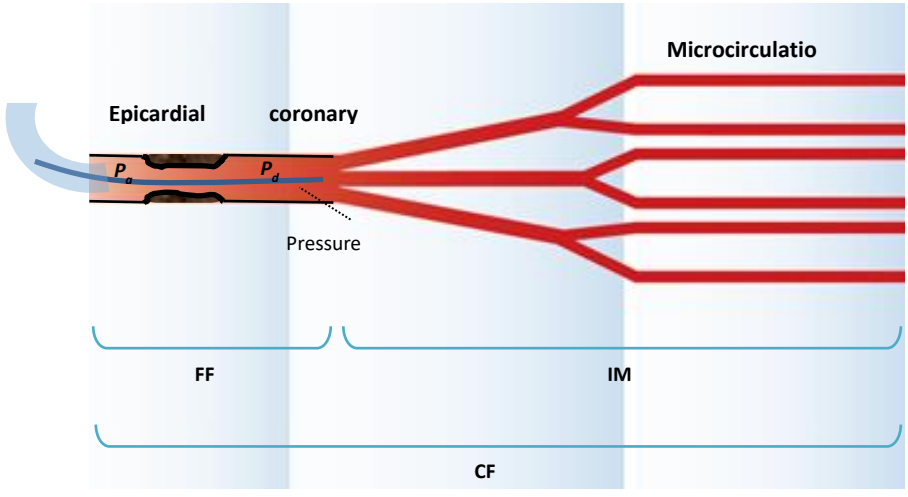



**Figure 2 – Schematic of physiological assessment (FFR, CFR and IMR) using a coronary pressure and temperature wire**

IMR measurement was made possible by the development of a wire with both pressure and temperature sensors on its tip.[158,175] The distal sensor of the wire measures pressure and temperature and the shaft of the wire can be used as a second thermistor.

By injecting room-temperature saline down a coronary artery, an indicator-dilution curve is obtained (according to the decrease in temperature) and the mean transit time ($\boldsymbol{Tmn}$) can be determined. $\boldsymbol{Tmn}$ has been shown to strongly correlate inversely with absolute flow and therefore provides an easily measurable surrogate, both *in vitro*, in animal models[158] and in humans.[162]

According to Ohm's law, the resistance ($\boldsymbol{R}$) in a given circuit is related to the pressure gradient ($\Delta\boldsymbol{P}$) and absolute flow ($\boldsymbol{Q}$), according to the formula:

$$\boldsymbol{R} = \frac{\Delta \boldsymbol{P}}{\boldsymbol{Q}}$$

In the coronary microcirculation, the pressure gradient ($\Delta\boldsymbol{P}$) is determined by distal pressure (distally in the epicardial vessel) minus venous pressure; at maximal hyperaemia, however, venous pressure can be assumed to be zero, implying that $\Delta\boldsymbol{P}$ approximates distal pressure (***Pd***), measured with the pressure-wire.

Similarly, at maximal hyperemia, absolute flow ($\boldsymbol{Q}$) has been shown to have a linear relationship with the inverse of flow velocity ($^{\boldsymbol{1}}/_{\boldsymbol{Tmn}}$). Therefore, the microcirculation resistance can be derived from the simplified formula[176]:

$$\boldsymbol{IMR} = \frac{\boldsymbol{Pd}}{(^{\boldsymbol{1}}/_{\boldsymbol{Tmn}})}$$

Or, to make it even simpler,

$$\boldsymbol{IMR} = \boldsymbol{Pd} * \boldsymbol{Tmn}$$

Importantly, IMR is derived from the assumption that, at peak hyperaemia, the variability of resting vascular tone and hemodynamic will be eliminated, and the minimum microvascular resistance will be achieved.

In the presence of a severe epicardial stenosis, myocardial flow is the composite of both the coronary and collateral flows. Accordingly, distal coronary pressure decreases to a lesser degree, given the contribution of collaterals – this in turn will produce an overestimation of IMR if not corrected for collateral flow. Therefore, to calculate the true IMR ($IMR_{true}$) in this setting, a more complex formula has been developed, which includes measurement of the coronary wedge pressure as a measure of collateral pressure.[177] However, in the absence of significant collateral flow, it is reasonable to use IMR as a surrogate for $IMR_{true}$, avoiding the measurement of coronary wedge pressure.

IMR evaluation was validated in animals and showed a strong correlation with true microvascular resistance, being independent on epicardial stenosis presence and severity (since both distal

pressure and flow drop in the presence of an epicardial stenosis).[166] This was also validated in humans.[177,178]

Despite the relatively complex theoretical background, IMR is easy to evaluate and a very reproducible measurement.[179] A dedicated console, equipped with dedicated software for IMR evaluation makes the procedure simple and easy to learn. The complete description of the procedure is given in detail in Population and Methods, Section 3.5 – Index of microcirculatory resistance (IMR), page 90.

It is a safe technique to perform in STEMI patients[180] and has been used in several studies (described in the next section).

## 5.2. Clinical evidence on the use of IMR in STEMI patients

The evaluation of coronary microcirculation involvement and its consequences in patients with acute STEMI treated with primary angioplasty is a logical application of IMR. Indeed, several studies were performed in ST elevation acute myocardial infarction patients, immediately after the primary PCI. These studies, summarized in Tables 3-6, showed that IMR is related with:

- Left ventricular remodelling (either evaluated by wall motion score index, improvement in left ventricular volumes or improvement in left ventricle ejection fraction) between the acute phase and follow-up, using either echocardiography or contrast enhanced cardiac magnetic resonance (ceCMR),
- Infarct size, measured either by the amount of cardiac biomarkers released, by single-photon emission computed tomography (SPECT) or by ceCMR,
- The presence of microvascular obstruction, evaluated by ceCMR, and
- Myocardial viability, assessed by positron emission tomography (PET).

Additionally, recent evidence suggests that IMR measured immediately after the primary PCI in STEMI patients is a strong and independent predictor of major events, including death (Table 5). Although almost all of these studies were unicenter and small (the majority included less than 50-60 patients), their results, coming from different groups of investigators, are remarkably consistent. Therefore, in the last years, IMR has also been used as a tool to evaluate both pharmacological strategies (like IIb/IIIa inhibitors, nicorandil or nitroprusside) and devices (like thrombus aspirators or distal protection devices) in the treatment of STEMI patients (Table 6).

Finally, IMR has been widely used in stable patients, for several indications[176,181]: as an adjuvant of FFR in patients with equivocal clinical presentation and intermediate coronary stenosis[182], in patients with probable microvascular angina[183], to evaluate drugs[184–186] or devices[187], to assess the impact on microcirculation of percutaneous revascularization[188,189], and in other specific clinical conditions (apical ballooning syndrome[190], assessment of steam cell therapy[191,192], transplant arteriopathy[193] etc.). However, to the date, there are no studies relating IMR to endothelial-dependent tests in patients with acute myocardial infarction.

**Table 3 – Studies using IMR to evaluate LV remodelling in patients with ST elevation myocardial infarction**

| Author (Year) | No. of patients | Comparator | Outcome | Results |
|---|---|---|---|---|
| **LV remodelling** | | | | |
| Fearon (2008)[238] | 28 | Echocardiography | WMS at 3 months | Significant correlation (r = 0.59, p = 0.002)<br>IMR>32 associated with significantly worse WMS<br>IMR was the only independent predictor of WMS |
| Lim (2009)[249] | 40 | Echocardiography | Change in anterior wall motion score at 6 month | Significant correlation (r = -0.464, p = 0.003)<br>Cut-off 33U IMR: AU ROC for predicting LV recovery: 0.89 (CI 95% 0.888-0.894) |
| Sezer (2010)[250] | 35 | Echocardiography | LV volumes and LVEF at 5 months | (IMR measured acutely and at 5 months); significant superior improvements in LV volumes and LVEF in patients that increased more IMR between the 2 evaluations |
| McGeoch (2010)[251] | 47 | Contrast enhanced CMR | LV volumes and LVEF<br>(2 days and 3 months) | IMR was an independent predictor of LVEF at 2 days and 3 months |
| Yoo (2012)[252] | 34 | Echocardiography | Change in WMSI at 6 months<br>Change in LVEF at 6 months | Significant correlation (r = -0.61, P < 0.01) with change in WMSI<br>Significant correlation (r = -0.52, P < 0.01) with change in LVEF |
| Faustino (2016)[56] | 40 | Echocardiography | Change in WMSI, LVEF and E/e' between acute and 3 months | All parameters significantly improved more in patients with lower IMR<br>Lower IMR was associated with lower acute global longitudinal strain values |
| Carrick (2016)[290] | 259 | Contrast enhanced CMR | Change in LV end-diastolic volume and in LVEF at 6 months | IMR was an independent predictor of LV end-diastolic and LVEF improvement between ceCMR performed at 2 days and 6 month, but CFR was not |
| Park (2016)[257] | 89 | Echocardiography | WMSI at 3 months | Improvement in patients with lower IMR and higher FFR groups, but not on patients with both higher IMR and lower CFR |
| Palmer (2016)[258] | 31 | Echocardiography | Change in WMSI and LVEF at 3 months (treatment = thrombolysis) | IMR correlated with LVEF (R = 0.652, p=0.005) and WMSI (R = 0.452, p=0.011)<br>Only patients with lower IMR showed LV recovery at 3 months |

**Table 4 – Studies using IMR to evaluate the size of the infarction, microvascular obstruction and myocardial viability in patients with ST elevation myocardial infarction**

| Author (Year) | No. of patients | Comparator | Outcome | Results |
|---|---|---|---|---|
| **Extension of the infarction** | | | | |
| Fearon (2008)[238] | 32 | Cardiac biomarkers | CK peak values | Significant correlation with peak CK (R = 0.61, p = 0.0005)<br>IMR>32 associated with significantly higher peak CK values<br>IMR was the strongest independent predictor of peak CK |
| Sezer (2010)[250] | 35 | Tec-99m sestamibi SPECT | Infarct size (IS)<br>(at 2 days and 5 months) | IMR was a predictor of IS at 5 months (β = 0.280, P = 0.013)<br>IMR was a predictor of change in IS between 1st and 2nd SPECT (r=0.55, P=0.001)<br>In patients with an improvement in IMR >33%, IS decreased significantly |
| McGeoch (2010)[251] | 47 | Contrast enhanced CMR | Infarct volume<br>(2 days and 3 months) | IMR was an independent predictor of infarct volume at 2 days and 3 months |
| Payne (2012)[253] | 96 | Contrast enhanced CMR | Infarct volume and salvage index<br>(2 days and 3 months) | IMR was an independent predictor of infarct volume and salvage index (the difference between infarct size and area at risk) at 2 days, but not at 3 month |
| Carrick (2016)[290] | 281 | Contrast enhanced CMR | Infarct size, % LV mass | Higher IMR values were associated with larger infarct size |
| **Microvascular obstruction** | | | | |
| McGeoch (2010)[251] | 53 | Contrast enhanced CMR | Microvascular obstruction (MVO) | IMR significantly higher in patients with MVO |
| Yoo (2012)[252] | 34 | Contrast enhanced CMR | Microvascular obstruction (MVO) | MVO correlated with IMR (r = 0.754; P < 0.001) |
| Payne (2012)[253] | 96 | Contrast enhanced CMR | Microvascular obstruction (MVO) | IMR was an independent predictor of MVO (at 2 days) |
| Fukunaga (2014)[254] | 88 | Contrast enhanced CMR | Microvascular obstruction (MVO) | Patients with bimodal shape IMR curve had significantly higher MVO |
| Ahn (2016)[248] | 40 | Contrast enhanced CMR | Microvascular obstruction (MVO) | IMR and CFR both good predictors of MVO, but the combination of both was even better for predicting MVO (AUC 0.941) |
| Carrick (2016)[290] | 219 | Contrast enhanced CMR | Microvascular obstruction (MVO) | IMR was an independent predictor of MVO (at 2 days) |
| **Myocardial Viability** | | | | |
| Lim (2009)[249] | 38 | PET (18Ffluorodeoxyglucose) | Regional myocardial FDG uptake at 6 months | Significant correlation (R = -0.738, p < 0.001) |

**Table 5 – Studies using IMR to evaluate clinical outcomes in patients with ST elevation myocardial infarction**

| Author (Year) | No. of patients | Comparator | Outcome | Results |
|---|---|---|---|---|
| **Clinical Outcomes** | | | | |
| Fearon (2013)[64]<br><br>(multicentre registry) | 253 | IMR | Mortality & hospitalization for heart failure (MACE) | Significantly higher rate of MACE in patients with IMR>40 (HR 2.1, p=0.034))<br>Significantly higher rate of death in patients with IMR>40 (HR 3.95, p=0.028)<br>IMR >40 was the only independent predictor of death alone (HR 4.3, p=0.02) |
| Fukunaga (2014)[254] | 88 | IMR curve shape (bimodal vs. other) | Mortality & hospitalization for heart failure (MACE) | Higher risk of MACE at 6 month in patients with a bimodal curve<br>Bimodal curve was the only independent predictor of cardiac death at 6 months |
| Carrick (2016)[290] | 283 | IMR and CFR | Mortality & hospitalization for heart failure (MACE) | IMR was an independent predictor of MACE, but CFR was not |
| Park (2016)[257] | 89 | IMR and CFR | Cardiovascular death, target vessel failure, heart failure, and stroke | Patients in the group with high IMR and low CFR had significantly more events, as compared to patients with lower IMR and/or higher CFR |

**Table 6 – Studies using IMR as a tool to evaluate the effect on coronary microcirculation function of drugs or devices in patients with ST elevation myocardial infarction**

| Author (Year) | No. of patients | Treatment | Type of Study | Results |
|---|---|---|---|---|
| **IMR values** | | | | |
| Sezer (2007)[312] | 41 | Streptokinase (intracoronary) after P-PCI | Randomized<br>IMR evaluated 48h post P-PCI | Significantly lower IMR in treatment group<br>Lower cTFC<br>No difference in LEFV and volumes in 6 month echo |
| Sezer (2009)[313] | 95 | Streptokinase (intracoronary) after P-PCI | Randomized<br>IMR evaluated 48h post P-PCI | Significantly lower IMR in treatment group<br>Smaller infarct size (22.7 vs. 32.9%; p = 0.003) by SPECT<br>Smaller LV end-systolic and end-diastolic volumes (Echo)<br>LVEF significantly higher in SK patients (Echo) |
| Bonello (2010)[297] | 45 | Several | Observational | Lower IMR values in patients treated with thrombus aspiration, direct stenting and GP IIb/IIIa inhibitors (univariate analysis) |
| Ito (2010)[314] | 40 | Nicorandil (intracoronary) | Non-randomized, prospective | Significant decreases in IMR after nicorandil administration |
| Ito (2011)[315] | 36 | Distal protection (Filtrap) | Randomized | Significantly lower IMR patients in the distal protection group |
| Morimoto (2012)[316] | 18 | Nitroprusside (intracoronary) | Observational | IMR measured after nitroprusside significantly lower than IMR measured before |
| Ito (2013)[317] | 60 | Nicorandil vs. nitroglycerin | Randomized | IMR significantly decreased after nicorandil, but not after nitroglycerin intracoronary administration |
| Woo (2014)[318] | 63 | Thrombus aspiration | Randomized | IMR significantly lower in patients treated with thrombus aspiration<br>LVEF and WMSI improved significantly more in these patients |
| Ahn (2014)[255] | 40 | Thrombus aspiration vs. abciximab vs. both | Randomized | IMR values lower in combination treatment group<br>MVO (assessed by ceCMR) lower in the combination treatment group |
| Orlic (2015)[319] | 128 | Thrombus aspiration | Randomized | No difference in IMR (after adjustment for confounders) between patients treated or not with thrombus aspiration; CK release, ST resolution, WMSI and LVEF were also similar. |
| De Maria (2015)[320] | 85 | Stent implantation | Observational | IMR globally improved after stent implantation, but remained elevated in 32.9% of patients (mainly late-comers and patients with high thrombotic burden) |
| Hoole (2015)[321] | 41 | Thrombus aspiration vs. balloon | Randomized | IMR similar in both groups, thrombus aspiration did not improve results; MVO (assessed by ceCMR) and final infarct size were also similar |
| Kostic (2015)[322] | 32 | Nicorandil (intracoronary) | Observational | After nicorandil, IMR significantly decreased. Likewise, a significant reduction in ST elevation was documented after intracoronary administration of the drug |

# 6. Methods for the evaluation of endothelial function

When the endothelial function is altered, any of its functions could be impaired. Yet, for practical reasons, the current standard is to measure endothelial function by studying of its vasomotor regulation function. It is also customary to use the generic term "endothelial function" as an equivalent for endothelium-dependent vascular reactivity.

The first demonstration of endothelial dysfunction in atherosclerotic coronary arteries using intracoronary infusion of acetylcholine and quantitative coronary angiography dates back to 1986 by Ludmer and colleagues.[194] Later, less invasive techniques were developed using mainly the forearm circulation as a surrogate for coronary arteries.[195–197]

All approaches have their advantages and disadvantages and it is important to keep in mind that different vascular beds are examined. The basic principle, however, is similar: healthy arteries such as the coronary or brachial arteries dilate in response to reactive hyperaemia (flow-mediated vasodilatation) or after pharmacological stimuli, including intra-arterial infusion of endothelium-dependent vasodilators such as acetylcholine, bradykinin, or serotonin, via release of NO and/or other endothelium-derived vasoactive substances.[198,199] In disease states, such endothelium-dependent dilatation is reduced or absent. Impaired endothelial-independent function, on the other hand, is associated with structural vascular alterations and alterations in smooth muscle cells rather than changes in the endothelium.

A comprehensive assessment of the coronary microcirculation requires information on both the endothelium-dependent and independent coronary microcirculatory responses. CFR and IMR (described in the previous section), which require maximal hyperaemia, obtained primarily through adenosine vasodilator action on vascular smooth muscle cells, are potentially an incomplete measurement of coronary microcirculatory function, due to their inability to distinguish between endothelium-dependent and independent microcirculatory responses. In fact, the poor correlation between adenosine derived CFR and coronary endothelium-dependent microvascular function has been already demonstrated[200].

In the following pages, the main techniques available for the evaluation of both coronary and peripheral endothelial dysfunction are briefly described. EndoPAT, used in the current study, will be further detailed in Section 7.

## 6.1. Invasive evaluation of coronary endothelial function

### 6.1.1. Epicardial Endothelial Function

The first studies of endothelial function *in vivo* evaluated endothelium-dependent reactivity of large epicardial vessels.[194] In this model, different concentrations of acetylcholine and the

endothelium-independent vasodilator nitroglycerin were infused into the coronary arteries, followed by angiographic measurements of coronary vessels diameter. In patients with coronary atherosclerosis, acetylcholine infusion resulted in paradoxical vasoconstriction of epicardial vessels in contrast to coronary vasodilation observed in patients without documented cardiovascular disease. A normal vasodilator response to nitroglycerin suggests that a defect in endothelial vasodilator function, and not in smooth muscle cell reactivity, is responsible for the abnormal acetylcholine-induced coronary vasorelaxation.

In addition to endothelium-dependent pharmacological agents (like acetylcholine, bradykinin or substance P), increase in blood flow can be used to investigate the responsiveness of coronary arteries. For this purpose, endothelium-independent agents (such as adenosine and papaverine, which act primarily on the coronary microvasculature to induce its vasodilation and thereby increase blood flow) can be used, resulting in an increase in shear stress, endothelial nitric oxide release and consequent proximal (epicardial) arterial vasodilation (flow-mediated dilation). By infusing either adenosine or papaverine into the midportion of the coronary artery of interest, followed by the angiographic measurement of its diameter, at the site proximal to the infusion, flow mediated dilation can be assessed in the coronary vasculature.[201,202]

Finally, more “physiological” stimuli can be used, such as the cold pressor test and dynamic exercise. In the cold pressor test, sympathetic activation is achieved by immersing a patient’s hand and forearm in a slurry of iced water, followed by intracoronary blood velocities measurements and quantitative angiography.[203] The supine dynamic exercise test has been performed using a bicycle ergometer with continuous hemodynamic monitoring and obtaining repeated coronary angiograms at baseline (before exercise), at peak exercise, after exercise, and after intracoronary nitroglycerin infusion.[204]

### 6.1.2. Coronary microvascular function

The just described angiographic evaluation of changes in epicardial vessel diameter after infusion of acetylcholine (or other pharmacological agents) or flow mediated dilation (induced by adenosine or papaverine) primarily evaluates large coronary conduit vessels.

This assessment does not reflect the functional status of the coronary microcirculation that determines vascular resistance and, thus, blood flow to the myocardium. Endothelial function of the coronary microvasculature can be assessed by measuring changes in coronary blood flow, as described in Section 4 of this chapter.

This method involves placement of a Doppler wire into a coronary artery and measuring blood flow velocities after the infusion of endothelium-dependent (acetylcholine) and endothelium-independent (adenosine or papaverine) agents. Relative changes in coronary blood flow can be calculated by multiplying changes in mean coronary blood flow velocity by changes in the estimated vessel cross-sectional area (determined by quantitative angiography). This is a rather cumbersome and technically challenging procedure and

therefore rarely used outside a research setting. A pressure wire-based thermodilution technique was also validated for this[200], but its use both in clinical and reearch settings is also limited.

## 6.2. Non-invasive evaluation of peripheral endothelial function

Although measurements of the acetylcholine-induced and flow-mediated variations in coronary artery diameter allow the direct assessment of endothelial function in coronary arteries, several limitations restrict their widespread use. The invasive nature of these studies confines their use to patients undergoing coronary angiography for clinical reasons, but also limits the possibility of repeated evaluations.

Furthermore, measurements of coronary diameter, used in the assessment of endothelial function of large epicardial vessels, as well as in calculating changes in blood flow, are limited by the accuracy of coronary angiography and may pose technical difficulties in patients with atherosclerosis. Finally, and most importantly for the current study, this may not be a safe test to perform in acute patients, since the acetylcholine induced vasoconstriction would aggravate the ischemia and increase the risk of severe complications.

Alternatively, endothelial function can be assessed non-invasively by measuring vasodilator responses to interventions known to stimulate endothelial release of nitric oxide. Several methodologies have been developed to measure endothelial vasomotor function in humans [41,205], including ultrasound flow-mediated dilatation (that evaluates the change in brachial artery diameter), pulse wave analysis (change in augmentation index), pulse contour analysis (change in reflective index) and pulse amplitude tonometry (change in pulse amplitude).

Importantly, impaired endothelial responses, characteristically found in coronary arteries of patients with cardiovascular risk factors, have also been confirmed in different peripheral circulatory territories in these patients.[206–208] This has led to the concept of a generalized nature of endothelial dysfunction and has facilitated endothelial function testing in more accessible vascular beds.

### 6.2.1. Plethysmography of the forearm circulation

With this technique (which is actually semi-invasive, since it requires an arterial puncture), a catheter is placed into the brachial artery, and drugs are infused, in small concentrations, directly into the forearm circulation. Blood flow is measured noninvasively, by means of strain gauge plethysmography.[41]

It allows quantification of endothelium-dependent and endothelium-independent vasodilation (through infusion of acetylcholine or nitroglycerin). The dosages required have

limited systemic effects, allowing the contralateral limb to serve as an internal control. The results are expressed as the ratio of the changes in flow measured in both arms and are reproducible.[209] The technique is well suited to measure differences in blood flow to various stimuli or inhibitors in a single patient. However, because of different initial arterial pressures, forearm blood flow, different sizes of the forearm, and other factors, comparisons between groups or serial studies in the same patient are of limited value.[210]

### 6.2.2. Flow-mediated dilation (FMD) of brachial artery

The technique measures the ability of the arteries to respond with endothelial NO release during reactive hyperaemia (flow mediated) after a 5-minute occlusion of the brachial artery with a blood pressure cuff inflated, either above the antecubital fossa or on the forearm, to suprasystolic pressure (usually to ≥50 mm Hg above systolic pressure).

Two-dimensional images of the brachial artery and Doppler signals are acquired at baseline, before cuff inflation, and for 1 minute after cuff release (time of maximum vasodilation). FMD is the change in poststimulus diameter, usually expressed as a percentage of baseline diameter. In most studies, subjects are given a systemic vasodilator (usually a single dose of sublingual nitroglycerin) as a parallel experiment to assess endothelium-independent vasodilation[41].

Peripheral endothelial function as assessed by FMD correlates with coronary artery endothelial function.[206,208]

However, although the principle of this technique seems simple, its application is technically challenging. Study preparation, image acquisition and site selection, sphygmomanometer probe position, cuff occlusion time, accurate use of edge-detection software, and correct characterization of the FMD response are crucial.[211,212]

This technique is therefore highly dependent on the skills of the examiner. High-quality ultrasound images are essential for accurate analysis, typically requiring several months of hands-on training by experienced individuals, as well as continuous performance of the technique, to maintain optimal quality and consistency of the data.

### 6.2.3. Pulse wave analysis – applanation tonometry

Applanation tonometry is a method that involves positioning a tonometer over the maximal arterial pulsation of the artery under study (typically a superficial artery, such as a radial, brachial, and femoral) to minimally flatten or applanate the arterial wall. This normalizes the circumferential stresses in the arterial wall thereby allowing accurate recording of the pressure waveform through the changes of the electrical resistance of a piezoelectric crystal

within the tonometer.[213] The pulse-waveform shape obtained provides information about arterial compliance (including the augmentation index, a ratio between the pulse pressure at the second systolic peak and the pulse pressure at the first systolic peak, which is commonly used as a measure of arterial stiffness) and about endothelial function (by measuring the changes in the peripheral pressure waveform in response to β-2 adrenergic stimulation).[214,215] However, this technique has rarely been used to investigate endothelial dysfunction in clinical settings.

### 6.2.4. Peripheral (pulse) arterial tonometry

In addition to applanation tonometry, pulse wave amplitude of the peripheral microvasculature can be assessed by measuring changes in digital pulse volume using a finger photopletysmograph (pulse contour analysis).[216]

Reactive hyperemia peripheral artery tonometry is a recent development of this technique, which uses plethysmography to record digital volume changes accompanying pulse waves.[217] Its fundaments and clinical evidence will be further detailed in the next section.

### 6.2.5. Laser Doppler flowmetry of the skin

Laser Doppler flowmetry (LDF) is a technique that enables the monitoring of skin microvascular blood flow.[218] The assumption is that the response observed in the cutaneous circulation is a window towards the responses that would be observed in other vascular beds.[219] During LDF, the original beam of coherent light changes in contact with moving tissues (red blood cells) and a photodiode measures the emerged beam. The fraction of shifted light depends on the concentration of moving red blood cells, whereas the magnitude of the frequency broadening depends on their average velocity.[220]

LDF has been used to evaluate endothelial function of the skin microvasculature using postocclusive hyperemia, local thermal hyperemia, and acetylcholine iontophoresis. Despite being noninvasive and, therefore, attractive for routine clinical and research use, these techniques have certain limitations. Firstly, because the skin is a critical thermoregulatory organ, there are extreme variations in basal blood flux, which, in turn, dictate the need to use maximal vasodilatation (by either local warming of the skin or local sodium nitroprusside infusion) to normalize submaximal flux values. Secondly, poor interassay and intra-assay reproducibility and lack of standardization (e.g., site of the skin measurement) limit within-patient and across-studies comparisons. Thirdly, and most importantly, recent insights into the mechanisms of the postocclusive hyperemia and acetylcholine-mediated dilatation indicate that these phenomena are not primarily NO mediated, suggesting that they might represent a summation of complex, microvascular responses involving sensory nerves and

metabolic and endothelial vasodilators (independent from NO). Therefore, rather than representing specific markers of endothelial function, these tests provide a more global form of assessing microvascular function.[41]

### 6.2.6. Biochemical biomarkers

**Markers of coagulation/thrombosis**

The plasma levels of several procoagulant mediators have been shown to increase with endothelial damage, suggesting that they could represent reliable markers of endothelial dysfunction. Furthermore, a change in the production of these molecules by the endothelium could directly contribute to atherotrombotic disease. These markers include, among others, von Willebrand factor, tissue plasminogen activator (t-PA) and PAI-1. However, prospective epidemiological studies aiming to evaluate the association between plasma levels of different hemostatic markers and the risk for cardiovascular disease are relatively sparse and often have inconclusive results.[41]

**Markers of inflammation**

Strong evidence suggests that atherosclerotic risk factors are often associated with systemic inflammation, which is a key player in the development and progression of atherosclerosis.[106] C-reactive protein, in particular, has emerged as a potential marker for cardiovascular risk. It can be measured with several standardized, validated, and inexpensive high-sensitivity assays and is the only biomarker ready for clinical use, adding predictive value above the currently established risk factors, both in stable and unstable patients.[41] However, it is affected by several mechanisms and factors, which limits its use as a marker of endothelial function.

**Other markers of endothelial dysfunction**

Asymmetrical dimethylarginine (ADMA) and oxidized low-density lipoprotein (oxLDL) are two other markers which have been investigated as indicators of endothelial function. However, despite initial promising results, suggesting an association with cardiovascular disease and risk, both had more recent conflicting results and there are doubts if they represent reliable substitutes for direct endothelial function measurement.[211]

Endothelial microparticles and bone-marrow derived endothelial progenitor cells have also been widely investigated as markers of endothelial dysfunction, with promising results, but they remain mostly investigational tests.[211]

Finally, baseline plasma levels of several cellular adhesion molecules (CAMs), including intercellular adhesion molecule-1 and E-selectin, have been shown to be associated with increased cardiovascular risk in generally healthy population. In patients with coronary artery

disease, elevated circulating vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 values were also predictors of adverse outcome. However, the results from other large studies indicated that, after adjustments for other cardiovascular risk factors, the association of CAMs with coronary heart disease is not statistically significant.[41]

# 7. Peripheral (pulse) arterial tonometry (EndoPAT)

## 7.1. Principle and methodology

Reactive hyperemia peripheral artery tonometry uses plethysmography to record digital volume changes accompanying pulse waves.[217] On the basis of this principle, a finger pneumatic plethysmographic cuff, providing a ‘‘beat to beat’’ blood flow volume assessment by recording finger (peripheral) arterial pulsatile (PAT) volume changes, was developed by Itamar Medical, and made commercially available under the name EndoPAT 2000® (Figure 9, page 95).

It consists of a fingertip plethysmograph capable of sensing volume changes in the digit with each arterial pulsation. The fingertip probe has a rigid external casing containing inflatable chambers and the uniformly applied pressure field across the finger prevents venous pooling and partially unloads arterial wall tension (Figure 10, Page 96). Volume changes in the fingertip are recorded digitally as pulse amplitude that can be tracked over time.

Endothelial function testing with PAT is based on the same physiological mechanisms as the FMD technique, inducing transient ischemia in the upper limb as a stimulus for reactive vasodilatation:

- A pressure cuff is placed around the arm and inflated above systolic pressure after a baseline recording
- The cuff is then deflated after 5 minutes to induce reactive hyperemia in one arm (a main advantage of the system is that the contralateral arm serves as an internal control).
- The ratio between hyperaemic and baseline pulse volume analysis (PAT ratio) is normalized for the same ratio in the contralateral arm, thus obtaining the reactive hyperemia index (RHI), or its natural logarithm (L_RHI).[221]

This index is a validated marker of endothelial function – a value below 1.67 (or ≤0.51 for L_RHI) is considered suggestive of endothelial dysfunction. The reproducibility of the procedure was also clearly established.[222–224]

The technical details of the EndoPAT procedure are further detailed in Section 3.9 of the Methods Chapter (Page 95).

Unlike flow-mediated dilation evaluation, the PAT technique is operator-independent, and the pulse amplitude recordings are digitized and analysed by an automated, proprietary algorithm.

## 7.2. Clinical evidence on the use of EndoPAT in coronary artery disease

The role of EndoPAT in the evaluation of patients with coronary artery disease is supported by the study performed by Bonetti and colleagues, in which they showed that a lower PAT hyperaemic response is significantly correlated with the presence of coronary endothelial dysfunction measured by acetylcholine response[207] in patients undergoing coronary angiography. This study confirms previous evidence endorsing the concept of a generalized nature of endothelial dysfunction[206,208] and supports the use of endothelial dysfunction evaluated by EndoPAT as a surrogate for coronary endothelial dysfunction.

Additionally, EndoPAT has been evaluated in different stages of coronary artery disease:

- In patients **without known/suspected coronary artery disease**, PAT hyperaemic ratio is progressively lower with increasing burden of cardiovascular risk factors[225–227] and several studies confirmed that endothelial dysfunction evaluated with EndoPAT is related both to the risk of developing coronary disease and the risk of cardiac events. In fact, as described in Section 3.3 above and also confirmed with this technique, the presence of endothelial dysfunction appears to have an incremental prognostic value, after control for these classical risk factors, which may be explained by the fact that it reflects the overall burden of risk and significantly predicts the risk of cardiovascular events.

  The main prognostic studies performed with EndoPAT to evaluate the risk of events in patients without coronary artery disease are presented in Table 7.

- In patients with **known stable coronary artery disease**, endothelial dysfunction evaluated by EndoPAT was related with the risk and severity of the disease and to the characteristics of coronary plaques (Table 8).

  EndoPAT was also used to evaluate the effect of enhanced external counter pulsation in patients with stable angina and refractory complaints.

  Additionally, three long-term studies performed with EndoPAT confirmed its additional and independent value in predicting events in patients with coronary artery disease (Table 9).

- Finally, in patients with **acute coronary syndromes and ST elevation myocardial infarction**, evidence with EndoPAT includes studies predicting the risk of in-stent restenosis, initial patency of the culprit artery, angiographic severity of the coronary artery disease and the extension of infarction in STEMI patients (Table 10). These two last studies were performed in our centre, and they will be further described below, since they were essential for the design of this thesis protocol.

**Table 7 – Prospective studies investigating the predictive role of peripheral arterial tonometry in patients with suspected coronary artery disease or other clinical settings**

| Author, Year | No. of patients | Clinical Context | FUP duration | Clinical endpoints | Results |
|---|---|---|---|---|---|
| Rubinstein (2010)[129] | 270 | Suspected CAD | 5.8 years | CV death, nonfatal MI, coronary revascularization or CV hospitalization | Low L_RHI(<0.4) associated with higher rate of events (48% vs. 28%, p=0.03)<br>L_HRI was an independent predictor of adverse events (HR 1.79 CI95 1.16-2.76, *P*=0.008) |
| Akiyama (2012)[275] | 321 | Heart failure with preserved ejection fraction | 20 months | CV death, nonfatal MI, unstable angina pectoris, nonfatal ischemic stroke, coronary revascularization, hospitalization for HF | RHI (HR 0.80, 95% CI 0.67-0.94, *P*=0.007, for an increase in RHI of 0.1) was an independent predictor of events.<br>RHI improved the prediction of events (C-statistics from 0.671 to 0.712) when added to 5 other risk factors (age, diabetes, NYHA classification, heart failure hospitalization history, and LVEF) |
| Matsue (2013)[276] | 159 | Heart failure with preserved ejection fraction | 300 days | HF-related death, hospitalization for HF | L_RHI (HR 0.56, 95% CI: 0.39-0.80 for an increase of 0.1) was an independent predictor of HF-related events.<br>AUC of ROC curve for L_RHI was 0.73 (95% CI: 0.62–0.83)<br>L_RHI 0.49 was suggested as the optimal cut-off value for prediction of adverse events in this population. |
| Matsuzawa (2013)[277] | 528 | Suspected CAD | 2.8 years | CV death, nonfatal MI, unstable angina, nonfatal ischemic stroke, coronary revascularization, hospitalization for HF, nonfatal aortic disease, and peripheral arterial disease | RHI (HR 0.761 95%CI 0.673-0.859; P<0.0001, for an increase of 0.1) was an independent predictor of events<br>RHI improved the prediction of events (C-statistics from 0.728 to 0.766) when added to 3 other risk factors (FRS, SYNTAXsc and BNP) |
| Hirata (2014)[278] | 383 | Chronic kidney disease | 30 months | CV death, non-fatal MI, unstable angina, nonfatal ischemic stroke, coronary revascularization, hospitalization for HF | Low L_RHI (HR 2.70, 95% CI: 1.62-4.51, p<0.001) was an independent predictor of events<br>RHI improved the prediction of events (C-statistics from 0.49 to 0.62) when added to FRS. |

**Table 8 – Prospective studies investigating peripheral arterial tonometry in patients with stable coronary artery disease**

| Author, Year | No. of patients | Clinical Context | Objectives | Results |
|---|---|---|---|---|
| Bonetti (2003)[222] | 23 | Stable CAD, refractory angina | Effect of enhanced external counterpulsation (EECP) | RHI significantly increased only in patients that clinically improved (less symptoms) with EECP |
| Bonetti (2010)[323] | 140 | Stable CAD | Prediction of obstructive and non-obstructive CAD | RHI significantly attenuated in both obstructive (median 1.57, IQR 1.42-1.76) and non-obstructive CAD (median 1.58, IQR 1.41-1.78), as compared to non-IHD (2.15, IQR 1.85-2.48, p < 0.001).<br>RHI (HR 0.51 95%CI 0.38-0.68; P<0.001) was an independent predictor of CAD<br>AUC ROC curve for prediction of CAD: 0.86, p<0.001 |
| Heffernan (2010)[324] | 42 | Stable CAD | Identification of patients with high risk CAD (elevated hs-CRP and Lp-PLA2) | RHI significantly lower in the high-risk group (1.3 ± 0.04) compared to the moderate-risk (1.6± 0.07, P < 0.05) and low-risk (2.0± 0.1, P < 0.05).<br>RHI was a significant independent predictor of high risk CAD |
| Schonenberger (2012)[267] | 362 | Stable CAD | IVUS-assessed coronary plaque structure. | Patients with RHI<1.67 had greater plaque burden (41% vs 39%, p = 0.047)<br>RHI significantly associated with fibrous and fibrofatty plaques and with necrotic core and dense calcium volumes |

**Table 9 – Prospective studies investigating the predictive role of peripheral arterial tonometry in patients with established coronary artery disease**

| Author, Year | No. of patients | Clinical Context | FUP duration | Clinical endpoints | Results |
|---|---|---|---|---|---|
| Suessenbacher (2014)[325] | 96 | Stable CAD | 44 months | Revascularization, acute coronary syndrome, ischemic stroke, cardiovascular death, repeat coronary angiography due to chest pain | Risk similar in patients below median RHI. However, the combined endpoint occurred earlier in the patients with an RHI within the 1st tertile (11.3±11.0 vs. 27.5±18.6 months for patients in the 2nd/3rd tertile, p=0.03) |
| Matsue (2014)[326] | 213 | Stable CAD, under statin treatment, LDL-C< 100 mg/dl | 2.7 years | Angina pectoris requiring coronary revascularization, recurrent angina pectoris with proven myocardial ischemia, non-fatal MI, and death from CAD | L_RHI (HR 0.79, 95% CI: 0.66-0.95, p=0.012) was an independent predictor of events<br>RHI improved the prediction of events (C-statistics from 0.60 to 0.77) when added to risk factors and estimated glomerular filtration rate. |
| Tabata (2015)[327] | 435 | PCI patients with chronic kidney disease (CKD) | 2.5 years | Cardiovascular death, nonfatal myocardial infarction, ischemic stroke, hospitalization due to unstable angina pectoris, and coronary revascularization | L_RHI lower in patients with events in the non-CKD group (0.46 ± 0.18 versus 0.60 ± 0.25; P = 0.002)<br>L_RHI (HR: 0.096; 95 % CI 0.02–0.47; P = 0.004) was an independent predictor of cardiovascular events in the non-CKD group |

**Table 10 – Prospective studies investigating peripheral arterial tonometry which include patients with acute coronary syndromes or ST elevation myocardial infarction**

| Author, Year | No. of patients | Clinical Context | Objectives | Results |
|---|---|---|---|---|
| Baptista (2013)[19] | 231 | Stable angina and ACS patients | Severity of CAD | RHI was progressively lower as CAD severity increased (from no disease to 3 vessel disease: 1.98±0.46 -> 1.86±0.46 in 1 vessel -> 1.85±0.43 in 2V -> 1.60±0.39 in 3V, p=0.003)<br>RHI (HR=0.16 for each unit of RHI reduction , 95%CI 0.04-0.68, p=0.013) was independent predictor of severity of CAD |
| Yamamoto (2014)[268] | 86 | STEMI patients | Risk of in-stent restenosis (ISR) | RHI at 8 months significantly lower in the patients with ISR (1.75 vs. 2.12; p=0.03).<br>RHI (OR: 4.23, 95% CI 1.25-14.28, p=0.02) was a significant risk factor for ISR |
| Kandhai-Ragunath (2014)[269] | 71 | STEMI patients | Initial patency of culprit artery | RHI significantly higher if culprit vessel patent before P-PCI (2.08±0.34 vs. 1.75±0.35; p<0.007).<br>RHI (OR 7.1, 95% CI 2.1-23.6) was an independent predictor of culprit vessel patency |
| Baptista (2016)[18] | 58 | STEMI patients | Extension of infarction (peak TnI) | Patients with an RHI<1.67 had significant larger infarcts (TnI 73.5 [IQR 114.42] vs. 33.2 [IQR 65.2 ng/mL]; p =0.028)<br>RHI was an independent predictor of peak TnI values |
| Komura (2016)[328] | 249 | Stable angina and ACS patients | Risk of in-stent restenosis | RHI_L at follow-up lower in patients with ISR (0.52±0.23 versus 0.65±0.27, P<0.01)<br>RHI_L (OR 0.13; 95% CI: 0.04-0.48; P=0.002) was a significant independent predictor of ISR |

### 7.2.1. Severity of coronary artery disease and endothelial dysfunction evaluated by peripheral arterial tonometry[19]

With the purpose of evaluating the prevalence of endothelial dysfunction by severity of coronary artery disease, 231 patients referred for diagnostic angiography and 39 control subjects, were evaluated by peripheral arterial tonometry (EndoPAT) in our department. The severity of coronary artery disease was defined as the number of vessels with disease (lesions >70%).

Of the 231 patients, 92 (39.8%) had no relevant disease ("normal" coronaries), 78 (33.8%) had 1 vessel disease, 37 (16.0%) had 2 vessel disease and 24 (10.4%) had 3 vessel disease. Mean RHI values in the control group were 2.10±0.63. In catheterised patients, RHI was progressively lower as CAD severity increased: 1.98±0.46, 1.86±0.46, 1.85±0.43 and 1.60±0.39, respectively in patients with "normal" coronaries, 1, 2 and 3 vessels disease (p=0,003). Since patients with 1 and 2 vessels had similar RHI results, they were merged in one group for the analysis performed (Figure 3, Table 11).

**Table 11 – Patient characteristics according to the severity of coronary artery disease**

| | Controls | "Normal" coronaries | 1-2 vessel disease | 3 vessel disease | *P* value [c] |
|---|---|---|---|---|---|
| **Patients** [a] | **39** | **92 (39.8%)** | **115 (49.8%)** | **24 (10.4%)** | - |
| **Reactive hyperemia index** [b] | 2.08 ± 0.63 | 1.98 ± 0.46 [d] | 1.85 ± 0.45 | 1.60 ± 0.39 | 0.001 |
| **Physical characteristics** | | | | | |
| Age (years) [b] | 39.5± 12.8 | 59.5 ± 13.7 [†] | 60.3 ± 13.7 | 64.2 ± 13.1 | ns |
| Male gender [a] | 17 (40.5%) | 44 (47.8%) [††] | 87 (75.7%) | 20 (83.3%) | <0.001 |
| Body mass index [b] | 25.4 ± 5.6 | 27.2 ± 4.1 [†] | 27.2 ± 4.4 | 28.3 ± 3.9 | ns |
| Heart rate [b] | 66.2 ± 9.2 | 65.0 ± 11.7 [†] | 65.6 ± 11.2 | 69.1 ± 14.4 | ns |
| Systolic blood pressure [b] | 114.1 ± 14.4 | 121.7 ± 17.3 [†] | 116.7 ± 20.8 | 118.4 ± 22.6 | ns |
| **Risk Factors** [a] | | | | | |
| Diabetes | 0 (0.0%) | 13 (14.2%) | 28 (24.3%) | 8 (33.3%) | ns |
| Hypertension | 6 (14.3%) | 66 (72.5%) | 77 (67.0%) | 18 (75.0%) | ns |
| Dyslipidaemia | 12 (28.6%) | 48 (52.2%) | 54 (47.0%) | 14 (58.3%) | ns |
| Smoking | 9 (21.4%) | 20 (22.2%) [‡] | 47 (40.9%) | 9 (21.4%) | 0.018 |

[a] presented as N (%); [b] Presented as mean±standard deviation: [c] *p*-value for the comparison normal coronaries vs. 1-2 vessel disease vs. 3 vessel disease: One-Way ANOVA for continuous variables and Chi-Square for categorical variables. [d] *p*=0.002 (One-Way ANOVA; Controls vs. normal coronaries vs. CAD)

Three vessel disease was more prevalent in male patients (in the unadjusted analysis). Patients with multivessel disease also tended to be older and more often diabetics. The other characteristics were similar between groups.

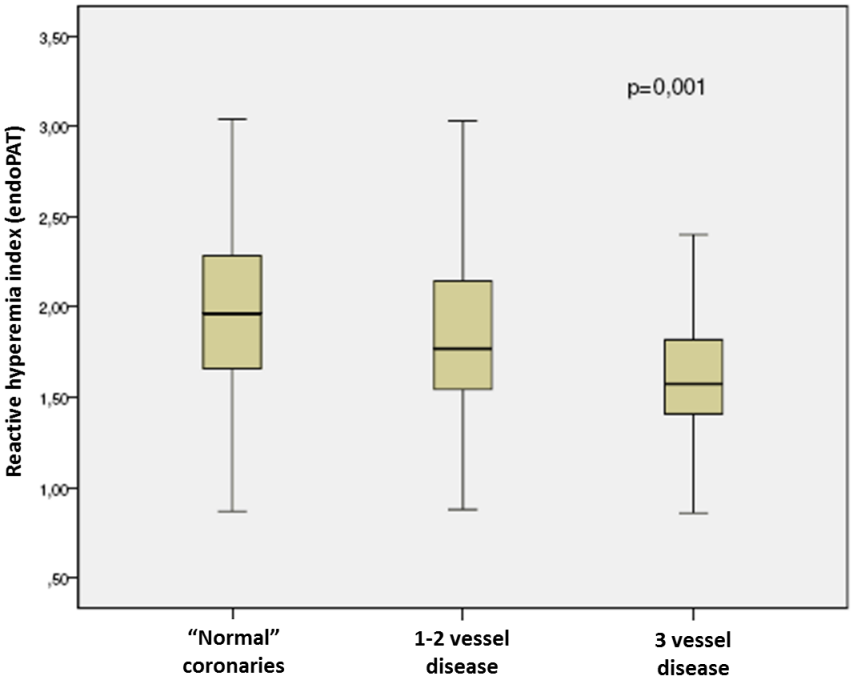



**Figure 3 – Reactive hyperemia index according to the severity of the coronary artery disease (number of major vessels with lesions >70%)**

In the univariate analysis, comparing 3-vessel disease with 1 or 2-vessel disease, RHI and non-ST acute coronary syndrome as indication for the angiography (vs. ST elevation myocardial infarction) were the strongest predictors of the severity of disease (Table 12).

However, when this analyses was adjusted (for age, gender, previous diabetes mellitus or dyslipidaemia, BMI and waist circumference), only RHI was independently associated with the diagnosis of 3 vessel disease (Table 13).

**Table 12 – Univariate analysis of predictors of 3 vessel disease in the population with documented coronary artery disease**

| Univariate analysis | 1-2 Vessels | 3 Vessels | RR | 95% CI | *P* value |
|---|---|---|---|---|---|
| Reactive hyperemia index | 1,85±0,45 | 1,60±0,39 | | | 0,014 |
| nST-ACS (vs STEMI) | 44,3% | 70,8% | 2,54 | 1,12-5,73 | 0,016 |
| Gender (male) | 75,7% | 83,3% | 1,61 | 0,51-5,11 | 0,301 |
| Dyslipidaemia | 47,0% | 58,3% | 1,58 | 0,65-3,85 | 0,215 |
| Diabetes mellitus | 24,3% | 33,3% | 1,55 | 0,60-4,02 | 0,251 |
| Hypertension | 67,0% | 75,0% | 1,48 | 0,54-4,03 | 0,304 |
| Age (per year) | 60,3±13,6 | 64,2±13,1 | | | 0,193 |
| Waist circumference (cm) | 98,5±13,0 | 104,6±16,3 | | | 0,051 |

nST-ACS: non-ST acute coronary syndrome; STEMI: ST elevation myocardial infarction

**Table 13 – Multivariable analysis of predictors of 3 vessel disease in the population with documented coronary artery disease**

| Multivariate analysis | RR | 95% CI | *P* value |
|---|---|---|---|
| RHI (each unit) | 0,18 | 0,04-0,73 | 0,016 |
| Gender (male) | 3,27 | 0,86-12,4 | 0,082 |
| Presentation = nST ACS | 2,27 | 0,78-6,63 | 0,132 |
| Dyslipidaemia | 1,29 | 0,46-3,61 | 0,630 |
| Diabetes mellitus | 1,23 | 0,42-3,60 | 0,708 |
| Waist circumference (each cm) | 1,03 | 0,99-1,07 | 0,106 |
| Age (each year) | 1,02 | 0,98-1,06 | 0,431 |

nST-ACS: non-ST acute coronary syndrome;

Based on these results, we conclude that endothelial dysfunction assessed by PAT is related with the severity of coronary artery disease. Therefore, we hypothesized that endothelial function may have a continuous role in the atherogenic process and, consequently, its noninvasive evaluation might be useful not only to predict the risk of CAD, but also in patients with disease already established.

### 7.2.2. Endothelial dysfunction evaluated by peripheral arterial tonometry is related with peak TnI values in patients with ST elevation myocardial infarction treated with primary angioplasty[18]

This study was published in Microvascular Research. A copy of the complete published paper can be found in the Appendix.

In summary, our purpose was to evaluate the impact of endothelial dysfunction on peak Troponin I (TnI) values, as a surrogate for the extension of myocardial infarction, in patients with ST elevation myocardial infarction treated with primary angioplasty.

Fifty-eight patients (mean age 59.0±14.0 years, 46 males) were included. Endothelial function was assessed by reactive hyperaemia index (RHI) as determined by PAT. Patients were divided in two groups according to the previous reported RHI threshold for high risk (1.67). The extension of myocardial necrosis was evaluated by peak TnI levels.

RHI median value was 1.78 (IQR 0.74); 25 patients had endothelial dysfunction (RHI<1.67). The two groups had no significant differences in age, gender, main risk factors and pain-to-balloon time.

Patients with an RHI<1.67 had significant larger infarcts: TnI 73.5 ng/mL (IQR 114.42 ng/mL) versus TnI 33.2 ng/mL (IQR 65.2 ng/mL); p=0.028. Significant differences were also found in peak Creatine-kinase and its Mb fraction (Figure 4, Table 14). On multivariate analysis, the presence of an RHI<1.67 kept a significant impact on TnI peak values (p=0.02).

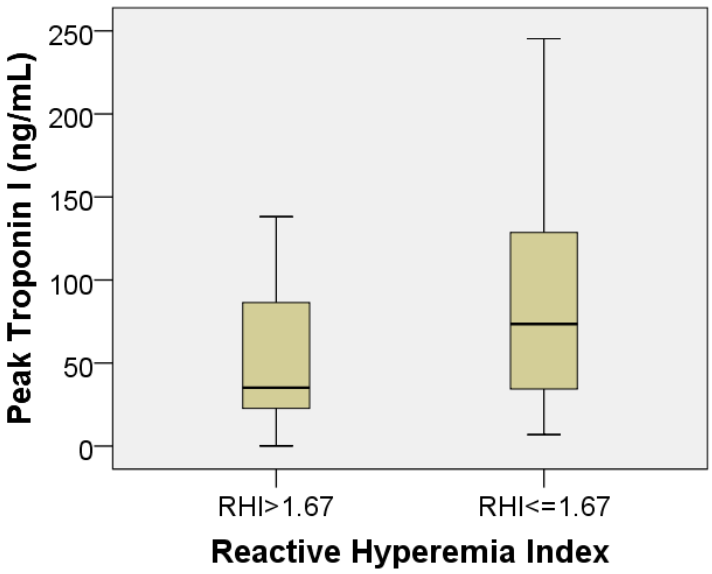



**Figure 4 – Peak Troponin I values according to the presence of endothelial dysfunction (RHI<1.67) in patients with ST elevation myocardial infarction treated with primary PCI**

Importantly, the presence of endothelial-dependent dysfunction was related to peak TnI values even when other factors that affect the extension of myocardial infarction (e.g. age, risk factors, pain-to-balloon time, culprit artery and Killip class at admission) were taken into account. Therefore, since endothelial-dependent function plays a crucial role in vascular tone and coronary blood flow regulation, we hypothesized that endothelial dysfunction can play a role not only in the development of coronary artery disease, but also in the pathophysiological processes occurring in the microcirculation before and during STEMI.

**Table 14 – Cardiac enzymes peak values, according to the presence of endothelial dysfunction (RHI<1.67)**

| | Total Population (n=58) | RHI<1.67 (n=25) | RHI ≥ 1.67 (n=33) | *P* value |
|---|---|---|---|---|
| **Peak TnI (ng/mL)** | 50.3 (68.1) | 73.5 (107.1) | 35.2 (64.3) | 0.028 † |
| **Peak CK (UI/L)** | 1.586 (1.938) | 1.909 (2.181) | 1.227 (1.758) | 0.045 † |
| **Peak CK-Mb (UI/L)** | 252 (240) | 303 (312) | 224 (186) | 0.032 † |

Data presented as median (interquartile range); † Mann-Whitney

# 8. Summary of the evidence underlying this research – evaluation of endothelial dysfunction in the early acute phase in STEMI patients

Primary angioplasty is a very effective treatment for ST elevation myocardial infarction and major improvements were obtained with dedicated primary PCI programs that significantly reduced ischemic times. However, despite a normal epicardial coronary artery flow is almost always achieved after primary PCI in a timely fashion, a significant proportion of patients has a worse outcome due to microvascular malperfusion.

The negative prognostic implications (both on the risk of left ventricle remodelling and on the risk of hard endpoints, including death) associated with coronary microvascular damage has been repeatedly confirmed, with several invasive and non-invasive indicators. However, it is not clear if this microvascular dysfunction is a *consequence* of myocardial infarction (by distal embolization of thrombi, secondary endothelial dysfunction and/or reperfusion injury) or if it is *part of the pathophysiological process* that leads to and aggravates myocardial infarction.

Endothelial function seems to be at the core of all the events occurring in myocardial infarction patients. In fact, there is accumulating evidence that endothelial dysfunction is not just a risk factor and precursor of coronary artery disease, but it also plays a central role leading to acute coronary syndromes and ST elevation myocardial infarction. It is therefore licit to speculate that previous endothelial dysfunction or a blunted reaction of the endothelial mechanisms during ST elevation myocardial infarction may be a central component of coronary microvascular dysfunction in these patients and, as a consequence, a determinant of microvascular reperfusion, infarction extension and left ventricle remodelling.

Ideally, this hypothesis would best be proved if endothelial function evaluation had been performed *before* the myocardial infarction, which evidently is not possible. Actually, most studies evaluating endothelial function in acute coronary syndrome patients were performed relatively *late* (several days or weeks) after the onset of the event. As such, the worse results documented in patients with endothelial dysfunction may just be a consequence of larger and more complicated infarcts.

In fact, endothelial function testing is particularly challenging in acute patients: the unpredictable nature of the acute event onset, the unstable condition of the patient and the early dramatic impairment in microvascular function make it difficult to obtain and interpret data concerning vascular reactivity in this context.

Still, measuring endothelial function as early as possible after the onset of the ST elevation myocardial infarction could help clarify this question. Assuming peripheral endothelial function (which is much more easy to measure) as a surrogate of coronary endothelial function, we would expect, if the above theory is true, worse acute endothelial dysfunction, worse microvascular reperfusion and worse left ventricle remodelling in patients with larger infarctions. This is the central idea of this thesis.

The recent availability of a non-invasive, reproducible and non-operator dependent technique for endothelial function evaluation – peripheral arterial tonometry (EndoPAT®), together with the development of a new invasive tool for the assessment of coronary microcirculation – the index of microcirculatory resistance – opened new opportunities to try to understand what is happening in the microcirculation in ST elevation myocardial infarction.

# STUDY OBJECTIVES

## Study Hypothesis

The study´s **main hypothesis** is: In patients with acute ST elevation myocardial infarction treated with primary PCI, endothelial dysfunction (evaluated by peripheral arterial tonometry) is related to the extent of microvascular damage and, consequently, to the extent of myocardial necrosis.

Based on this hypothesis, the study **primary aim** is to evaluate the relation between endothelial dysfunction (evaluated with EndoPAT) and coronary microvascular dysfunction (evaluated by IMR immediately after primary revascularization).

**Secondary aims** include both *confirming* IMR and *evaluating* endothelial dysfunction (as measured by peripheral arterial tonometry) as predictors of microvascular dysfunction and extension of the myocardial infarction.

## Study Outcome Measures

The **primary outcome measure** is the IMR value. Since there are no clearly defined values for abnormal endothelial function in coronary artery disease patients, two pre-specified evaluations of this primary outcome measure were defined:

1. IMR value in patients with endothelial dysfunction according to the prevailing threshold defined for EndoPAT (RHI <1.67 versus ≥1.67)
2. IMR value in patients according to the tertile of RHI

**Secondary outcomes** of the study are:

1. The relation between RHI values and
   a) The extent of myocardial necrosis, evaluated by troponin release (in the first 48 hours), echocardiographic parameters (both measured acutely and at 3 months) and ceCMR;
   b) The extent of microvascular reperfusion indicators, including angiographic indicators (cTFC and TMPG), ECG (ST resolution), and ceCMR (microvascular obstruction).

2. The relation between IMR values and
   a) The extent of myocardial necrosis, evaluated by troponin release (in the first 48 hours), echocardiographic parameters (both measured acutely and at 3 months) and ceCMR;
   b) The extent of microvascular reperfusion indicators, including angiographic indicators (cTFC and TMPG), ECG (ST resolution), and ceCMR (microvascular obstruction)

# POPULATION AND METHODS

# 1. Type and Location of the Study

Observational, prospective, single centre, cohort study, performed in the Cardiology Department of Hospital Prof. Doutor Fernando da Fonseca (Amadora, Portugal).

# 2. Population

All patients admitted to Hospital Prof. Doutor Fernando da Fonseca with a first acute ST elevation myocardial infarction, treated with primary angioplasty, were considered for inclusion in the study, according to the inclusion and exclusion criteria defined below.

## 2.1. Inclusion Criteria

- Age > 18 years.
- First ST elevation acute myocardial infarction, defined by:
  - Chest pain lasting at least 20min and /or
  - ST segment elevation of at least 1 mm in two or more contiguous leads
- Pain to balloon time < 6 hours (or between 6 and 12 hours if clearly with ongoing pain)
- Primary PCI performed with success on the culprit lesion (with no significant residual stenosis, independently of the final TIMI flow) in a native coronary artery.
- Informed consent obtained

## 2.2. Exclusion Criteria

- Patients presenting with left bundle-branch block and patients with implanted pacemaker – excluded since ST resolution could not be evaluated.
- History of previous of myocardial infarction (either ST elevation or non ST elevation MI) – excluded in order to reduce the bias in the ceCMR and in the microvascular tests.
- Patients with clear retrograde circulation to the infarct related artery (Rentrop ≥2).
- Killip class IV (cardiogenic shock) on presentation or during the primary PCI procedure
- Patients with known myocardial diseases (such as hypertrophic cardiomyopathy or restrictive cardiomyopathies) and patients with severe left ventricular hypertrophy (wall thickness > 15 mm) – were excluded in order to reduce the bias in the ceCMR and in the microvascular tests.
- Previous coronary artery bypass surgery.
- Percutaneous revascularization in the last 3 months.

- Long QT syndrome, 2nd or 3rd degree heart block and sick sinus syndrome (due to the risk of severe bradycardia/arrhythmias induced by adenosine)
- Patients in atrial fibrillation (if ceCMR was to be performed) or with other arrhythmias considered by the investigator as serious enough to contra-indicate the use of adenosine immediately after the primary angioplasty.
- Severe asthma or chronic pulmonary obstructive disease (due to the risk of bronchospasm induced by adenosine)
- Previous severe reaction to adenosine or any other contraindication to adenosine, including systolic blood pressure < 90 mmHg or bradycardia deemed to be significant by the operator.
- Presence of any serious non-cardiac disease associated with a life expectancy of less than 12 months
- Inclusion in other trials/studies

## 2.3. Sample

Since there were no clearly defined "normal" values both for RHI and IMR in patients with acute myocardial infarction, sample calculation was a difficult task to accomplish. Our previous work with EndoPAT in patients with ST elevation myocardial infarction[18], showed that RHI values lower than 1.67 are associated with larger infarcts (measured by peak troponin I release). Values lower than 1.67 were present in approximately 30% of patients (unpublished data), which translates into an "exposed/non-exposed" ratio of 2.3. In patients with RHI lower than 1.67, approximately 70% had larger infarcts (peak troponin I > 50 ng/dL).

Using the OpenEpi sample size calculator (Kelsey method) [228], for a two-sided significance level (α) of 0,05, a power (1-β, % chance of detecting) of 80 and assuming a proportion with disease (larger infarcts) in patients with RHI > 1.67 ("non-exposed") of 30%, the sample size would be 58 patients (18 "exposed" + 40 "non-exposed").

# 3. METHODS

## 3.1. Clinical information

Data concerning patient physical characteristics (age, gender, weight, height, body mass index, waist circumference), major risk factors (high blood pressure, diabetes, dyslipidaemia, smoking habits) and previous coronary/non-coronary atherosclerotic history (previous angina, previous percutaneous revascularization were collected in all patients included. Data on previous drug treatment was also registered.

## 3.2. Primary Percutaneous Coronary Intervention

Primary percutaneous coronary angioplasty was performed according to the recommended standards. The time of symptoms onset, time of hospital admission and time of first coronary intervention (thrombus aspiration, balloon dilation or stenting, whichever was first) was recorded, in order to allow the determination of ischemic times:

- "pain-to-hospital": time between the onset of symptoms and the first medical contact;
- "hospital-to-balloon": time between the first medical contact and the first coronary intervention; and,
- "pain-to-balloon": time between symptom onset and the first coronary intervention.

The treatment strategy (thrombus aspiration, direct stenting versus balloon pre-dilatation, balloon post-dilatation, type and number of stents, dose of non-fractionated heparin, use of GP IIb/IIIa inhibitors, dose and timing of clopidogrel, etc.) was left to the discretion of the primary operator. All these technical parameters were collected.

After the primary PCI, patients were admitted to the Intensive Cardiac Unit and offered standard care for patients with acute STEMI, including recommended pharmacological therapy (aspirin, clopidogrel/ticagrelor, beta-blockers, ACE inhibitors and statins), according to Portuguese[229] and European[1] guidelines.

## 3.3. Coronary angiography and area-at-risk scores

Angiography was performed according to usual procedures, by one of the 4 senior operators of the Interventional Cardiology Unit of Hospital Prof. Doutor Fernando da Fonseca. All operators were largely experienced in treating patients with acute ST elevation myocardial infarction.

The severity of coronary artery disease was evaluated by the Syntax Score.[230] Lesion segments were classified (as in the Syntax Score) based on the 16 segment definition proposed by the American Heart Association and modified for the ARTS I and II trials.[230]

The presence of collateral flow to the culprit artery was evaluated, using the Rentrop collateral flow classification[231]:

- Grade 0: None
- Grade 1: Filling of side branches of the artery to be dilated via collateral channels without visualization of the epicardial segment;
- Grade 2: Partial filling of the epicardial segment via collateral channels;
- Grade 3: Complete filling of the epicardial segment of the artery being dilated via collateral channels

Patients with a Rentrop flow 3 or 4 in the infarct related artery were not included, in order to reduce the bias in IMR measurement in this territory.

For each lesion, the area-at-risk was calculated, according to the segment involved and using two different scores: the APPROACH score and the BARI score. These 2 scores have been widely validated in clinical practice.[232–235]

### 3.3.1. Modified APPROACH Score

The Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease (APPROACH) score[236] is an angiographic score in which the left ventricle is divided into regions according to the percentage of myocardium supplied by a vessel or its branches. The area-at-risk for a given lesions is calculated taking into account the location of the culprit lesion, dominance and size of the secondary branches. The modified APPROACH score[232] simplifies this evaluation, using a table with values defined for each lesion location (Figure 5).

| Culprit lesion location | Infarct related artery side branches ➡ ⬇ | | Diagonal for LAD occlusion only or Posterolateral for all others | | |
|---|---|---|---|---|---|
| | | | Small or absent | Medium | Large |
| LAD (RD or LD) | | Distal | 13.75 | 14.8 | 15.9 |
| | | Mid | 27.5 | 29.7 | 31.8 |
| | | Proximal | 41.25 | 44.5 | 47.75 |
| Proximal LCx (RD) | OM | Small or absent | 9.25 | 12.5 | 15.75 |
| | | Medium | 15.25 | 18.5 | 21.75 |
| | | Large | 21.25 | 24.5 | 27.75 |
| Proximal LCx (LD) | PDA | Small or absent | 23.5 | 28 | 32.5 |
| | | Medium | 29.5 | 34 | 38.5 |
| | | Large | 35.5 | 40 | 44.5 |
| Mid LCx (LD) or RCA (RD) | PDA | Small or absent | 9.25 | 12.5 | 15.75 |
| | | Medium | 15.25 | 18.5 | 21.75 |
| | | Large | 21.25 | 24.5 | 27.75 |
| Mid LCx (RD) | | | 3.25 | 6.5 | 9.75 |

**Figure 5 – Modified APPROACH score for evaluating the area-at-risk**
(adapted from Ortiz-Perez et al[233])

### 3.3.2. BARI Score

The *Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index* (BARI) score was developed based on angiographic data from the BARI trial.[237] It assigns a score to all terminal arteries (terminal portion of the left anterior descending, left circumflex, and right coronary artery, as well as the ramus, diagonals, obtuse marginals, posterior descending and posterolateral branches) based on their length and calibre according to specific criteria. A value of 0 represents an almost insignificant vessel size, whereas a value of 3 defines a large-size artery with a length of two thirds the distance between the base and cardiac apex. Right ventricular marginals and posterior descending artery septal branches are not taken into account. The final score is obtained by dividing the resulting value from the infarct-related artery by the overall score of all arteries supplying the LV, which finally permits estimation of the percentage of myocardial muscle at risk.

An example of the calculation of both the APPROACH and BARI scores for a given patient is presented in Figure 6. The patient had a STEMI due to a proximal left anterior descending artery (LAD) occlusion. Since there was a reperfused diagonal branch of medium importance, the area-at-risk according to the APPROACH score (Figure 5) was 44.5%. For the BARI score, all left and coronary artery branches were classified from 1 to 3, according to their importance. The total value of branches distal to the occluded proximal LAD (total 11 points, corresponding to: distal LAD = 3 points, major diagonal branch = 3 points, 2 minor diagonal branches = 1 point each, 3 septal branches = 1 point each) was divided by the total points of the patient (26), resulting in an area-at-risk of 42.3%.

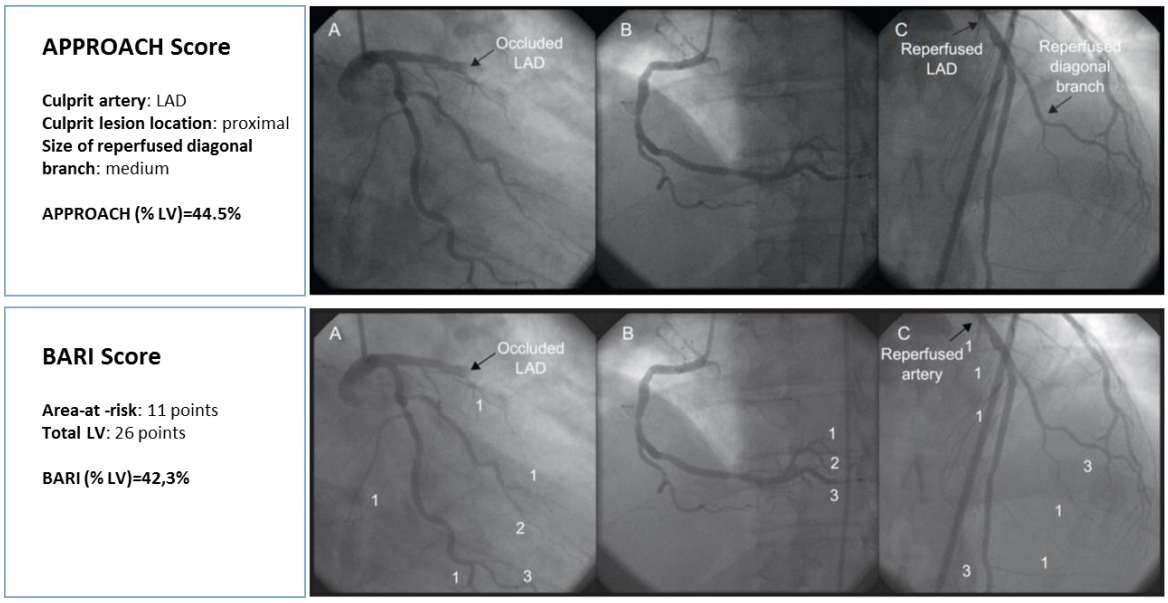



**Figure 6 – Area-at-risk calculation according to the APPROACH and BARI scores**
(adapted from Moral et al[234])

## 3.4. TIMI Flow, corrected TFC and TIMI myocardial perfusion grade

At the end of the primary PCI procedure, a final run of images of the culprit artery was registered, at 30 frames per second, in order to measure TIMI flow, TIMI frame count and TIMI perfusion grade. If necessary, the view was adjusted, so that the culprit vessel territory was not superimposed. The duration of cine filming was prolonged at least 3 cardiac cycles, to make sure that the entire washout phase was included. These three measures of flow were analysed offline by an operator blinded to other evaluations of the patient.

### 3.4.1. TIMI flow grade

TIMI flow grade was classified according to the standard definition[152]:

- **TIMI 3** (complete reperfusion): Anterograde flow into the terminal coronary artery segment through a stenosis is as prompt as anterograde flow into a comparable segment proximal to the stenosis. Contrast material clears as rapidly from the distal segment as from an uninvolved, more proximal segment. If there are difficulties in reproducibly assessing myocardial flow relative to other vessels, the modified definition of TIMI grade 3 flow[153] can be used (opacification of the distal coronary artery within three cardiac cycles)
- **TIMI 2** (partial reperfusion): Contrast material flows through the stenosis to opacify the terminal artery segment. However, contrast enters the terminal segment perceptibly more slowly than more proximal segments. Alternatively, contrast material clears from a segment distal to a stenosis noticeably more slowly than from a comparable segment not preceded by a significant stenosis.
- **TIMI 1** (penetration with minimal perfusion): A small amount of contrast flows through the stenosis but fails to fully opacify the artery beyond.
- **TIMI 0** (no perfusion): No contrast flow through the stenosis.

### 3.4.2. Corrected TIMI frame count

The corrected TIMI frame count (cTFC) was measured as the number of frames required for epicardial contrast to reach standardized distal landmarks, as previously described.[154] The first frame used for TIMI frame counting was defined as the frame in which a column of dye touched both borders of the coronary artery and moved forward, and the last frame was defined as the frame in which dye begins to enter (but does not necessarily fill) a standard distal landmark in the artery.

The standard distal landmarks for each epicardial vessel were:

- The first branch of the posterolateral artery for the right coronary artery;
- The most distal branch of the obtuse marginal branch beyond the culprit lesion in the circumflex system; and,
- The distal bifurcation in the left anterior descending coronary artery. These frame counts were corrected for the longer length of the left anterior descending coronary artery by dividing the TFC by 1.7, to arrive at the corrected TIMI frame count (cTFC).

### 3.4.3. TIMI myocardial perfusion grade (TMPG)

TIMI myocardial perfusion grade (TMPG) was classified according to the standard definition[155]:

- **Grade 3 TMPG**: Normal entry and exit of dye from the microvasculature: There is a ground-glass appearance ("blush") or opacification of the myocardium in the distribution of the culprit lesion that clears normally, and is either gone or mildly or moderately persistent at the end of the washout phase (approximately three cardiac cycles), similar to an uninvolved artery. Blush that is of only mild intensity throughout the washout phase but fades normally is also classified as grade 3.
- **Grade 2 TMPG**: Delayed entry and exit of dye from the microvasculature: There is a ground-glass appearance ("blush") or opacification of the myocardium in the distribution of the culprit lesion that is strongly persistent at the end of the washout phase (i.e., dye is strongly persistent after three cardiac cycles of the washout phase and either does not diminish or only minimally diminishes in intensity during washout).
- **Grade 1 TMPG**: Dye slowly enters but fails to exit the microvasculature: There is a ground-glass appearance ("blush") or opacification of the myocardium in the distribution of the culprit lesion that fails to clear from the microvasculature, and dye staining is present on the next injection (approximately 30 sec between injections).
- **Grade 0 TMPG**: Failure of the dye to enter the microvasculature: Either minimal or no ground-glass appearance ("blush") or opacification of the myocardium in the distribution of the culprit artery, indicating lack of tissue-level perfusion.

## 3.5. Index of microcirculatory resistance (IMR)

The index of microcirculatory resistance was measured immediately after successful P-PCI. All interventional cardiologists recruiting patients were trained in the evaluation of this index.

The measurements were made with a 0.014 mm Certus pressure-wire, together with a RadiAnalyzer™ Xpress console (St. Jude Medical; Figure 7), equipped with dedicated software for IMR calculation.

**Figure 7 – The Certus Pressure-wire, with a pressure and temperature sensor, and the RadiAnalyzer Xpress (St. Jude Medical®)**

The procedure was done as previously defined[238]:

- The coronary pressure wire was calibrated outside the body, and equalized to the pressure reading from the guide catheter with the pressure sensor positioned at the ostium of the guide catheter, after intracoronary administration of 1-2 ml of nitroglycerin;
- After calibration and equalization, the pressure wire was advanced to the distal two-thirds of the culprit vessel.
- Three ml of room-temperature saline were injected down the culprit vessel 3 times at rest, and the resting transit times, which are inversely proportional to flow, were recorded and averaged (Figure 8).
- Maximal hyperemia was induced using intravenous adenosine, 140 μg/kg/min.
- Three millilitres of room-temperature saline were again injected down the culprit vessel, and the hyperaemic transit times were recorded and averaged (Figure 8).
- The mean aortic and distal coronary pressures were recorded during peak hyperemia.
- The IMR was defined as distal coronary pressure divided by flow during peak hyperemia and calculated by dividing the mean distal coronary pressure by the inverse of the hyperaemic transit time, or, more simply, by multiplying the mean distal coronary pressure by the hyperaemic transit time (these values were given automatically by the RadiAnalyzer console).

Coronary flow reserve (CFR), defined as the mean resting transit time divided by the mean hyperaemic transit time, was also automatically calculated by the software.

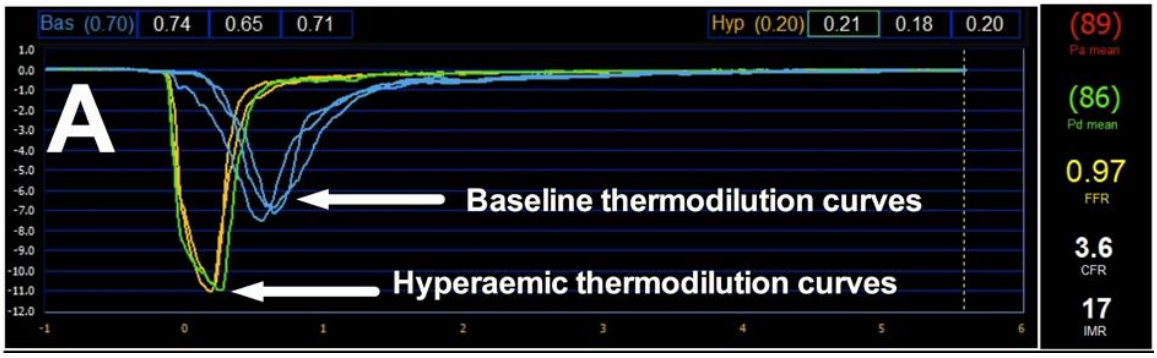



**Figure 8 – Example of baseline and hyperaemic thermodilution curves**
(IMR = mean hyperaemic transit time x distal pressure = 0.20 x 89 = 17.8)

## 3.6. Laboratory Tests

On admission (before P-PCI), a blood sample was collected, for evaluation of:

- Cardiac biomarkers (creatine kinase [CK], creatine kinase-MB [CK-Mb], troponin I [TnI])
- Other relevant risk markers in myocardial infarction (N-terminal pro b-type natriuretic peptide [NT-Pro-BNP], high-sensitivity C-reactive protein [hs-CRP], complete blood count, glucose, glycated haemoglobin [HbA1c], creatinine).

Blood samples were also collected every 6 hours for the first 24 hours after admission (i.e., 0, 6, 12, 18, 24), and every 12 hours thereafter until 48 hours after admission (i.e., 36 and 48 hours), in order to determine the peak CK, CK-Mb and TnI values and to calculate the areas under the curve (AUC) for these markers, as previously described.[239] Troponin I values were used to quantify the extent of the myocardial infarction. In order to account for lesion location, these values (AUC of TnI and peak TnI values) were indexed to area-at-risk scores (BARI and modified APPROACH scores).

The following laboratory tests were used:

- Troponin I: sandwich chemiluminescent immunoassay based LOCI™ technology, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- Creatine kinase: NAC activated, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- Creatine kinase-Mb fraction: Immunoinhibition, enzymatic, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- NT-pro-BNP: sandwich chemiluminescent immunoassay based LOCI™ technology, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- hs-CRP: Immunonephelometric, fixed-time kinetic, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).

- Creatinine: Alkaline picrate, fixed-time kinetic with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- Glucose: Hexokinase, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™).
- HbA1c: High performance liquid chromatography, with ADAMS A1C HA-8180V

## 3.7. ECG ST segment resolution

Twelve-lead ECGs were performed:

- Immediately before P-PCI (baseline ECG),
- Immediately after the end of the procedure (post-PCI ECG),
- 90 minutes after reperfusion (90-min ECG), and
- 180 minutes after reperfusion (180-min ECG).

In the baseline ECG, the following baseline measurements were performed:

- Total ST elevation: the sum of ST elevation in all leads, measured 20 milliseconds after the end of the QRS complex, with the PR segment as reference baseline, in leads I, aVL, and V1–V6 for anterior, and leads II, III, aVF, and V5–V6 for non-anterior myocardial infarction.[240]
- Total ST deviation: the sum of ST deviation in all leads, measured 20 milliseconds after the end of the QRS complex.[139]
- ST elevation in the lead with maximal ST elevation: measured the same way as described above.[135,136]

In the 3 post-revascularization ECGs (post-PCI, 90-min and 180-min), reperfusion was evaluated through the following ST resolution parameters, in comparison with the baseline ECG:

- Percentage of total ST-segment-elevation resolution.
- Percentage of total ST-segment-deviation resolution.
- Percentage of maximal ST-segment-elevation resolution in the lead showing the maximum elevation in the baseline ECG.
- Sum of residual ST-segment-elevation.
- Sum of residual ST-segment-deviation.
- Residual ST-segment elevation in the lead showing maximum elevation in the baseline ECG.

## 3.8. Echocardiographic Evaluation

A transthoracic echocardiogram was performed within 12 hours of presentation and a follow-up exam was scheduled approximately 3 months after the acute event.

The exams were performed using a commercially available ultrasound system (Vivid 7 GE Healthcare, with a M4S GE probe). Measures were performed offline with EchoPAC version 113 GE Healthcare by two observers.

Parameters were measured several times and then averaged. To minimize bias, operators performing the measurements were blinded to the invasive (including IMR) and non-invasive (including EndoPAT) results.

The following echocardiographic parameters were evaluated in both exams:

- Left ventricular end-diastolic, end-systolic volumes and left ventricular ejection fraction (measured in apical 4-chamber and 2-chamber views), using a semiautomatic border detection based on feature tracking imaging[241];
- Wall motion score index (WMSI), by 2D imaging, according to the European Society of Echocardiography's Recommendations, using the 17-segment model on a 1–5 scale: 1-normal, 2-hypokinesia, 3-akinesia, 4-dyskinesia, 5-aneurysmal. The final WMSI was calculated by adding the points for each segment (a lower score implies better left ventricular function)[242];
- Left atria volume (indexed to body surface area) by 2D imaging;
- E/A ratio in the mitral inflow, obtained by pulsed-wave Doppler-echocardiography with the sample volume between mitral leaflet tips during diastole;
- Ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/e´), using mitral annulus velocities obtained from the media of septal and lateral annulus by tissue Doppler imaging;
- Global longitudinal strain (GLS), assessed using speckle-tracking analysis with automated function imaging. Peak longitudinal strain was defined as the change in length of the myocardium from end-diastole to end-systole and expressed as a percentage: longitudinal strain (%) = (L end-systole - L end-diastole)/L end-diastole x 100%, where L is the length of the region of interest. GLS was obtained from 2D grey scale images of the apical four-chamber, two-chamber, and long-axis view with optimized frame rate (50–90 frames/sec). Software identified the endocardial border, and myocardial motion was automatically tracked in each imaging view. In segments with poor tracking, the observer readjusted the endocardial trace line until a better tracking was achieved. The mean of the peak systolic longitudinal strain values from the 17 left ventricle segments was calculated to determine left ventricular GLS.

In patients with the 2 exams available, these parameters were compared.

## 3.9. Digital peripheral arterial tonometry (EndoPAT)

Noninvasive measurements of endothelial function were done with EndoPAT 2000 (Itamar Medical®, Caesarea, Israel; Figure 9). The system includes a fingertip plethysmograph capable of sensing volume changes in the digit with each arterial pulsation.

Measurements were performed in a thermoneutral environment, after admission to the Intensive Cardiac Intensive Care Unit, immediately after the primary PCI.

As described below (see point 1.4, under Results), after an interim analysis of the results, patients also did a second evaluation 24 hours after the first, using the same methodology.

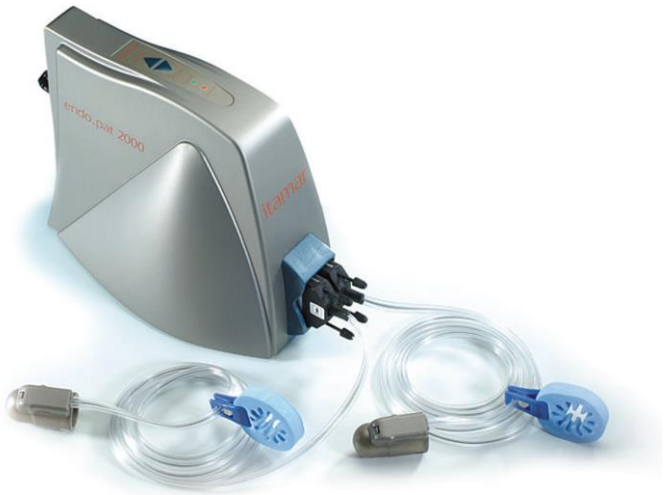



**Figure 9 – The EndoPAT 2000**

The protocol was the same followed in previous studies.[222] Briefly, a complete digital peripheral arterial tonometry (PAT) endothelial function test includes three phases: baseline, occlusion, and hyperemia:

- A blood pressure cuff is placed on one upper arm (study arm), while the other arm serves as a control (control arm).
- A PAT probe is positioned on one finger of each hand and set by the computer to inflate to 10 mm Hg below diastolic pressure or 70 mm Hg (the lower value is selected). Recordings are taken simultaneously from both fingers throughout the study. The response in the control finger not experiencing hyperemia can be used to adjust for systemic effects (Figure 10).
- After baseline data acquisition, the blood pressure cuff is inflated on one arm to suprasystolic pressures for 5 minutes. During the occlusion period, signals are absent from the hyperaemic finger but continue from the control finger.

- After cuff release, pulse amplitude increases in the hyperaemic finger. The pulse amplitude recordings are digitized and analysed by an automated, proprietary algorithm. Average pulse amplitude is calculated for each 30-second intervals after cuff occlusion for up to 5 minutes.
- As a measure of reactive hyperemia, the pulse amplitude tonometry is then calculated as the ratio of the average amplitude of the PAT signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the PAT signal of a 3.5-min time period before cuff inflation (baseline).
- Subsequently, PAT index values from the study arm are normalized to the control arm. All these data are analysed by a computer in an operator-independent manner, to get the reactive hyperemia index (RHI) and its logarithmic transformation (L_RHI).

In patients with two exams, the same study arm (left or right) was used in both tests.

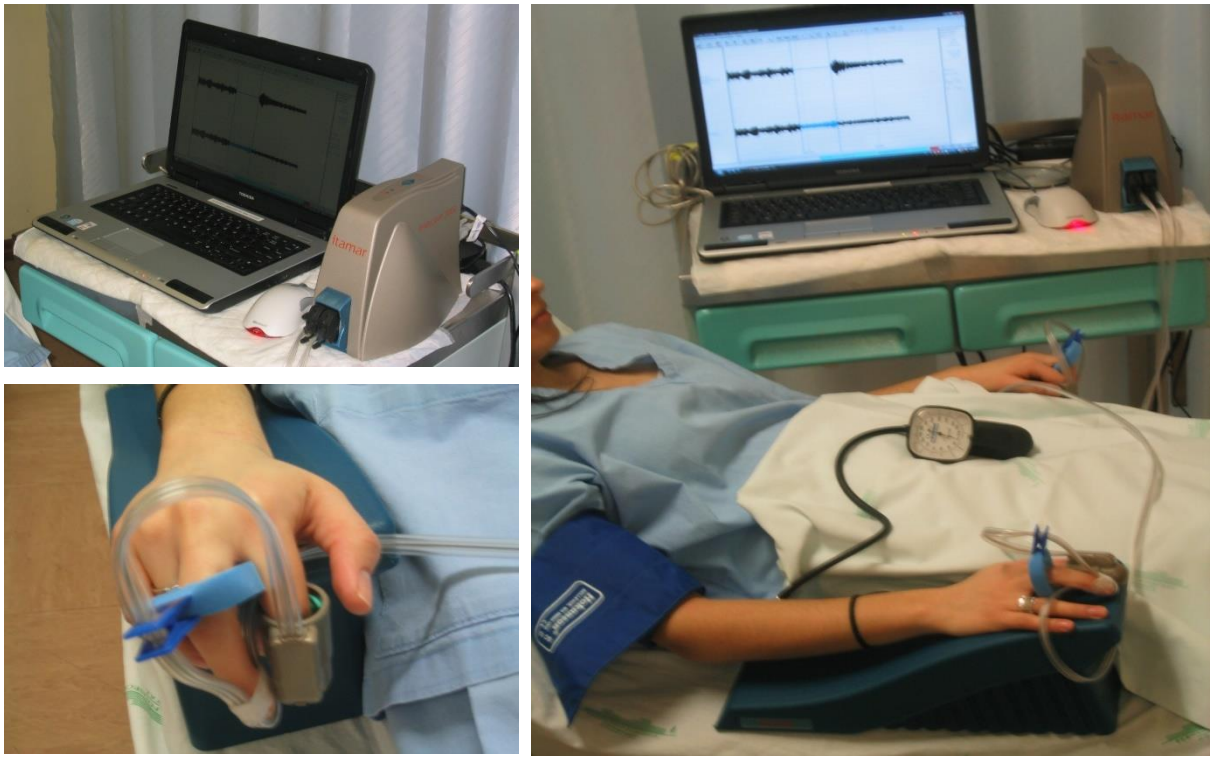


**Figure 10 – EndoPAT procedure**

## 3.10. Contrast Enhanced Cardiac Magnetic Resonance

Cardiac magnetic resonance was performed in a subset of patients on the 7-8$^{th}$ day post-MI.[243], using a 1.5-T magnetic resonance imaging system (Avanto, Siemens Medical System, Erlangen, Germany) equipped with a dedicated cardiac software package and 8 available independent radiofrequency receiver channels, cardiac coil, and vectorcardiogram.

After the acquisition of localizing images, long- and short-axis cine images were obtained, using retrospectively ECG-gated breath-hold segmented K-space balanced steady-state free precession pulse sequence (trueFISP) technique. The short-axis cine scans of 6-mm slices were used to determine the left ventricular mass, volume, and function (in-plane resolution 1.6x1.6mm; gap 2mm). STIR technique, a triple-IR black-blood turbo spin echo pulse sequence was used for oedema quantification (area at risk).

A bolus of contrast medium (gadopentetate dimeglumine - Magnevist, Schering AG, Berlin, Germany) was injected at a dose of 0.2 mmol/kg. Early enhancement images for microvascular obstruction assessment were obtained by acquiring an inversion-recovery segmented gradient echo T1-weighted sequence with a high inversion time (approximately 500 ms), 2-4 min after gadolinium injection. Delayed enhancement images were then obtained by acquiring an inversion-recovery segmented gradient echo T1-weighted sequence, 10 to 15 min after the bolus.

All post-processing and analyses of the left ventricular mass, volume, function, area at risk, myocardial infarct size, and presence of microvascular obstruction were performed using CVI 42 Version 5 Software (Circle Cardiovascular Imaging Inc, Calgary, Canada) by a cardiologist experienced in CMR and blinded to all clinical and invasive physiological data.

Area-at-risk was manually quantified on short-axis STIR sequences slices, delineating higher intensity areas (no threshold definition) at each slice, with subsequent computation for mass estimation. Infarct size was also assessed manually by planimetry on each short-axis slice, delineating the hyperenhanced area, including areas of hypoenhancement surrounded by the hyperenhanced area, the latter being considered microvascular obstruction. Infarct size, as a percentage of left ventricular mass, was computed from the sum of hyperenhanced pixels from each of the 10 short-axis images divided by the total number of pixels within the left ventricular myocardium multiplied by 100% (21)[244]. Microvascular obstruction mass was also manually quantified as the sum of hypoenhanced pixels at delayed enhancement sequences as better spatial resolution was found when compared for early enhancement sequences[245].

## 4. STATISTICAL ANALYSIS

Continuous variables are presented as mean ± standard deviation (normal distribution) or as median and interquartile range (non-normal distribution); categorical variables are presented as frequencies.

All analysis of categorical dependent variables (i.e., RHI<1.67 vs. >1.67, IMR<24 vs >24, etc.) were performed using independent sample T-Test for continuous variables with a normal distribution, Mann-Whitney Test, for continuous variables with a non-normal distribution and Chi-square for categorical variables (with Fisher correction when applicable).

Analysis according to RHI tertiles was performed using one-way ANOVA for continuous variables with a normal distribution and Kruskal-Wallis for continuous variables with non-normal distribution.

Analysis of IMR or RHI as continuous variables were performed using Pearson's correlation for continuous variables with a normal distribution and Spearmans's rho correlation for continuous variables with a non-normal distribution

The analysis of IMR predictors was adjusted for confounding variables by linear regression analyses, including the variables identified as relevant on univariate analysis and also all variables considered clinically relevant.

For paired comparisons, paired sample T test, Wilcoxon or signs test was used, as indicated.

Statistical tests and corresponding p-values were two-sided and a $p$ value <0.05 was considered as statistically significant. IBM SPSS version 21.0 was used for all statistical analyses.

## 5. ETHICAL ASPECTS

The study complied with all ethical international standards, including the World Medical Association's Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

It was submitted to and approved by Hospital Prof. Doutor Fernando da Fonseca´s Ethics Committee, Reseaerch Committee and Hospital Administration and to Nova Medical School Ethics Committee.

A detailed, written informed consent was obtained from each patient. Inclusion in the study did not affect patient's care and all patients were treated with the best available resources and knowledge, as usual.

## 6. FINANTIAL ASPECTS

The study was funded by the following companies/foundations:

- Astra-Zeneca (unrestricted grant)
- Merck Foundation (unrestricted grant)
- S. Jude Medical Portugal (pressure-wire offer)
- Medtronic (unrestricted grant)
- Cordis, Cardinal Health (unrestricted grant)
- Bayer (unrestricted grant)

# RESULTS

# 1. Population included

Between June 2012 and June 2015, a total of 543 ST elevation myocardial infarction patients treated by primary percutaneous coronary intervention were admitted to Hospital Prof. Doutor Fernando da Fonseca. Of these, 60 patients fulfilled all the inclusion criteria, accepted to participate in the study and were considered eligible for the procedure, according to the operator. The flowchart of patient inclusion is presented in Figure 11**Error! Reference source not found.**.

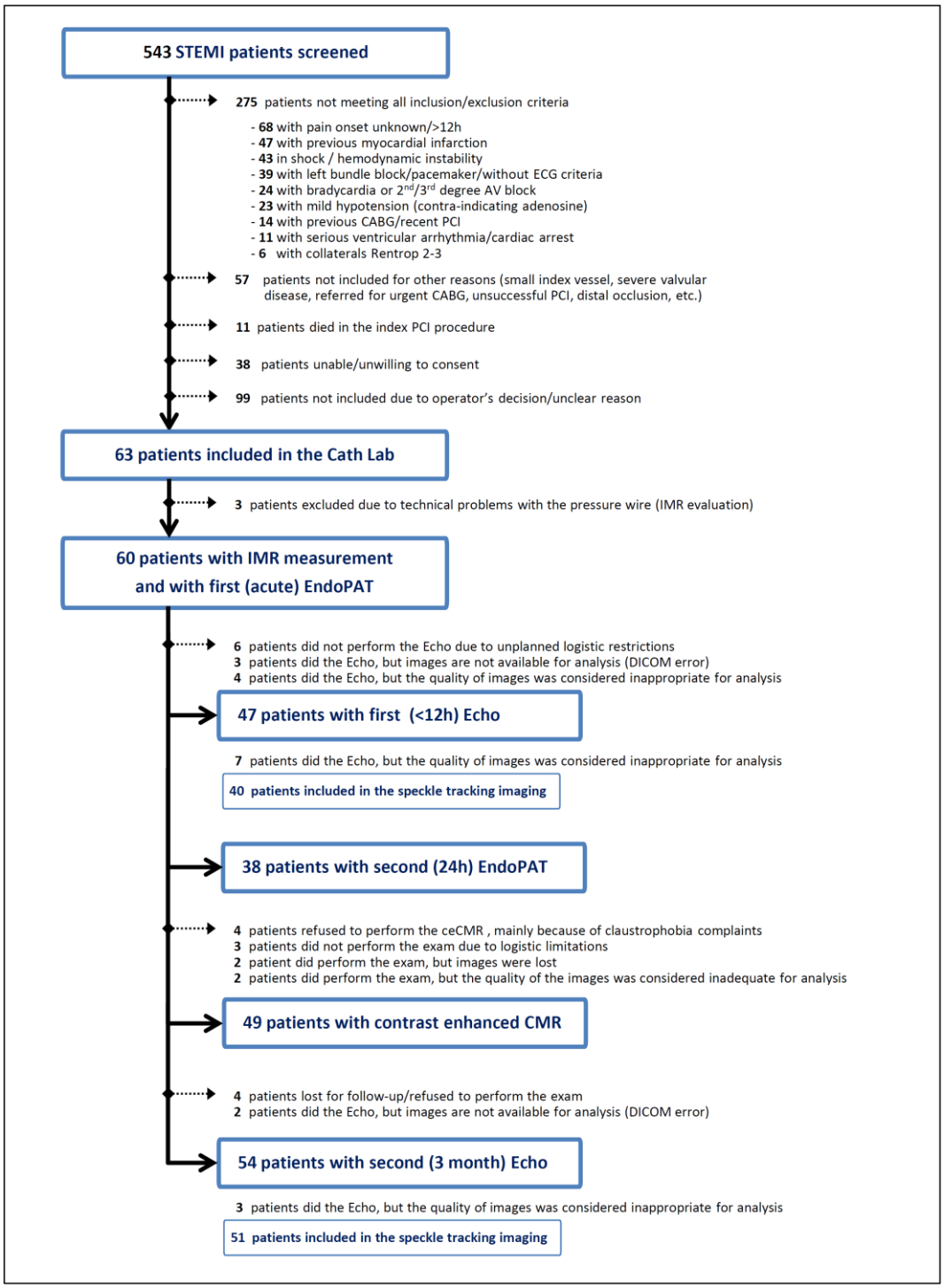



**Figure 11 - Flow diagram of the cohort study**

## 1.1. Main epidemiological characteristics

Of the 60 patients included, 48 (80.0%) were male. Mean age was 59.6±12.7 years (58.5±12.0 for male and 63.9±14.7 for female patients). The histogram for age is presented in Figure 12. Half of the patients had a previous history of dyslipidaemia and one fourth were diabetics. The main risk factors in the population included are presented in Figure 13. Mean body mass index (BMI) was 27.5±4.0 kg/m$^2$ (28.0±3.8 kg/m$^2$ for males and 25.4±4.2 kg/m$^2$ for females). Mean waist circumference was 99.2±12.2 cm (100.2±12.0 for male and 92.5±12.6 cm for female patients).

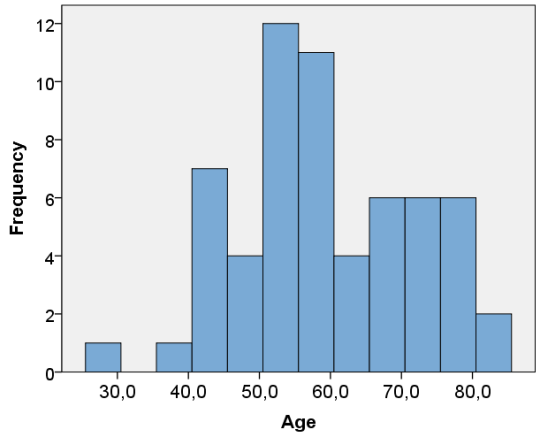



**Figure 12 – Age distribution of patients included**

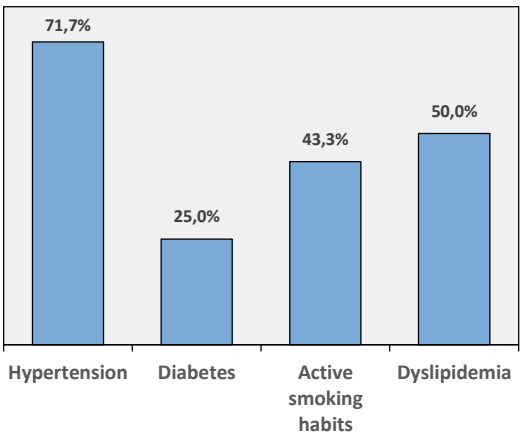



**Figure 13 – Prevalence of main risk factors**

Most patients had no history of coronary artery disease: only 9 patients (15.0%) had previous angina complaints and 2 (3.3%) had previously undergone coronary angiography and percutaneous coronary intervention. Accordingly, only 6 patients (10.0%) were on anti-platelet (aspirin or clopidogrel) therapy. Statins were used by 9 patients (15.0%). ACE inhibitors/angiotensin antagonists, beta-blockers and calcium channel antagonists were being used, respectively, by 23 (38.3%), 4 (6.7%) and 4 (6.7%) patients prior to hospital admission.

## 1.2. Angiographic and angioplasty-related characteristics

### 1.2.1 Out-of-hospital and in-hospital time delays

The median time between the onset of symptoms and the first medical contact ("pain-to-door" time) was 130 minutes (IQR 126 minutes). The median time between the first medical contact and the first balloon dilation ("door-to-balloon" time) was 78 minutes (IQR 45 minutes). The

median total ischemic time (time between the onset of symptoms and first balloon dilation, or "pain-to-balloon" time) was 209 minutes (IQR 148 minutes).

### 1.2.2 Angiographic data

The culprit artery was the left anterior descending artery (LAD) in 28 patients (46.7%), the left circumflex artery (LCx) in 13 patients (21.7%) and the right coronary artery in 19 patients (31.7%). Multivessel disease (classified as lesions >70% in major coronary arteries) was present in 25 patients (41.7%). The median Syntax score was 15.5 (IQR 10.0).

TIMI flow on the first injection was 0 (no flow beyond the point of occlusion) or 1 (faint coronary flow beyond the occlusion with incomplete filling of the distal coronary bed) in 50 patients (83.8%).

### 1.2.3 Area-at-risk scores

Mean APPROACH and BARI scores were similar: 28.5%±6.6% and 28.0%±6.3%, respectively, with a slight tendency for higher areas with the APPROACH score. Correlation between the 2 scores was very high (R=0.90, p<0.001), suggesting that both scores perform similarly well in identifying the area at risk for each lesion location (Figure 14).

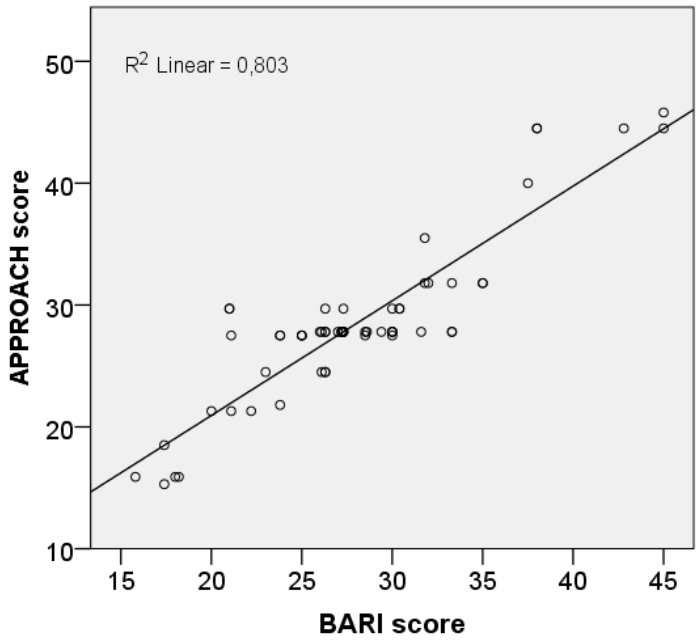



**Figure 14 – Correlation between APPROACH and BARI scores in identifying the area-at-risk for each lesion location**

### 1.2.4 Angioplasty procedure

All patients received intravenous non-fractionated heparin and both aspirin (intravenous) and clopidogrel/ticagrelor (oral). Intracoronary nitrates were also administrated in all patients, since it is mandatory for the IMR measurement. Fourteen patients (23.3%) received intravenous abciximab during angioplasty.

Mechanical thrombectomy (aspiration) was performed in 26 cases (43.3%). Stents were implanted in 57 (95.0%) and a direct stenting technique was used in 25 (41.7%), with balloon post-dilation in 28 (38.3%).

### 1.2.5 Angiographic indicators of microvascular perfusion

A normal (TIMI 3) flow was obtained in all patients. Corrected TIMI frame count (cTFC) and TIMI myocardial perfusion grade (TMPG) were measured in all patients immediately after the P-PCI. cTFC median value was 17.0 (IQR 7.0). A normal 3 TMPG was obtained in 37 patients (61.7%). Patients with lower TMPG had, as expected, higher cTFC values (Figure 15).

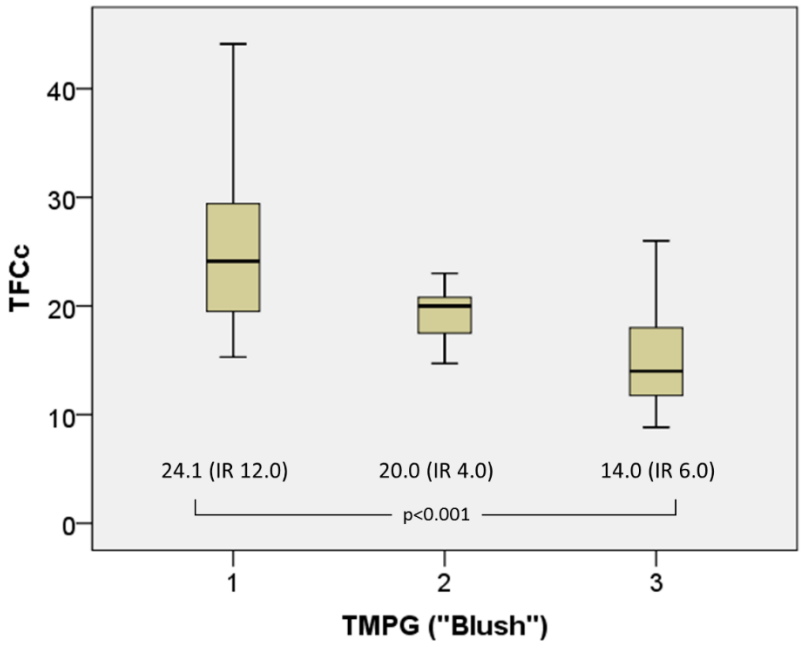


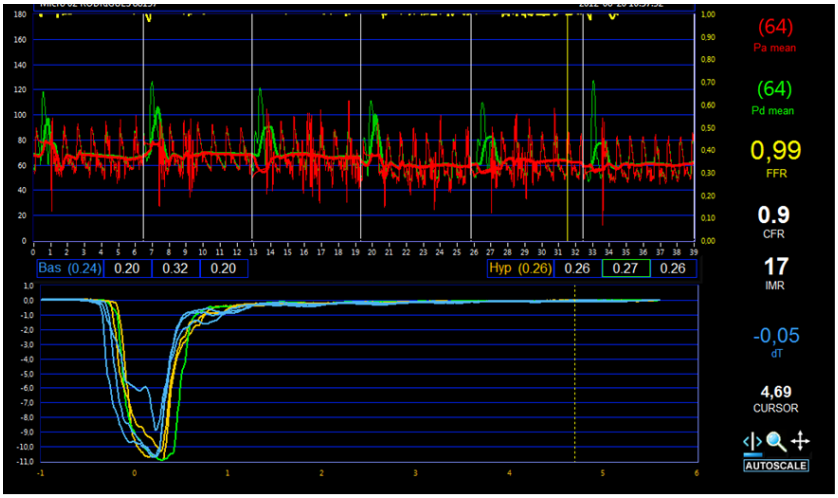

**Figure 15 – Corrected TIMI frame count, according to TIMI myocardial perfusion grade**
(values expressed as median and interquartile range, p value for Kruskal-Wallis test)

## 1.3. Index of microcirculatory resistance (IMR)

IMR was measured in all patients. Median IMR value was 23.9 (IQR 32.9). An IMR value higher than 40 was measured in 21 patients (35.0%).

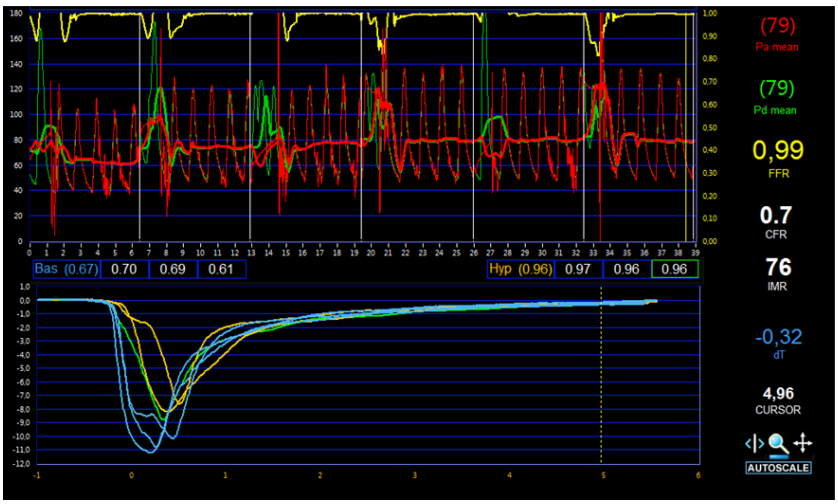
Two examples of IMR measurements are presented in Figure 16.

- Example 1 corresponds to a young 28 years old male patient with an inferior myocardial infarction, due to a proximal RCA occlusion, revascularized (pain-to-balloon time) 155 minutes after symptoms onset. His IMR was 17.
- Example 2 corresponds to a 75 years old female patient, with an inferior myocardial infarction revascularized 192 minutes after symptoms onset; she had a proximal LCx occlusion successfully revascularized and her IMR after the procedure was 76.




**Example 1**

28 years ♂
Inferior STEMI
Proximal RCA occlusion
Successful P-PCI
IMR = 17




**Example 2**

75 years ♀
Inferior STEMI
Proximal LCx occlusion
Successful P-PCI
IMR = 76

**Figure 16 – Examples of IMR measurement**

## 1.4. Endothelial function (EndoPAT)

The first EndoPAT evaluation was performed immediately after the P-PCI in all patients. Since these were seriously ill patients, with an acute ST elevation myocardial infarction, *endothelial dysfunction* (defined as an RHI< 1.67) was expected to be present in a majority of patients. However, when the first 20 patients were included, an interim analysis showed that exactly the opposite was apparently happening, since RHI values in these first patients were actually higher than the ones we previously reported, both in normal and in coronary artery disease patients.[18,20]

Following this interim analysis, it was decided to perform a second EndoPAT evaluation 24 hours after the first. This second EndoPAT exam is available in 38 patients. The mean values of the reactive hyperaemia index (RHI) and logarithmic RHI (L-RHI) are presented in Table 15, together with the percentage of patients with *endothelial dysfunction* (RHI<1.67). When only patients with 2 EndoPAT evaluations were considered (n=38), RHI measured in the first EndoPAT (2.16±0.52) was similar to the total population (n=60, 2.15±0.58), suggesting this subgroup with 2 evaluations is similar to the whole sample population.

**Table 15 – First and second EndoPAT main results (complete cohort)**

| Parameter | 1st EndoPAT (n=60) | 1st EndoPAT in patients with 2nd EndoPAT (n=38) | 2nd EndoPAT (n=38) | *p* value[c] |
|---|---|---|---|---|
| RHI [a] | 2.15±0.58 | 2.16±0.52 | 1.87±0.60 | 0.006 |
| Endothelial dysfunction (RHI<1.67)[b] | 11 (18.3) | 6 (15.8) | 16 (42.1) | 0.011 |
| L_RHI [a] | 0.73±0.28 | 0.74±0.24 | 0.61±0.25 | 0.006 |

[a] Presented as mean±standard deviation; [b] Presented as N(%). [c] p-value for the comparison between first and second EndoPAT only in patients with 2 evaluations; paired samples T-Test for continuous variables and Chi-square test for categorical variables; RHI: reactive hyperaemia index; L_RHI: logarithmic RHI.

In patients with 2 EndoPAT evaluations, RHI values were significantly lower in the second evaluation (1.87±0.60 vs. 2.16±0.52, p=0.006). Accordingly, the number of patients with *endothelial dysfunction* (RHI<1.67) increased from 15.8% to 42.1% (p=0.011).

Another important question, as shown in the boxplot of RHI values in the first EndoPAT (Figure 17) is that there were 6 outliers – one extremely low value (0.64, in a female patient with vasculitis and Raynaud syndrome), and 5 extremely high values.

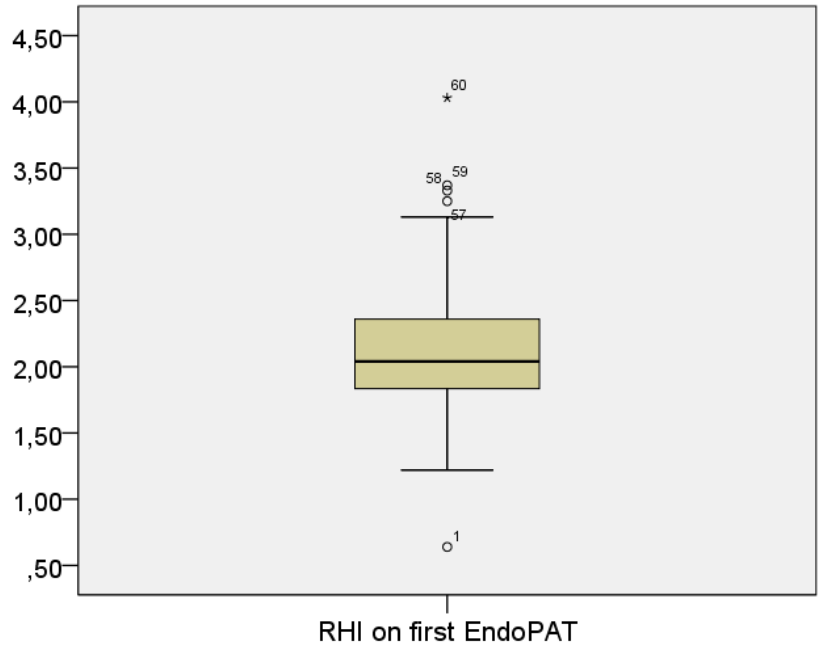



**Figure 17 – Reactive hyperaemia index (RHI) values on the 1st EndoPAT (complete cohort)**

When these extreme values were excluded from the analysis (Figure 18, Table 16), mean value of RHI was naturally lower (2.08±0.42).

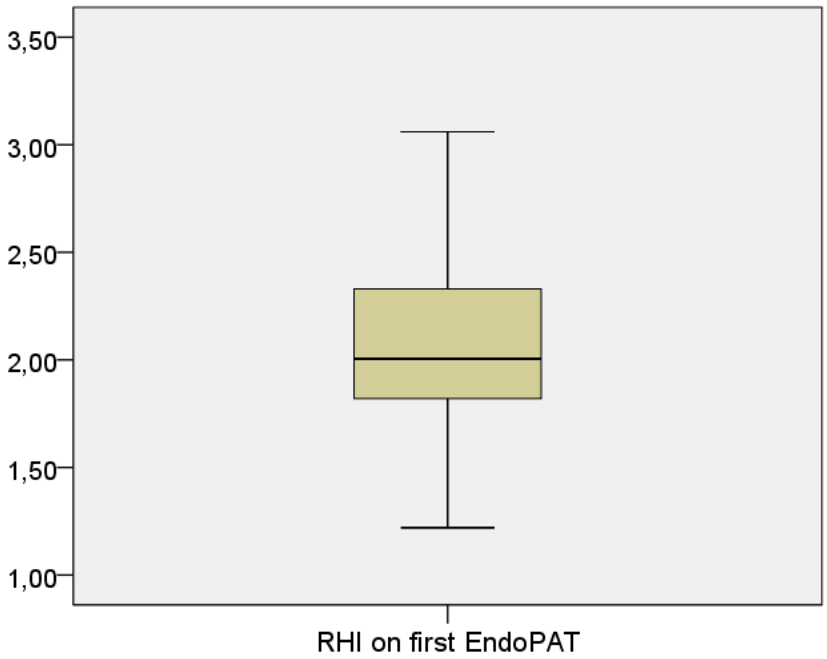



**Figure 18 – Reactive hyperaemia index (RHI) values on the 1st EndoPAT (excluding outliers)**

Importantly, the same observations described above for the complete cohort were confirmed in this "purified" population:

- When only patients with 2 EndoPAT evaluations were considered (n=35), RHI measured in the first EndoPAT (2.06±0.41) was similar to the one measured in the global population (n=54, 2.08±0.42).
- In these patients with 2 EndoPAT evaluations, RHI values were significantly lower in the second evaluation (1.81±0.56 vs. 2.06±0.41, p=0.018), and the number of patients with *endothelial dysfunction* (RHI<1.67) increased from 15.8% to 45.7% (p=0.01).

In this population, values of RHI on the second EndoPAT evaluation did not correlate well with values on the first EndoPAT (r=0.260, p=0.131). Among the 6 patients with *endothelial dysfunction* (RHI<1.67) on the first EndoPAT, 3 (50.0%) evolved to normal endothelial function on the second one. On the other hand, 13 of the 29 patients (44.8%) with a normal endothelial function on the first EndoPAT ended up with *endothelial dysfunction* (RHI<1.67) on the second evaluation. Although patients with *endothelial dysfunction* (RHI<1.67) on the second EndoPAT had a tendency for lower RHI values on the first evaluation (1.92±0.36, vs. 2.17±0.42), this difference was not statistically significant (p=0.07). Altogether, this data suggests that the first EndoPAT results are apparently not related to the second EndoPAT results.

**Table 16 – First and Second EndoPAT main results (excluding outliers)**

| Parameter | 1st EndoPAT (n=54) | 1st EndoPAT in patients with 2nd EndoPAT (n=35) | 2nd EndoPAT (n=35) | *p* value[a] |
|---|---|---|---|---|
| RHI [a] | 2.08±0.42 | 2.06±0.41 | 1.81±0.56 | 0.018 |
| *Endothelial dysfunction* (RHI<1.67)[b] | 10 (16.7) | 6 (15.8) | 16 (45.7) | 0.01 |
| L_RHI | 0.70±0.20 | 0.70±0.20 | 0.59±0.24 | 0.006 |

[a] Presented as mean±standard deviation; [b] Presented as N(%). [c] p-value for the comparison between first and second EndoPAT only in patients with 2 evaluations; paired samples T-Test for continuous variables and Chi-square test for categorical variables; RHI: reactive hyperaemia index; L_RHI: logarithmic RHI.

Two examples of EndoPAT measurements are presented in Figure 19. Panel A corresponds to a patient with normal reactive hyperaemic response (RHI 2.14), with an increase of signal amplitude after unilateral cuff occlusion (bottom graph), corrected with contralateral finger signal (top graph). Panel B corresponds to a patient with an abnormal response (RHI 1.40) with a blunted increase in signal amplitude after unilateral cuff occlusion.

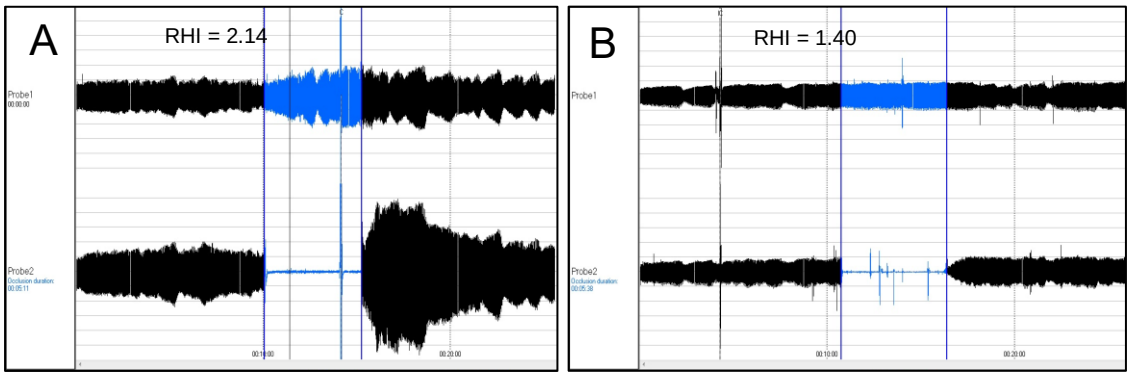



**Figure 19 - Examples of peripheral endothelial function measured by digital pulse amplitude with endothelial peripheral arterial tonometry**

## 1.5. Laboratory tests

### 1.3.1 Laboratory tests on admission

A blood sample was collected on admission in all patients. Among others, glucose, HbA1c, creatinine, high sensitive C-reactive protein and NT-pro-BNP were measured, along with cardiac enzymes (see below). In a few cases, not all are available. Median values are presented in Table 17.

**Table 17 – Admission laboratory values**

| Test | Available in | Median (IQR) | Median ± standard deviation |
|---|---|---|---|
| Creatinine (mg/dL) | 60 | 0.90 (0.31) | 0.95±0.30 |
| hs-CRP (mg/dL) | 59 | 0.44 (0.59) | 0.78±0.93 |
| Glucose (mg/dL) | 56 | 133.5 (61.0) | 158.8±85.5 |
| HbA1c | 49 | 5.7 (1.1) | 6.4±1.9 |
| NT-pro-BNP (pg/mL) | 54 | 137.5 (255.6) | 334.0±728.5 |

### 1.3.2 Cardiac enzymes

Peak values and the area under the curve of creatine kinase (CK), Mb fraction of creatine kinase (CK-Mb) and Troponin I (TnI), measured on admission and at 6, 12, 18, 24, 36 and 48 hours are

presented in Table 18. In order to account for the area-at-risk for each lesion, these values were indexed to APPROACH and BARI scores.

**Table 18 – Total and indexed values for maximal and AUC values of cardiac biomarkers**

| Test | Total | Indexed | | *Correlation* | |
|---|---|---|---|---|---|
| | | APPROACH | BARI | *R* | *p* value [a] |
| **Creatine Kinase (UI/L)** | | | | | |
| Peak value | 2370±1539 | 692±568 | 673±516 | 0.989 | <0.001 |
| AUC | 43398±24921 | 12645±9171 | 12296±8282 | 0.987 | <0.001 |
| **Mb Fraction of Creatine Kinase (CK-Mb)** | | | | | |
| Peak value | 255±141 | 74±48 | 72±46 | 0.981 | <0.001 |
| AUC | 4291±1834 | 1232±622 | 1208±612 | 0.975 | <0.001 |
| **Troponin I (ng/mL)** | | | | | |
| Peak value | 117±82 | 34±27 | 33±25 | 0.994 | <0.001 |
| AUC | 1938±1283 | 565±441 | 548±405 | 0.993 | <0.001 |

[a] Spearman correlation between APPROACH and BARI indexed values; AUC – area under the curve

## 1.6. ECG ST segment resolution

ECGs were performed in all patients, before and immediately after the P-PCI, and 90 and 80 min after the procedure. Total ST elevation, total ST deviation and ST elevation in the lead with largest ST-segment-elevation, along with the respective percent resolution, are presented in Table 19.

**Table 19 – ECG parameters before and after primary PCI**

| | Pre-PCI | Post-PCI | | 90 min | | 180 min | |
|---|---|---|---|---|---|---|---|
| | | Residual | % resolution | Residual | % resolution | Residual | % resolution |
| Total ST elevation | 9.5(11.6) | 3.3 (6.0) | 74.5 (39.0) | 1.5 (4.0) | 79.5 (32.0) | 1.5 (3.0) | 84.5 (33.0) |
| Total ST deviation | 13.3 (17.5) | 4.8 (6.0) | 73.5 (44.0) | 2.0 (4.0) | 82.5 (33.0) | 1.5 (4.0) | 88.0 (26.0) |
| ST max lead [a] | 3.0 (3.9) | 1.0 (2.0) | 68.0 (47.0) | 0.5 (2.0) | 77.5 (34.0) | 0.5 (2.0) | 83.0 (33.0) |

Values expressed as median (Interquartile range), in mV; [a] ST in lead with initial largest ST elevation.

Classical ECG criteria suggesting reperfusion (i.e., ST resolution >70% of the changes observed in the ECG performed before revascularization) were present in around 80% of the patients at 180

minutes (Table 20). Remarkably, in this group of patients with adequate (final TIMI 3 flow) and relatively early reperfusion, about 1/5 maintained significant ST changes, suggesting microcirculatory damage.

**Table 20 – ST resolution >70% in ECGs performed after the primary PCI**

| | Post-PCI | 90 min | 180 min |
|---|---|---|---|
| Total ST elevation | 34 (56.7) | 41 (68.3) | 49 (81.7) |
| Total ST deviation | 32 (53.3) | 41 (68.3) | 46 (76.7) |
| ST max lead [a] | 29 (48.3) | 33 (55.0) | 41 (68.3) |

Values expressed as number (%); [a] ST at lead with initial largest ST elevation.

## 1.7. Echocardiographic evaluation

The first (acute) echocardiographic evaluation was not performed in 3 patients, due to unplanned logistic restrictions. In the remaining 57 patients:

- In 6 cases, the echo was performed, but the images are not available due to errors in the DICOM files;
- In 4 patients the quality of the images was considered inappropriate for analysis;
- The remaining 47 patients were included in the morphological (2D) analysis;
- From these, only 40 had images with enough quality for the speckle tracking imaging analysis.

The second (3 month) echocardiographic evaluation was not performed in 4 patients, which were lost for follow-up or missed the schedule date for the exam. In the remaining 56 patients:

- In 2 cases, the exam was performed, but the images are not available due to errors in the DICOM files;
- The remaining 54 were included in the morphological (2D) analysis;
- From these, only 51 had images with enough quality for the speckle tracking imaging analysis.

In 45 patients, both exams were available, and 35 of these had images with enough quality for the speckle tracking imaging analysis to be performed.

Main results of both exams are presented in Table 21 (2D measurements) and Table 22 (Doppler and 2D speckle tracking imaging). Between the two exams, significant improvements were documented in LVEF, WMSI, E/A ratio and GLS (analysis performed only for patients with 2 exams). Importantly, when comparing mean values in patients with and without the 2 exams, results were quite similar, suggesting that populations are similar.

**Table 21 – 2D measurements in first (acute) and second (3 months) echocardiographic exams**

| | First (acute) echo | | Second (3 month) echo | | *p* value[c] |
|---|---|---|---|---|---|
| | All patients (n=47) | Patients w/2 echos (n=45) | All patients (n=54) | Patients w/ 2 echos (n=45) | |
| LVEdV (ml) [a] | 105.8±24.2 | 105.4±24.8 | 109.7±26.4 | 109.8±27.8 | 0.18 |
| LVEsV (ml) [a] | 54.7±12.7 | 54.1±12.5 | 52.9±18.8 | 53.0±19.6 | 0.60 |
| LVEF (%) [a] | 47.9±6.7 | 48.3±6.0 | 52.6±7.1 | 52.6±6.7 | 0.001 |
| WMSI [b] | 1.41 (0.35) | 1.41 (0.35) | 1.24 (0.35) | 1.24 (0.35) | <0.001 |
| LA Volume (ml/m$^2$) [a] | 34.8±12.2 | 34.3±11.8 | 39.1±15.4 | 39.8±16.6 | 0.005 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [C] p-value for the comparison between the first and the second echo only in patients with 2 evaluations; paired samples T-Test for variables with normal distribution and Wilcoxon test for variables with non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index. LA – left atria

**Table 22 – Doppler and 2D speckle tracking measurements in first (acute) and second (3 months) echocardiographic exams**

| | First (acute) echo | | Second (3 month) echo | | *p* value[b] |
|---|---|---|---|---|---|
| | All patients (n=40) | Patients w/2 echos (n=35) | All patients (n=51) | Patients w/ 2 echos (n=35) | |
| **Doppler measurements** | | | | | |
| E/A ratio [a] | 1.00±0.34 | 0.99±0.34 | 1.20±0.56 | 1.14±0.51 | 0.022 |
| E/e' ratio [a] | 9.00±2.71 | 9.00±2.84 | 8.83±3.29 | 8.89±3.58 | 0.82 |
| **2D speckle tracking imaging** | | | | | |
| GLS [a] | -13.54±2.28 | -13.88±2.09 | -15.77±3.11 | -15.67±3.10 | <0.001 |

[a] Presented as mean±standard deviation; [b] p-value for the comparison between the first and the second echo only in patients with 2 evaluations; paired samples T-Test for variables. GLS – global longitudinal strain

## 1.8. Contrast enhanced cardiac magnetic resonance (ceCMR)

Of the 60 patients included in the study:

- 4 refused to perform the ceCMR, mainly because of claustrophobia;
- 3 did not perform the exam due to logistic limitations;
- 2 did the exam, but images were lost;
- 2 did the exam, but the quality of the images was considered inadequate for evaluation;
- 49 have the exam available for evaluation of the endpoints defined.

The exam was performed 8.8 days (6-13) after the primary PCI. Oedema was present in all but 1 patient. Microvascular obstruction was identified in 13 patients (26.5%) and complete transmural necrosis was found in 23 (46.9%).

Figure 20 depicts three examples of ceCMR exams, with one case of extensive no-reflow, one case of transmural infarction and one of subendocardial infarction.

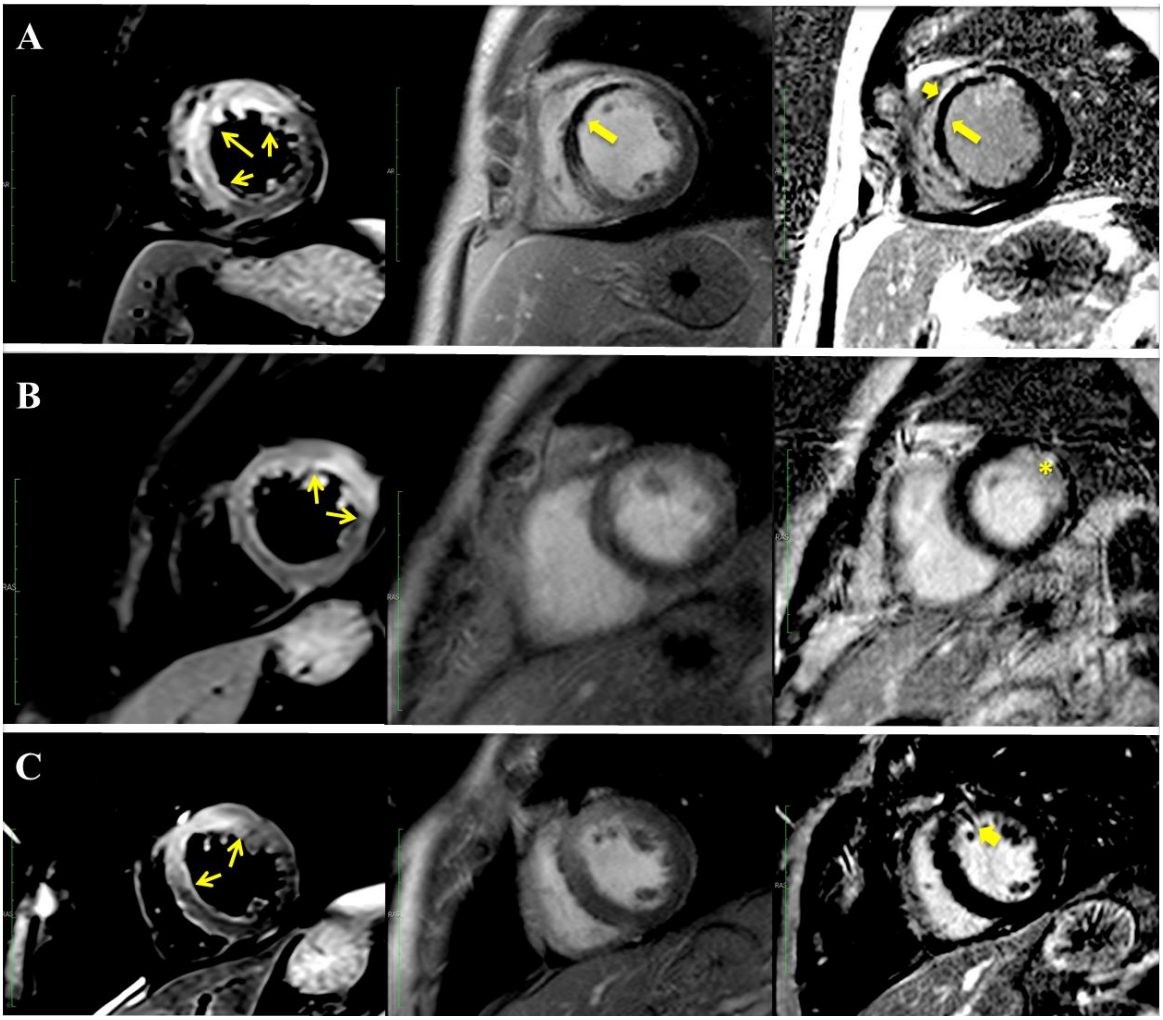



**Figure 20 – Three examples of ceCMR**

Left column images depict myocardial oedema (area at risk) as assessed by t2-weighted stir (*short tau inversion recovery*) sequences; the column in the centre shows early enhancement acquisition with a long time of inversion for microvascular obstruction detection; right column shows delayed enhancement sequences for infarct mass estimation. **Patient A**: large area at risk (between arrows) with extensive no-reflow (yellow arrow), clearly depicted across a transmural antero-septal infarction (short yellow arrow); **Patient B**: lateral oedema (between arrows) with absence of no-reflow despite the presence of a transmural infarction (*); **Patient C**: antero-septal oedema (between arrows), negative for the presence of no-reflow and subendocardial myocardial infarction (short yellow arrow)

## 2. Outcome measures - summary

The **primary outcome measures** were, as stated above, the IMR values according to the presence of endothelial dysfunction (RHI<1.67) and to RHI tertiles. Since a second EndoPAT measurement was performed, these 2 outcomes will be presented for each EndoPAT exam. Additionally, analysis of RHI as a continuous variable will also be presented for each EndoPAT evaluation. So, in summary, primary outcomes measures will be presented as follows:

**First EndoPAT**

1. IMR values in patients with and without endothelial dysfunction (RHI<1.67) in the first EndoPAT (Section 3.1).
2. IMR values according to RHI tertiles in the first EndoPAT (Section 3.2).
3. Relation of RHI as a continuous variable in the first EndoPAT to IMR values (Section 3.3).

**Second EndoPAT**

4. IMR values in patients with and without endothelial dysfunction (RHI<1.67) in the second EndoPAT (Section 4.1).
5. IMR values according to RHI tertiles in the second EndoPAT (Section 4.2).
6. Relation of RHI as a continuous variable in the second EndoPAT to IMR values (Section 4.3).

**Secondary outcome measurements** included the impact or relation between RHI and IMR and the extent of myocardial necrosis and microvascular reperfusion, evaluated by several different methods. These results will be presented as follows:

**First EndoPAT**

1. Impact of the presence of endothelial dysfunction (RHI<1.67) in the first EndoPAT on the extent of myocardial infarction, evaluated by troponin release, echocardiographic parameters and ceCMR (Section 5.1).
2. Impact of the presence of endothelial dysfunction (RHI<1.67) in the first EndoPAT on microvascular reperfusion indicators, including angiographic indicators (cTFC and TMPG), ECG (ST resolution), and ceCMR (microvascular obstruction) (Section 5.2).

**Second EndoPAT**

3. Impact of the presence of endothelial dysfunction (RHI<1.67) in the second EndoPAT on the extent of myocardial infarction, evaluated by troponin release, echocardiographic parameters and ceCMR (Section 6.1).
4. Impact of the presence of endothelial dysfunction (RHI<1.67) in the second EndoPAT on microvascular reperfusion indicators, including angiographic indicators (cTFC and TMPG), ECG (ST resolution) and ceCMR (microvascular obstruction) (Section 6.2).

**Index of microcirculatory resistance (IMR)**

5. Relation between IMR and patient baseline characteristics (Section 7.1)
6. Impact of the presence of coronary microvascular damage as evaluated by an IMR>24 (median value) on the extent of myocardial infarction, evaluated by troponin release, echocardiographic parameters and ceCMR (Section 7.2).
7. Relation between the presence of coronary microvascular damage as evaluated by an IMR>24 (median value) on microvascular reperfusion indicators, including angiographic indicators (cTFC and TMPG), ECG (ST resolution) and ceCMR (microvascular obstruction) (Section 7.3).

# 3. Primary outcome – IMR and RHI values on the first EndoPAT

## 3.1. IMR values in patients with and without endothelial dysfunction (RHI<1.67) on the first EndoPAT

Endothelial dysfunction, as evaluated by an RHI<1.67, was present in 11/60 patients (18.3%) in the first EndoPAT evaluation. In the following pages, the main patients characteristics according to the presence of endothelial dysfunction will be shortly described, followed by the IMR analysis.

### 3.1.1. Main characteristics of patients according to the presence of endothelial dysfunction (RHI<1.67)

Table 23 summarizes the main characteristics of patients with and without endothelial dysfunction (RHI<1.67) in the first EndoPAT evaluation.

**Table 23 – Main characteristics of patients according to the presence of endothelial dysfunction (RHI<1.67) in the first EndoPAT evaluation**

| Variable | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| **Physical characteristics** | | | | |
| Age (years) [a] | 59.6±12.7 | 59.7±13.4 | 59.1±9.5 | 0.89 |
| Male gender [b] | 48 (80.0) | 39 (79.6) | 9 (81.8) | 0.80 [d] |
| BMI [a] | 27.5±4.0 | 27.5±4.0 | 27.4±4.2 | 0.94 |
| Waist circumference [a] | 99.2±12.2 | 98.9±12.0 | 100.8±14.0 | 0.68 |
| **Risk Factors and previous coronary disease** [b] | | | | |
| Hypertension | 43 (71.7) | 34 (69.4) | 9 (81.8) | 0.21 [d] |
| Diabetes | 15 (25.0) | 10 (20.4) | 5 (45.5) | 0.18 [d] |
| Dyslipidaemia | 30 (50.0) | 25 (51.0) | 5 (45.5) | 0.74 |
| Active smoking | 26 (43.3) | 17 (34.7) | 9 (81.8) | 0.01 [d] |
| Previous angina | 9 (15.0) | 7 (14.3) | 2 (18.2) | 0.89 [d] |
| Previous revascularization | 2 (3.3) | 1 (2.0) | 1 (9.1) | 0.80 [d] |
| **Previous medication** [b] | | | | |
| Aspirin | 5 (8.3) | 3 (6.2) | 2 (18.2) | 0.50 [d] |
| Clopidogrel | 1 (1.7) | 1 (2.1) | 0 (0.0) | 0.42 [d] |
| ACEi/ARBs | 23 (38.3) | 17 (35.4) | 6 (54.5) | 0.41 [d] |
| Beta-blockers | 4 (6.7) | 1 (2.1) | 3 (27.3) | 0.04 [d] |
| Nitrates | 1 (1.7) | 0 (0.0) | 0 (0.0) | - |
| Statins | 9 (15.0) | 6 (12.5) | 3 (27.3) | 0.44 [d] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Independent t-test for continuous variables, Chi-Square for categorical variables; [d] Yates correction

Patients whit endothelial dysfunction were more frequently active smokers (81.8% vs. 34.7%, p=0.01), but there were no other significant differences in physical characteristics or in other risk factors between the two groups. Patients with endothelial dysfunction were more likely to be on beta-blocker treatment, but the numbers were too small (1 vs. 3 patients) to allow for any valid conclusion. There were no other significant differences in previous treatment, including anti-platelets and statins.

Blood tests on admission, including creatinine, NT-pro-BNP, hsCRP, glucose and HbA1c, were also similar between both populations (Table 24).

**Table 24 – Laboratory results on admission according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| Creatinine (mg/dL) [b] | 0.90 (0.31) | 0.90 (0.35) | 0.89 (0.16) | 0.51 |
| NT-pro-BNP (pg/mL) [b] | 137.5 (255.5) | 137.5 (256.3) | 191.3 (265.8) | 0.99 |
| hsCRP (mg(dL) [b] | 0.44 (0.59) | 0.40 (0.58) | 0.50 (1.11) | 0.46 |
| Glucose (mg/dL) [b] | 133.5 (61.0) | 131.0 (60.0) | 139.0 (64.0) | 0.77 |
| HbA1c (%)[b] | 5.7 (1.1) | 5.7 (0.7) | 5.7 (1.4) | 0.91 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution.

### 3.1.2. Angiography and angioplasty variables according to the presence of endothelial dysfunction (RHI<1.67)

There were no significant differences in ischemic (pain-to-balloon) or hospital-to-balloon times between patients with and without endothelial dysfunction. Main angiographic characteristics, including culprit artery, presence of multivessel disease, Syntax score and initial TIMI flow were also similar in both groups (Table 25).

The area-at-risk, measured both by the APPROACH and BARI scores, was also similar.

Finally, treatment options (use of mechanical aspiration, stent implantation technique and use of abciximab) were similar in patients with and without RHI<1.67 (Table 26).

**Table 25 – Ischemic times and angiographic characteristics according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| Pain-to-balloon time (min)[c] | 209 (148) | 210 (156) | 174 (128) | 0.52 |
| Door-to-balloon time (min) [c] | 78 (45) | 76 (49) | 80 (59) | 0.37 |
| Culprit artery [b] | | | | |
| Left anterior descending | 28 (46.7) | 24 (49.0) | 4 (36.4) | |
| Left circumflex | 13 (21.7) | 10 (20.4) | 3 (27.3) | 0.95 [e] |
| Right coronary artery | 19 (31.7) | 15 (30.6) | 4 (36.4) | |
| Multivessel disease [b] | 25 (41.7) | 22 (44.9) | 3 (27.3) | 0.54 [e] |
| Syntax score [c] | 15.5 (10.0) | 15.5 (11.0) | 16.5 (9.0) | 0.72 |
| Area at risk scores [a] | | | | |
| APPROACH score | 28.5±6.6 | 28.1±6.9 | 30.2±5.5 | 0.33 |
| BARI score | 27.7±6.3 | 27.4±6.4 | 30.6±5.4 | 0.13 |
| Initial TIMI flow 0-1 [b] | 50 (83.3) | 41 (83.7) | 9 (81.8) | 0.77 [e] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction

**Table 26 – Angioplasty treatment options according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable [a] | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value [b] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| Mechanical aspiration | 26 (43.3) | 21 (42.9) | 5 (45.5) | 0.86 |
| Balloon pre-dilatation | 35 (58.3) | 29 (59.2) | 6 (54.5) | 0.96 |
| Stent implantation | 57 (95) | 46 (93.9) | 11 (100.0) | 0.94 |
| Balloon post-dilatation | 23 (38.3) | 20 (40.5) | 3 (27.3) | 0.62 |
| Abciximab treatment | 14 (23.3) | 9 (18.4) | 5 (45.5) | 0.13 |

[a] Presented as number (%); [b] Chi-Square test, with Yates correction

### 3.1.3. IMR according to the presence of endothelial dysfunction (RHI<1.67)

IMR median values did not differ (and actually tended to be lower) between patients with and without endothelial dysfunction on the first EndoPAT evaluation (Figure 21, Table 27).

There was also no difference in the prevalence of microvascular coronary damage, whether this was defined as an IMR >24 (median value) or an IMR >40 (value published in the literature as prognostic marker in STEMI patients). Finally, coronary flow reserve (CFR) values were also similar in both groups (Table 27)

**Table 27 – Invasive hemodynamic measurements according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable [a] | Total Population (n=60) | Endothelial Dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| IMR [c] | 23.9 (32.9) | 24.0 (31.2) | 16.0 (37.3) | 0.17 |
| IMR < 24 (median) [b] | 30 (50) | 23 (46.9) | 7 (63.6) | 0.32 |
| IMR < 40 [b] | 21 (35) | 31 (63.9) | 8 (72.7) | 0.55 |
| Coronary flow reserve [c] | 1.1 (0.8) | 1.1 (0.8) | 1.2 (2.0) | 0.45 |
| Basal SBP [a] | 111.4±28.3 | 108.7±28.1 | 122.5±27.6 | 0.15 |
| Basal DBP [a] | 61.5±13.8 | 60.3±14.3 | 66.2±10.3 | 0.21 |
| Hyperaemic SBP [a] | 91.6±22.5 | 91.0±23.6 | 94.2±18.2 | 0.68 |
| Hyperaemic DBP [a] | 51.3±13.2 | 50.1±13.2 | 56.2±12.3 | 0.63 |
| Aortic pressure [a] | 65.3±15.7 | 64.1±16.0 | 69.8±14.2 | 0.13 |
| Distal pressure [a] | 71.8±23.1 | 69.5±22.8 | 81.1±22.8 | 0.29 |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. Chi-Square for categorical variables

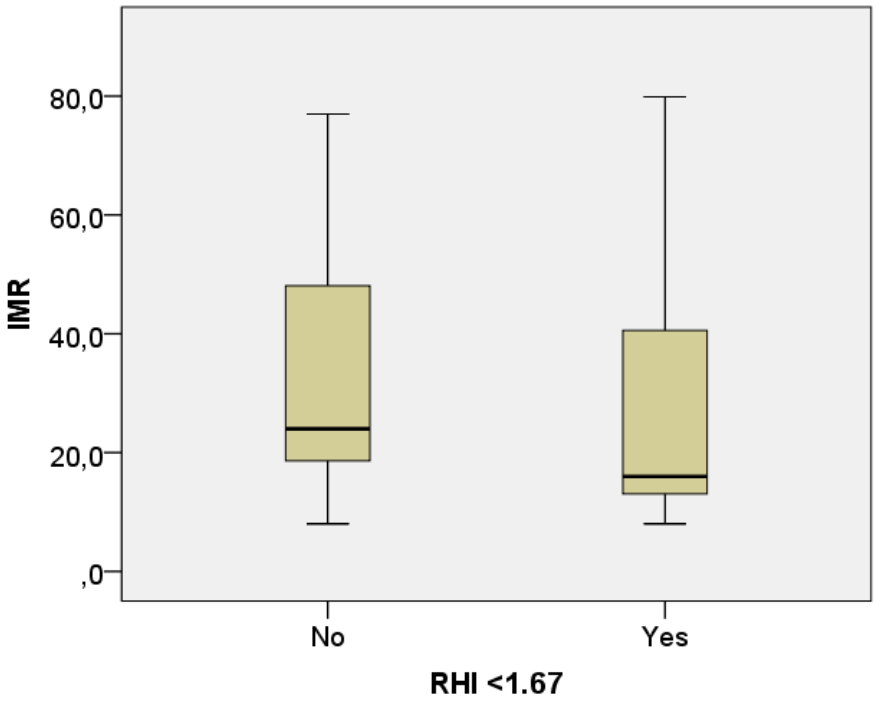



**Figure 21 – Boxplot of IMR values according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

**In summary**, there were no significant differences in IMR values in patients with and without RHI<1.67 on the first EndoPAT evaluation. With the exception of active smoking and previous beta-blocker therapy, endothelial dysfunction also did not relate to any patient baseline characteristics, including age, risk factors, blood tests on admission, coronary anatomy or procedural aspects of the P-PCI.

## 3.2. IMR values according to RHI tertiles on the first EndoPAT

According to the defined protocol, RHI was divided in tertiles:

- Tertile 1 (n=20): RHI< 1.92
- Tertile 2 (n=20): RHI 1.92 – 2.30
- Tertile 3 (n=20): RHI> 2.30.

In the following pages, the main patient characteristics according to RHI tertiles will be shortly described, followed by the IMR analysis in these groups.

### 3.2.1. Main characteristics of patients according to tertiles of RHI

Table 28 summarizes the main characteristics of patients according to RHI tertiles on the first EndoPAT evaluation. There were no significant differences in any of the analysed variables, including physical characteristics, risk factors and previous pharmacological treatment. Blood tests results on admission were also similar (Table 29).

**Table 28 – Main characteristics of patients according to RHI tertiles on the first EndoPAT evaluation**

| Variable | Total population (n=60) | RHI | | | *p* value[c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=20) | Tertile 2 (n=20) | Tertile 3 (n=20) | |
| **Physical characteristics** | | | | | |
| Age (years) [a] | 59.6±12.7 | 58.9±11.6 | 58.8±15.1 | 61.0±11.4 | 0.83 |
| Male gender [b] | 48 (80.0) | 16 (80.0) | 15 (75.0) | 17 (85.0) | 0.89 [d] |
| BMI [a] | 27.5±4.0 | 27.6±3.93 | 28.4±4.02 | 26.5±3.99 | 0.33 |
| Waist circumference [a] | 99.2±12.2 | 100.4±14.1 | 102.4±13.4 | 99.2±12.2 | 0.25 |
| **Risk factors and previous coronary disease** [b] | | | | | |
| Hypertension | 43 (71.7) | 16 (80.0) | 13 (65.0) | 14 (70.0) | 0.56 |
| Diabetes | 15 (25.0) | 6 (30.0) | 5 (25.0) | 4 (20.0) | 0.77 |
| Dyslipidaemia | 30 (50.0) | 9 (45.0) | 10 (50.0) | 11 (55.0) | 0.82 |
| Active smoking | 26 (43.3) | 12 (60.0) | 7 (35.0) | 7 (35.0) | 0.18 |
| Previous angina | 9 (15.0) | 4 (20.0) | 2 (10.0) | 3 (15.0) | 0.86 [d] |
| Previous revascularization | 2 (3.3) | 1 (5.0) | 1 (5.0) | 0 (0.0) | 0.94 [d] |
| **Previous medication** [b] | | | | | |
| Aspirin | 5 (8.3) | 2 (10.0) | 1 (5.0) | 2 (10.0) | 0.97 [d] |
| ACEi/ARBs | 23 (38.3) | 9 (45.0) | 7 (35.0) | 7 (35.0) | 0.79 |
| Beta-blockers | 4 (6.7) | 3 (15.0) | 1 (5.0) | 0 (0.0) | 0.44 [d] |
| Statins | 9 (15.0) | 4 (20.0) | 3 (15.0) | 2 (10.0) | 0.88 [d] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] One-way ANOVA for continuous variables, Chi-Square for categorical variables; [d] Yates correction

**Table 29 – Laboratory results on admission according to RHI tertiles on the first EndoPAT evaluation**

| Variable | Total Population (n=60) | RHI | | | *p* value[c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=20) | Tertile 2 (n=20) | Tertile 3 (n=20) | |
| Creatinine (mg/dL) [b] | 0.90 (0.31) | 0.90 (0.17) | 0.90 (0.40) | 0.96 (0.36) | 0.62 |
| NT-pro-BNP (pg/mL) [b] | 137.5 (255.5) | 162.0 (269.0) | 113.0 (170.5) | 131.5 (298.0) | 0.64 |
| hs-CRP (mg(dL) [b] | 0.44 (0.59) | 0.64 (0.71) | 0.29 (0.35) | 0.44 (0.95) | 0.10 |
| Glucose (mg/dL) [b] | 133.5 (61.0) | 122.0 (54.0) | 141.5 (60.0) | 131.0 (61.0) | 0.63 |
| HbA1c (%)[b] | 5.7 (1.1) | 5.5 (0.9) | 5.8 (1.9) | 5.7 (2.3) | 0.44 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] One-way ANOVA for continuous variables with a normal distribution, Kruskal-Wallis test for continuous variables with a non-normal distribution.

### 3.2.2. Angiography and angioplasty variables according to RHI tertiles

There were no significant differences in pain-to-balloon or hospital-to-balloon times in RHI tertiles groups. Main angiographic characteristics, including culprit artery, presence of multivessel disease, Syntax score and initial TIMI flow were also similar (Table 30).

**Table 30 – Ischemic times and angiographic characteristics according RHI tertiles on the first EndoPAT evaluation**

| Variable | Total Population (n=60) | RHI | | | *p* value[d] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=20) | Tertile 2 (n=20) | Tertile 3 (n=20) | |
| Pain-to-balloon time (min)[c] | 209 (148) | 223 (149) | 201 (179) | 200 (102) | 0.85 |
| Door-to-balloon time (min)[c] | 78 (45) | 82 (45) | 67 (55) | 76 (43) | 0.43 |
| Culprit artery [b] | | | | | |
| Left anterior descending | 28 (46.7) | 10 (50.0) | 8 (40.0) | 10 (50.0) | |
| Left circumflex | 13 (21.7) | 4 (20.0) | 5 (25.0) | 4 (20.0) | 0.99 [e] |
| Right coronary artery | 19 (31.7) | 6 (30.0) | 7 (35.0) | 6 (30.0) | |
| Multivessel disease [b] | 25 (41.7) | 11 (55.0) | 8 (40.0) | 6 (30.0) | 0.28 |
| Syntax score [c] | 15.5 (10.0) | 16.0 (9.0) | 11.8 (12.0) | 15.8 (7.0) | 0.95 |
| Area at risk scores | | | | | |
| APPROACH score [a] | 27.8 (2.0) | 27.8 (4.0) | 27.8 (4.0) | 27.8 (2.0) | 0.63 |
| BARI score [a] | 27.7±6.3 | 29.3±7.3 | 28.0±5.7 | 26.7±6.0 | 0.46 |
| Initial TIMI flow 0-1 [b] | 50 (83.3) | 17 (85.0) | 19 (95.0) | 14 (70.0) | 0.23 [e] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] One-way ANOVA for continuous variables with a normal distribution, Kruskal-Wallis test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction

The area-at-risk, measured both by the APPROACH and BARI scores, was also similar in the three tertiles. Finally, treatment options (use of mechanical aspiration, stent implantation technique and use of abciximab) was again, similar (Table 31).

**Table 31 – Angioplasty treatment options according to RHI tertiles on the first EndoPAT evaluation**

| Variable [a] | Total population (n=60) | RHI | | | *p* value [b] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=20) | Tertile 2 (n=20) | Tertile 3 (n=20) | |
| Mechanical aspiration | 26 (43.3) | 8 (40.0) | 8 (40.0) | 10 (50.0) | 0.76 |
| Balloon pre-dilatation | 35 (58.3) | 12 (60.0) | 12 (60.0) | 11 (55.0) | 0.93 |
| Stent implantation | 57 (95) | 19 (95.0) | 19 (95.0) | 19 (95.0) | 0.67 [c] |
| Balloon post-dilatation | 23 (38.3) | 7 (35.0) | 8 (40.0) | 8 (40.0) | 0.93 |
| Abciximab | 14 (23.3) | 8 (40.0) | 2 (10.0) | 4 (20.0) | 0.17 |

[a] Presented as number (%); [b] Chi-Square test; [c] Yates correction

### 3.2.3. IMR according to RHI tertiles

IMR median values did not differ between tertiles of RHI. The number of patients with coronary microvascular dysfunction (IMR>24 or IMR>40) was also similar in the three groups. All other invasive measurements were similar across RHI tertiles (Table 32).

**Table 32 – Invasive hemodynamic measurements according to RHI tertiles on the first EndoPAT evaluation**

| Variable | Total population (n=60) | RHI | | | *p* value [c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=20) | Tertile 2 (n=20) | Tertile 3 (n=20) | |
| IMR [a] | 23.9 (32.9) | 19.4 (35.0) | 40.5 (31.2) | 23.3 (30.3) | 0.26 |
| IMR < 24 (median) [b] | 30 (50) | 9 (45.0) | 13 (65.0) | 8 (40.8) | 0.25 |
| IMR < 40 [b] | 21 (35) | 6 (30.0) | 10 (50.0) | 5 (25.0) | 0.22 |
| Coronary flow reserve [a] | 1.1 (0.8) | 1.3 (0.84) | 1.0 (0.50) | 1.1 (0.9) | 0.36 |

[a] Presented as median (interquartile range); [b] Presented as number (%); [c] Mann-Whitney test for continuous variables with a non-normal distribution. Chi-Square for categorical variables.

**In summary**, there were no significant differences in IMR values between tertiles of RHI measured on the first EndoPAT. RHI tertiles did not relate either to any baseline patient characteristics, including age, risk factors, blood test results on admission, coronary anatomy or procedural aspects of the P-PCI.

## 3.3. Relation between RHI as a continuous variable on the first EndoPAT and IMR values

### 3.3.1. RHI as a continuous variable and main baseline patient characteristics

No significant associations were found between RHI and any baseline patient characteristics, including age, physical characteristics, risk factors, previous medication and blood tests on admission (Table 33, Table 34).

**Table 33 – Correlation between RHI on the first EndoPAT and baseline continuous variables**

| Variables | Correlation (R) | *p* value [a] |
|---|---|---|
| **Physical characteristics** | | |
| Age | 0.110 | 0.402 |
| BMI | -0.116 | 0.379 |
| Waist circumference | -0.186 | 0.210 |
| **Laboratory parameters on admission** | | |
| Creatinine (mg/dl) | 0.120 | 0.360 |
| NT-pro-BNP (pg/mL) | -0.123 | 0.376 |
| hs-CRP (mg/dL) | 0.007 | 0.960 |
| Glucose (mg/dL) | -0.036 | 0.795 |
| HbA1c (%) | 0.072 | 0.622 |

[a] Spearmans's rho

**Table 34 – RHI values on the first EndoPAT according to baseline categorical variables**

| Variables | Variable present? [a] | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| **Physical characteristics** | | | |
| Male gender | 1.99±0.61 | 2.19±0.57 | 0.280 |
| **Risk factors and previous coronary disease** | | | |
| Hypertension (43/60) | 2.16±0.53 | 2.15±0.60 | 0.91 |
| Diabetes (15/60) | 2.17±0.59 | 2.09±0.56 | 0.62 |
| Dyslipidaemia (30/60) | 2.07±0.58 | 2.24±0.57 | 0.26 |
| Active smoking (26/609) | 2.27±0.52 | 1.99±0.62 | 0.06 |
| Previous angina (9/60) | 2.14±0.55 | 2.21±0.75 | 0.71 |
| Previous revascularization (2/60) | 2.16±0.58 | 1.83±0.18 | 0.42 |
| **Previous medication** | | | |
| Aspirin (5/60) | 2.15±0.58 | 2.18±0.73 | 0.92 |
| ACEi/ARBs (23/60) | 2.17±0.50 | 2.12±0.70 | 0.71 |
| Beta-blockers (4/60) | 2.18±0.59 | 1.70±0.23 | 0.11 |
| Statins (9/60) | 2.18±0.61 | 1.97±0.40 | 0.32 |

[a] RHI presented as mean±standard deviation; [b] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution

### 3.3.2. RHI as a continuous variable and angiography/angioplasty variables

No significant associations were found between RHI and angiographic or primary PCI variables (Table 35, Table 36).

**Table 35 – Correlation between RHI on the first EndoPAT and angiography and P-PCI related continuous variables**

| Variables | Correlation (R) | *p* value [a] |
|---|---|---|
| Pain-to-balloon time (min) | -0.012 | 0.925 |
| Door-to-balloon time (min) | -0.132 | 0.313 |
| Syntax score | -0.132 | 0.314 |
| Area at risk scores | | |
| APPROACH score | -0.140 | 0.287 |
| BARI score | -0.216 | 0.097 |

[a] Pearson's Correlation for continuous variables with a normal distribution; Spearmans's rho for continuous variables without a normal distribution

**Table 36 – RHI values on the first EndoPAT according to angiography and P-PCI categorical variables**

| Variables | Variable present? [a] | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| **Angiography** | | | |
| Culprit artery = LAD (28/60) | 2.11±0.50 | 2.20±0.66 | 0.57 |
| Multivessel disease (25/60) | 2.07±0.63 | 2.26±0.49 | 0.20 |
| Initial TIMI flow 0-1 (50/60) | 2.51±0.76 | 2.05±0.48 | 0.09 |
| **Primary PCI** | | | |
| Mechanical aspiration (26/60) | 2.15±0.51 | 2.16±0.67 | 0.96 |
| Balloon pre-dilatation (35/60) | 2.22±0.65 | 2.10±0.53 | 0.46 |
| Stent implantation (57/60) | 2.08±0.31 | 2.15±0.59 | 0.83 |
| Balloon post-dilatation (37/60) | 2.18±0.60 | 2.10±0.56 | 0.62 |
| Abciximab use (14/60) | 2.21±0.58 | 1.97±0.57 | 0.19 |

[a] RHI presented as mean±standard deviation; [b] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution.

### 3.3.3. RHI as a continuous variable and IMR

There was no relation between RHI measured on the first EndoPAT and IMR or any of the invasive measurements performed (Table 37). RHI values were similar in patients with and without coronary microvascular dysfunction, according to the IMR cut-offs of 24 and 40 (Table 38).

**Table 37 – RHI on the first EndoPAT and invasive hemodynamic continuous variables**

| Variables | Correlation (R) | *p* value [a] |
|---|---|---|
| IMR | 0.128 | 0.328 |
| Coronary flow reserve | -0.110 | 0.410 |
| Basal SBP | -0.022 | 0.875 |
| Basal DBP | -0.219 | 0.109 |
| Hyperaemic SBP | 0.093 | 0.500 |
| Hyperaemic DBP | -0.054 | 0.695 |
| Aortic pressure | -0.011 | 0.934 |
| Distal pressure | 0.007 | 0.958 |

[a] Pearson's Correlation for continuous variables with a normal distribution; Spearmans's rho for continuous variables without a normal distribution

**Table 38 – RHI values on the first EndoPAT according to IMR thresholds**

| Variables | Variable present? | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| IMR < 24 (median) (30/60) | 2.16±0.58 | 2.14±0.58 | 0.89 |
| IMR < 40 (21/60) | 2.18±0.68 | 2.10±0.34 | 0.64 |

[a] Presented as mean±standard deviation; [b] Mann-Whitney test for continuous variables with a non-normal distribution

**In summary**, there was no relation between RHI as a continuous variable measured on the first EndoPAT and IMR measured immediately after P-PCI. RHI did not relate either to any baseline patient characteristics, including age, risk factors, blood tests on admission, coronary anatomy or procedural aspects of the P-PCI

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# 4. Primary outcome – IMR and RHI values on the second EndoPAT

## 4.1. IMR values in patients with and without endothelial dysfunction (RHI<1.67) on the second EndoPAT

Endothelial dysfunction, as evaluated by an RHI<1.67, was present in 16/38 patients (42.1%) in the second EndoPAT evaluation. In the following pages, the main patient characteristics according to the presence of endothelial dysfunction will be shortly described, followed by the IMR analysis.

### 4.1.1. Main characteristics of patients according to the presence of endothelial dysfunction (RHI<1.67)

Table 39 summarizes the main characteristics of patients with and without endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation.

**Table 39 – Main characteristics of patients according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| **Physical characteristics** | | | | |
| Age (years) [a] | 60.0±13.7 | 60.1±12.4 | 59.8±15.7 | 0.94 |
| Male gender [b] | 29 (76.3) | 18 (81.8) | 11 (68.8) | 0.58 [d] |
| BMI [a] | 27.2±4.0 | 27.4±4.5 | 27.1±3.3 | 0.82 |
| Waist circumference [a] | 100.0±11.5 | 101.1±12.6 | 98.5±9.9 | 0.55 |
| **Risk factors and previous coronary disease** [b] | | | | |
| Hypertension | 26 (68.4) | 16 (72.7) | 10 (62.5) | 0.50 |
| Diabetes | 12 (31.6) | 8 (36.4) | 4 (25.0) | 0.46 |
| Dyslipidaemia | 19 (50.0) | 11 (50.0) | 8 (50.0) | 1.00 |
| Active smoking | 13 (34.2) | 9 (40.9) | 4 (25.0) | 0.31 |
| Previous angina | 7 (18.4) | 6 (27.3) | 1 (6.3) | 0.22 [d] |
| Previous revascularization | 2 (5.3) | 1 (4.5) | 1 (6.3) | 0.61 [d] |
| **Previous medication** [b] | | | | |
| Aspirin | 3 (7.9) | 2 (9.1) | 1 (6.3) | 0.73 [d] |
| Clopidogrel | 0 (0.0) | - | - | - |
| ACEi/ARBs | 13 (34.2) | 10 (45.5) | 3 (18.8) | 0.11 |
| Beta-blockers | 1 (2.6) | 1 (4.5) | 0 (0.0) | 0.85 [d] |
| Nitrates | 0 (0.0) | - | - | - |
| Statins | 4 (10.5) | 2 (9.1) | 2 (12.5) | 0.89 [d] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Independent t-test for continuous variables, Chi-Square for categorical variables; [d] Yates correction

There were no significant differences in physical characteristics, risk factors or previous medication between the two groups.

Blood tests on admission were also similar between both populations (Table 40), except for hs-CRP, which showed a tendency for higher values in patients with endothelial dysfunction.

**Table 40 – Laboratory results on admission according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| Creatinine (mg/dL) [b] | 0.88 (0.32) | 0.87 (0.54) | 0.90 (0.19) | 0.67 |
| NT-pro-BNP (pg/mL) [b] | 158.5 (305.0) | 154 (365) | 163 (250) | 0.95 |
| hs-CRP (mg(dL) [b] | 0.57 (0.71) | 0.29 (0.45) | 0.81 (0.98) | 0.06 |
| Glucose (mg/dL) [b] | 136.0 (63.0) | 131.0 (60.0) | 145.5 (81.8) | 0.51 |
| HbA1c (%)[b] | 5.8 (1.3) | 5.7 (1.4) | 5.9 (2.1) | 0.69 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution.

### 4.1.2. Angiography and angioplasty variables according to the presence of endothelial dysfunction (RHI<1.67)

There were no significant differences in ischemic (pain-to-balloon) time between patients with and without endothelial dysfunction, although there was a trend for lower door-to-balloon times in patients with endothelial dysfunction.

Patients with endothelial dysfunction had a prevalence of multivessel disease that was almost twice the one observed in patients without endothelial dysfunction (68.8% vs. 36.4%), although this difference was not statistically significant (p=0.10). Syntax score also tended to be higher in patients with endothelial dysfunction (Table 41).

There was a clear trend towards higher area-at-risk, both measured by APPROACH and BARI scores (p=0.08 and 0.07, respectively) in patients with RHI<1.67. These patients also showed a trend for worse initial TIMI score (TIMI 0-1: 93.8% vs. 72.7% in patients without endothelial dysfunction).

Finally, treatment options (use of mechanical aspiration, stent implantation technique and use of abciximab) were similar in patients with and without RHA<1.67 (Table 42).

**Table 41 – Ischemic times and angiographic characteristics according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| Pain-to-balloon time (min)[c] | 209 (173) | 209 (186) | 211 (167) | 0.94 |
| Door-to-balloon time (min) [c] | 75 (52) | 79 (46) | 57 (44) | 0.06 |
| Culprit artery [b] | | | | |
| Left anterior descending | 21 (53.3) | 11 (50.0) | 10 (62.5) | |
| Left circumflex | 7 (18.4) | 5 (22.7) | 2 (12.5) | 0.89 [f] |
| Right coronary artery | 10 (26.3) | 6 (27.3) | 4 (25.0) | |
| Multivessel disease [b] | 19 (50.0) | 8 (36.4) | 11 (68.8) | 0.10 [f] |
| SYNTAX score [c] | 17.8±6.2 | 16.8±6.2 | 19.0±6.0 | 0.29 |
| Area at risk scores [a] | | | | |
| APPROACH score | 27.8 (3.0) | 28.1 (5.0) | 29.7 (10.0) | 0.08 |
| BARI score | 28.5 (6.0) | 26.7 (7.0) | 30.2 (10.0) | 0.07 |
| Initial TIMI flow 0-1 [b] | 31 (81.6) | 16 (72.7) | 15 (93.8) | 0.22 |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [E] Yates correction

**Table 42 – Angioplasty treatment options according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable [a] | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [b] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| Mechanical aspiration | 16 (42.1) | 9 (40.9) | 7 (43.8) | 0.86 |
| Balloon pre-dilatation | 21 (55.3) | 13 (59.1) | 8 (50.0) | 0.58 |
| Stent implantation | 36 (94.7) | 20 (90.9) | 16 (100.0) | 0.61 |
| Balloon post-dilatation | 24 (63.2) | 14 (63.6) | 10 (62.5) | 0.94 |
| Abciximab treatment | 9 (23.7) | 4 (18.2) | 5 (31.5) | 0.58 |
| Mechanical aspiration | 16 (42.1) | 9 (40.9) | 7 (43.8) | 0.86 |

[a] Presented as number (%); [b] Chi-Square test, with Yates correction

### 4.1.3. IMR according to the presence of endothelial dysfunction (RHI<1.67)

There was a clear trend towards higher values of IMR in patients with endothelial dysfunction on the second EndoPAT evaluation: median values (IQR) 40.5 (54.4) vs. 22.0 (26.0) in patients without endothelial dysfunction (p=0.09) (Figure 22, Table 43). The prevalence of microvascular coronary damage, either defined as an IMR >24 (median value) or an IMR >40 (value published in the literature as prognostic marker in STEMI patients), was almost 2 times higher in patients with endothelial dysfunction, although differences did not reach statistical significance (Table 43). Other invasive variables, including coronary flow reserve (CFR) values, were similar in both groups.

**Table 43 – Invasive hemodynamic measurements according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable [a] | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| IMR [c] | 23.4 (35.2) | 22.0 (26.0) | 40.5 (54.4) | 0.09 |
| IMR < 24 (median) [b] | 18 (47.4) | 8 (36.4) | 10 (62.5) | 0.11 |
| IMR < 40 [b] | 14 (36.8) | 6 (27.3) | 8 (50.0) | 0.15 |
| Coronary flow reserve [c] | 1.00 (0.70) | 1.10 (0.60) | 1.00 (0.90) | 0.92 |
| Basal SBP [a] | 112.0±31.3 | 113.0±26.3 | 110.4±38.8 | 0.81 |
| Basal DBP [a] | 61.3±14.0 | 59.0±14.0 | 65.0±13.7 | 0.22 |
| Hyperaemic SBP [a] | 92.8±22.0 | 96.1±19.3 | 87.8±25.4 | 0.28 |
| Hyperaemic DBP [a] | 51.7±12.1 | 52.7±11.1 | 50.1±13.8 | 0.56 |
| Aortic pressure [a] | 75.7±25.1 | 74.7±20.3 | 77.2±31.7 | 0.72 |
| Distal pressure [a] | 64.6±14.9 | 67.1±14.3 | 60.9±15.5 | 0.24 |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. Chi-Square for categorical variables.

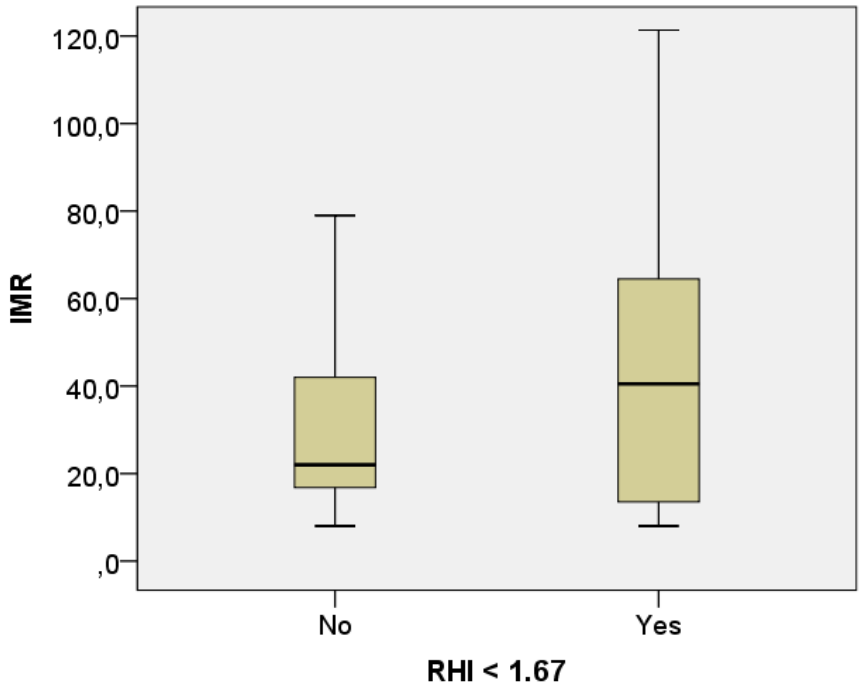


**Figure 22 – Boxplot of IMR values according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

**In summary**, the presence of endothelial dysfunction (defined as an RHI<1.67) on the second EndoPAT was associated with a trend for higher IMR values measured immediately after P-PCI. These patients also tended to have more severe coronary artery disease, higher areas-at-risk and worse initial TIMI flow.

## 4.2. IMR values according to RHI tertiles on the second EndoPAT

According to the defined protocol, RHI in the second endothelial dysfunction was divided in tertiles:

- Tertile 1 (n=13): RHI< 1.62
- Tertile 2 (n=13): RHI 1.62 – 1.96
- Tertile 3 (n=12): RHI> 1.96 .

In the following pages, main patient characteristics according to RHI tertiles will be shortly described, followed by the IMR analysis in these groups.

### 4.2.1. Main characteristics of patients according to tertiles of RHI

Table 44 summarizes the main characteristics of patients according to RHI tertiles on the second EndoPAT evaluation. There was a trend towards more male patients and more previous use of ACEi/ARBs for higher tertiles of RHI. There were no significant differences in any of the other variables, including physical characteristics, risk factors and other previous pharmacological treatment. Blood tests on admission (Table 45) were also similar.

**Table 44 – Main characteristics of patients according to RHI tertiles on the second EndoPAT evaluation**

| Variable | Total population (n=38) | RHI | | | *p* value[c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=13) | Tertile 2 (n=13) | Tertile 3 (n=12) | |
| **Physical characteristics** | | | | | |
| Age (years) [a] | 60.0±13.7 | 62.8±14.4 | 54.8±13.6 | 62.6±12.3 | 0.25 |
| Male gender [b] | 29 (76.3) | 8 (61.5) | 10 (76.9) | 11 (91.7) | 0.41 [d] |
| BMI [a] | 27.2±4.0 | 26.3±3.2 | 28.9±3.9 | 26.4±4.5 | 0.17 |
| Waist circumference [a] | 100.0±11.5 | 96.8±10.6 | 102.8±12.9 | 100.0±10.8 | 0.50 |
| **Risk Factors and previous coronary disease** [b] | | | | | |
| Hypertension | 26 (68.4) | 8 (61.5) | 9 (69.2) | 9 (75.0) | 0.93 [d] |
| Diabetes | 12 (31.6) | 3 (23.1) | 7 (53.8) | 2 (16.7) | 0.24 [d] |
| Dyslipidaemia | 19 (50.0) | 7 (53.8) | 5 (38.5) | 7 (58.3) | 0.58 |
| Active smoking | 13 (34.2) | 3 (23.1) | 6 (46.2) | 4 (33.3) | 0.69 [d] |
| Previous angina | 7 (18.4) | 1 (7.7) | 2 (15.4) | 4 (33.0) | 0.51 [d] |
| Previous revascularization | 2 (5.3) | 1 (7.7) | 0 (0.0) | 1 (8.3) | 0.94 [d] |
| **Previous medication** [b] | | | | | |
| Aspirin | 3 (8.1) | 1 (7.7) | 0 (0.0) | 2 (16.7) | 0.64 [d] |
| ACEi/ARBs | 13 (35.1) | 2 (15.4) | 5 (38.5) | 6 (50.0) | 0.43 [d] |
| Beta-blockers | 1 (2.7) | 0 (0.0) | 0 (0.0) | 1 (8.3) | 0.88 [d] |
| Statins | 4 (10.8) | 1 (7.7) | 3 (23.1) | 0 (0.0) | 0.46 [d] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] One-way ANOVA for continuous variables, Chi-Square for categorical variables; [d] Yates correction

**Table 45 –Laboratory results on admission according to RHI tertiles on the second EndoPAT evaluation**

| Variable | Total population (n=38) | RHI | | | *p* value[c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=13) | Tertile 2 (n=13) | Tertile 3 (n=12) | |
| Creatinine (mg/dL) [b] | 0.91±0.28 | 0.87±0.16 | 0.95±0.32 | 0.90±0.34 | 0.72 |
| NT-pro-BNP (pg/mL) [b] | 158.5 (305) | 163.0 (204.0) | 299.0 (290.0) | 103.0 (434.8) | 0.94 |
| hs-CRP (mg(dL) [b] | 0.57 (0.71) | 0.60 (0.99) | 0.95 (0.72) | 0.29 (0.14) | 0.12 |
| Glucose (mg/dL) [b] | 136.0 (63.0) | 136.0 (108.5) | 169.0 (132.5) | 122.0 (43.8) | 0.29 |
| HbA1c (%)[b] | 6.6 (1.3) | 5.8 (0.9) | 6.3 (4.3) | 5.7 (0.6) | 0.37 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] One-way ANOVA for continuous variables with a normal distribution, Kruskal-Wallis test for continuous variables with a non-normal distribution.

### 4.2.2. Angiography and angioplasty variables according to RHI tertiles

There were no significant differences in pain-to-balloon or hospital-to-balloon times in RHI tertiles groups. There was a clear trend towards more complex coronary disease in lower tertiles of RHI: multivessel disease was present in 75.0% of patients in the lower tertile, as compared to 30.8% in patients in the upper tertile. Syntax score also tended to be higher in lower tertiles of RHI (Table 46).

**Table 46 – Ischemic times and angiographic characteristics according RHI tertiles on the second EndoPAT evaluation**

| Variable | Total population (n=38) | RHI | | | *p* value[d] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=13) | Tertile 2 (n=13) | Tertile 3 (n=12) | |
| Pain-to-balloon time (min)[c] | 209 (173) | 257 (173) | 235 (185) | 247 (155) | 0.69 |
| Door-to-balloon time (min)[c] | 75 (52) | 55 (44) | 63 (51) | 82 (45) | 0.52 |
| Culprit artery [b] | | | | | |
| Left anterior descending | 21 (55.5) | 9 (69.2) | 8 (61.5) | 4 (33.3) | |
| Left circumflex | 7 (18.4) | 1 (7.7) | 3 (23.1) | 3 (25.0) | 0.61 [e] |
| Right coronary | 10 (26.3) | 3 (23.1) | 2 (15.4) | 5 (41.7) | |
| Multivessel disease [b] | 19 (50.0) | 9 (75.0) | 6 (46.2) | 4 (30.8) | 0.08 |
| Syntax score [c] | 17.8±6.1 | 20.4±5.7 | 17.0±6.0 | 15.8±6.4 | 0.15 |
| Area at risk scores | | | | | |
| APPROACH score [c] | 27.8 (3.0) | 29.7 (10.0) | 29.7 (10.0) | 27.7 (8.0) | 0.07 |
| BARI score [a] | 29.1±6.7 | 31.1±7.4 | 31.4±5.3 | 24.5±5.1 | 0.011 |
| Initial TIMI flow 0-1 [b] | 31 (81.6) | 12 (92.3) | 10 (76.9) | 9 (75.0) | 0.79 [e] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] One-way ANOVA for continuous variables with a normal distribution, Kruskal-Wallis test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction

The area-at-at risk evaluated by the BARI score was significantly lower in patients in the third tertile (higher values) of RHI and a similar trend was found when the APPROACH score was used (p=0.07). Initial unfavourable TIMI flow (0-1) was also more prevalent in the lowest RHI tertile, although the difference was not statistically significant (Table 46). Finally, treatment options (use of mechanical aspiration, stent implantation technique or use of abciximab) was similar between the 3 groups (Table 47).

**Table 47 – Angioplasty treatment options according to RHI tertiles on the second EndoPAT evaluation**

| Variable [a] | Total population (n=38) | RHI | | | *p* value [b] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=13) | Tertile 2 (n=13) | Tertile 3 (n=12) | |
| Mechanical aspiration | 16 (42.1) | 5 (38.5) | 5 (38.5) | 6 (50.0) | 0.80 |
| Balloon pre-dilatation | 21 (55.3) | 7 (53.8) | 6 (46.2) | 8 (66.7) | 0.58 |
| Stent implantation | 36 (94.7) | 13 (100.0) | 12 (92.3) | 11 (91.7) | 0.94 [c] |
| Balloon post-dilatation | 14 (36.8) | 4 (30.8) | 7 (53.8) | 3 (25.0) | 0.52 [c] |
| Abciximab | 9 (23.7) | 5 (38.5) | 2 (15.4) | 2 (16.7) | 0.59 [c] |

[a] Presented as number (%); [b] Chi-Square test; [c] Yates correction

### 4.2.3. IMR according to RHI tertiles

IMR median values were lower in the third tertile of RHI, although this difference was not statistically significant. The number of patients with coronary microvascular dysfunction (IMR>24 or IMR>40) also decreased from the first to the third tertile of RHI. CRF were similar between groups (Table 48).

**Table 48 – Invasive hemodynamic measurements according to RHI tertiles on the second EndoPAT evaluation**

| Variable | Total population (n=38) | RHI | | | *p* value [c] |
|---|---|---|---|---|---|
| | | Tertile 1 (n=13) | Tertile 2 (n=13) | Tertile 3 (n=12) | |
| IMR [a] | 23.4 (35.2) | 39.0 (43.4) | 23.8 (42.5) | 19.5 (30.6) | 0.64 |
| IMR < 24 (median) [b] | 18 (47.4) | 8 (61.5) | 6 (46.2) | 4 (33.3) | 0.37 |
| IMR < 40 [b] | 14 (36.8) | 6 (46.2) | 5 (38.5) | 3 (25.0) | 0.78 [c] |
| Coronary flow reserve [a] | 1.1 (0.8) | 1.3 (0.84) | 1.0 (0.50) | 1.1 (0.9) | 0.36 |

[a] Presented as median (interquartile range); [b] Presented as number (%); [c] Mann-Whitney test for continuous variables with a non-normal distribution. Chi-Square for categorical variables

**In summary**, there was a trend for lower IMR values in third tertile of RHI measured on the second EndoPAT. These patients also had lower areas-at-risk and a trend to less complex coronary artery disease (lower Syntax and lower prevalence of multivessel disease)

## 4.3. Relation between RHI as a continuous variable on the second EndoPAT and IMR values

### 4.3.1. RHI as a continuous variable and main baseline patient characteristics

Male patients had significantly higher RHI values on the second EndoPAT evaluation (Table 49).

**Table 49 – Correlations between RHI on the second EndoPAT and baseline continuous variables**

| Variable | Correlation (R) | *p* value [a] |
|---|---|---|
| **Physical characteristics** | | |
| Age | -0.006 | 0.970 |
| BMI | 0.033 | 0.970 |
| Waist circumference | 0.132 | 0.470 |
| **Admission laboratory parameters** | | |
| Creatinine (mg/dl) | 0.030 | 0.258 |
| NT-pro-BNP (pg/mL) | -0.193 | 0.259 |
| hs-CRP (mg/dL) | 0.249 | 0.131 |
| Glucose (mg/dL) | -0.107 | 0.528 |
| HbA1c (%) | 0.008 | 0.965 |

[a] Spearmans's rho

**Table 50 – RHI values on the second EndoPAT according to baseline categorical variables**

| Variable | Variable present? [a] | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| **Physical characteristics** | | | |
| Male gender (29/38) | 1.43±0.53 | 2.00±0.56 | 0.024 |
| **Risk factors and previous coronary disease** | | | |
| Hypertension (26/38) | 1.84±0.46 | 1.88±0.67 | 0.87 |
| Diabetes (12/38) | 1.92±0.68 | 1.76±0.38 | 0.46 |
| Dyslipidaemia (19/38) | 1.84±0.65 | 1.89±0.56 | 0.78 |
| Active smoking (13/38) | 1.86±0.70 | 1.87±0.37 | 0.99 |
| Previous angina (7/38) | 1.84±0.64 | 1.98±0.41 | 0.58 |
| Previous revascularization (2/38) | 1.89±0.59 | 1.50±0.99 | 0.38 |
| **Previous medication** | | | |
| Aspirin (3/38) | 1.85±0.53 | 2.16±1.35 | 0.40 |
| ACEi/ARBs (13/38) | 1.72±0.49 | 2.16±0.71 | 0.042 |
| Statins (9/38) | 1.71±0.10 | 1.89±0.64 | 0.57 |

[a] RHI presented as mean±standard deviation; [b] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution

No significant associations were found between RHI and any other baseline patient characteristics, including age, physical characteristics, risk factors and blood tests on admission (Table 49, Table 50). However, diabetic patients and patients with previous coronary revascularization tended to have lower RHI values.

Patients treated previously with ACEi/ARBs had significantly higher RHI values, and those treated previously with statins and aspirin also showed a trend for higher RHI values (Table 50).

### 4.3.2. RHI as a continuous variable and angiography/angioplasty variables

There were no significant associations between RHI measured on the second EndoPAT and ischemic times. RHI tended to be lower in patients with multivessel disease, lower initial TIMI flow and LAD as the culprit artery (Table 51, Table 52).

**Table 51 – Correlations between RHI on the second EndoPAT and angiography and P-PCI related continuous variables**

| Variable | Correlation (R) | *p* value [a] |
|---|---|---|
| Pain-to-balloon time (min) | -0.107 | 0.523 |
| Door-to-balloon time (min) | 0.263 | 0.111 |
| Syntax score | -0.241 | 0.144 |
| Area at risk scores | | |
| APPROACH score | -0.426 | 0.008 |
| BARI score | -0.361 | 0.026 |

[a] Pearson's Correlation for continuous variables with a normal distribution; Spearmans's rho for continuous variables without a normal distribution

**Table 52 – RHI values on the second EndoPAT according to angiography and P-PCI categorical variables**

| Variable | Variable present? [a] | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| **Angiography** | | | |
| Culprit artery = LAD (21/38) | 2.02±0.51 | 1.74±0.65 | 0.15 |
| Multivessel disease (20/38) | 2.00±0.61 | 1.71±0.58 | 0.13 |
| Initial TIMI flow 0-1 (29/38) | 2.05±0.43 | 1.81±0.64 | 0.30 |
| **Primary PCI** | | | |
| Mechanical aspiration (16/38) | 1.79±0.61 | 1.96±0.60 | 0.40 |
| Balloon pre-dilatation (21/38) | 1.84±0.52 | 1.89±0.68 | 0.79 |
| Stent implantation (36/38) | 2.71±1.24 | 1.82±0.54 | 0.21 |
| Balloon post-dilatation (14/38) | 1.80±0.58 | 1.98±0.65 | 0.39 |
| Abciximab use (9/38) | 1.90±0.68 | 1.76±0.25 | 0.57 |

[a] RHI presented as mean±standard deviation; [b] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution

There was a significant negative correlation between both area-at-risk-scores and RHI, suggesting that patients with higher areas at risk had lower RHI values on the second EndoPAT evaluation (Table 51).

### 4.3.3. RHI as a continuous variable and IMR

There was no relation between RHI measured on the second EndoPAT and IMR or any of the invasive measurements performed (Table 53). However, there was a trend for lower RHI values in patients with coronary microvascular dysfunction, according to the IMR cut-offs of 24 and 40 (Table 54).

**Table 53 – RHI on the second EndoPAT and invasive hemodynamic continuous variables**

| Variable | Correlation (R) | *p* value [a] |
|---|---|---|
| IMR | -0.090 | 0.593 |
| Coronary flow reserve | 0.018 | 0.917 |
| Basal SBP | 0.016 | 0.925 |
| Basal DBP | -0.227 | 0.184 |
| Hyperaemic SBP | 0.200 | 0.250 |
| Hyperaemic DBP | 0.037 | 0.833 |
| Aortic pressure | 0.181 | 0.298 |
| Distal pressure | -0.030 | 0.862 |

[a] Pearson's Correlation for continuous variables with a normal distribution; Spearmans's rho for continuous variables without a normal distribution

**Table 54 – RHI values on the second EndoPAT according to IMR thresholds**

| Variable | Variable present? | | *p* value [b] |
|---|---|---|---|
| | No | Yes | |
| IMR < 24 (median) (18/38) | 1.92±0.59 | 1.81±0.62 | 0.56 |
| IMR < 40 (14/38) | 1.92±0.68 | 1.77±0.45 | 0.48 |

[a] Presented as mean±standard deviation; [b] Mann-Whitney test for continuous variables with a non-normal distribution

**In summary**, there was a trend for lower RHI values in patients with coronary microvascular dysfunction (increased IMR). RHI values correlated with male gender and previous treatment with ACEi/ARBs and tended to be lower in diabetic and previously revascularized patients. Additionally, RHI values on the second EndoPAT correlated with the area-at-risk and tended to be lower in patients with more complex coronary artery disease)

# 5. Secondary outcome – Extent of myocardial infarction and microvascular reperfusion according to RHI on the first EndoPAT

## 5.1. Impact of the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT on the extent of myocardial infarction

The extent of infarction was evaluated by:

- The area under the curve and the peak values of 7 evaluations of troponin I in the first 48 hours after the P-PCI,
- The area of infarction in the contrast enhanced cardiac magnetic resonance,
- And, indirectly, by echocardiography parameters, including left ventricular volumes and ejection fraction, wall motion score index and global longitudinal strain, both on the initial and follow-up (3 month) echocardiograms.

### 5.1.1. Endothelial dysfunction and troponin release

The peak values and the area under the curve of the 7 values of troponin I in the first 48 hours, are presented in Table 55. Both total values and values indexed to the area-at-risk scores (APPROACH and BARI) are shown, in patients with and without endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation. There were no significant differences in these populations.

**Table 55 – Impact of endothelial dysfunction (RHI<1.67, measured on first EndoPAT) on Troponin I release**

| Variable [a, b] | Total Population (n=60) | Endothelial Dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| $TnI_{peak}$ | 117±82 | 117±87 | 115±55 | 0.96 |
| $TnI_{peak}$ (APPROACH) | 34±27 | 33±29 | 35±19 | 0.82 |
| $TnI_{peak}$ (BARI) | 33±25 | 32±26 | 36±20 | 0.62 |
| $TnI_{peak}$ (2 scores) | 33±26 | 33±27 | 36±19 | 0.72 |
| $TnI_{AUC}$ | 1938±1283 | 1951±1376 | 1883±787 | 0.88 |
| $TnI_{AUC}$ (APPROACH) | 565±441 | 564±472 | 574±275 | 0.94 |
| $TnI_{AUC}$ (BARI) | 548±405 | 541±43 | 584±282 | 0.75 |
| $TnI_{AUC}$ (2 scores) | 557±421 | 552±449 | 579±278 | 0.85 |

[a] Presented as mean±standard deviation; [b] Peak value and area under the curve (AUC) of 7 troponin I (TnI, in mg/dL) measurements performed in the first 48 hours after the primary angioplasty; total values and values indexed to the APPROACH, BARI or both are presented; [c] Independent t-test.

### 5.1.2. Endothelial dysfunction and echocardiography parameters

There were no significant differences in 2D, Doppler or 2D speckle tracking imaging measurements in the first echocardiogram between patients with and without endothelial dysfunction on the first EndoPAT (Table 56). Likewise, all measurements were similar in the second echocardiogram in patients with and without RHI<1.67 (Table 57).

**Table 56 – Impact of endothelial dysfunction (RHI<1.67, measured on first EndoPAT) on the first echocardiogram parameters**

| Variable [a, b] | Total population | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=60)** | **(n=49)** | **(n=11)** | |
| LVEdV (ml) [a] | 105.8±24.2 | 107.5±24.0 | 95.7±24.4 | 0.27 |
| LVEsV (ml) [a] | 54.7±12.7 | 55.6±12.9 | 49.5±11.1 | 0.28 |
| LVEF (%)[a] | 47.9±6.7 | 47.9±6.8 | 47.8±5.9 | 0.96 |
| Wall motion score index [b] | 1.41 (0.35) | 1.41 (0.35) | 1.53 (0.18) | 0.78 |
| Left atria volume (ml/m$^2$) [a] | 34.8±12.2 | 35.7±12.9 | 30.6±7.2 | 0.32 |
| **Doppler measurements** | **(n=40)** | **(n=34)** | **(n=7)** | |
| E/A ratio [a] | 1.00±0.34 | 0.99±0.34 | 1.06±0.36 | 0.68 |
| E/e' ratio [a] | 9.00±2.71 | 8.91±2.85 | 9.58±1.55 | 0.61 |
| **2D speckle tracking imaging** | **(n=40)** | **(n=34)** | **(n=7)** | |
| Global longitudinal strain [a] | -13.54±2.28 | -13.38±2.30 | -14.7±1.96 | 0.23 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

**Table 57 – Impact of endothelial dysfunction (RHI<1.67, measured on first EndoPAT) on the 3 month echocardiogram parameters**

| Variable [a, b] | Total population | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=54)** | **(n=43)** | **(n=11)** | |
| LVEdV (ml) [a] | 109.7±26.4 | 111.1±28.5 | 103.8±13.9 | 0.44 |
| LVEsV (ml) [a] | 52.9±18.8 | 54.2±20.4 | 47.6±8.8 | 0.32 |
| LVEF (%)[a] | 52.6±7.1 | 52.2±7.4 | 54.2±5.2 | 0.43 |
| Wall motion score index [b] | 1.24 (0.35) | 1.24 (0.35) | 1.29 (0.24) | 0.93 |
| Left atria volume (ml/m$^2$) [a] | 39.1±15.4 | 40.4±16.7 | 34.3±7.2 | 0.25 |
| **Doppler measurements** | **(n=51)** | **(n=40)** | **(n=11)** | |
| E/A ratio [a] | 1.20±0.56 | 1.21±0.54 | 1.15±0.66 | 0.75 |
| E/e' ratio [a] | 8.92±3.29 | 8.83±3.51 | 9.29±2.30 | 0.69 |
| **2D speckle tracking imaging** | **(n=40)** | **(n=40)** | **(n=11)** | |
| Global longitudinal strain [a] | -15.77±3.11 | -15.80±3.36 | -15.68±2.11 | 0.91 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

When the 2 echocardiograms were compared in patients with and without endothelial dysfunction, improvements in left ventricular ejection fraction, wall motion score index, E/A ratio and global longitudinal strain were seen in both groups. However, these changes were only significant in the group with RHI>1.67, since the group with endothelial dysfunction was very small (only 5 to 7 patients, depending on the parameters evaluated).

**Table 58 – Baseline and 3 month echocardiographic parameters according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Echo parameters | RHI > 1.67 | | | RHI < 1.67 | | |
|---|---|---|---|---|---|---|
| | Echo1 | Echo2 | P value[c] | Echo1 | Echo2 | P value[c] |
| **2D measurements** | **n=38** | | | **n=7** | | |
| LVEdV (ml) [a] | 107.2±24.8 | 111.4±29.7 | 0.26 | 95,7±24.4 | 101.0±12.9 | 0.75 |
| LVEsV (ml) [a] | 55.0±12.8 | 54.2±20.8 | 0.37 | 49.5±11.1 | 46.5±9.6 | 0.67 |
| LVEF (%)[a] | 48.4±6.1 | 52.3±6.7 | 0.001 | 47.8±5.9 | 54.0±6.8 | 0.12 |
| WMSI [b] | 1.41 (0.37) | 1.24±0.35 | <0.001 | 1.53 (0.18) | 1.29 (0.24) | 0.05 |
| Left atria (ml/m2) [a] | 35.1±12.5 | 41.3±17.6 | 0.007 | 30.6±7.2 | 32.3±7.5 | 0.80 |
| **Doppler measurements** | **n=31** | | | **n=6** | | |
| E/A' ratio [a] | 0.98±0.34 | 1.18±0.54 | 0.007 | 1.06±0.36 | 0.96±0.22 | 0.25 |
| E/e' ratio [a] | 8.90±3.02 | 8.87±3.83 | 0.68 | 9.58±1.55 | 8.96±1.87 | 0.50 |
| **2D speckle tracking imaging** | **n=30** | | | **n=5** | | |
| Global longitudinal strain [a] | -13.7±2.1 | -15.6±3.3 | 0.001 | -14.7±2.0 | -16.1±1.8 | 0.08 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [C] p-value for the comparison between the first and the second echo only in patients with 2 evaluations; Paired samples T-Test for variables with normal distribution and Wilcoxon test for variables with non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

### 5.1.3. Endothelial dysfunction and contrast enhanced cardiac magnetic resonance

Forty nine patients of the 60 patients had ceCMR performed during the index admission (**Error! Reference source not found.**). Of these, 10 (20.4%) had endothelial dysfunction, as defined by an RHI<1.67.

There were no significant differences between patients with and without endothelial dysfunction in left ventricular volumes, left ventricular ejection fraction or wall motion score index (Table 59).

Accordingly, the presence of transmural necrosis and the total and percent infarct mass were also similar between groups. Finally, the salvage mass was also similar.

**Table 59 – Impact of endothelial dysfunction (RHI<1.67, measured on first EndoPAT) on the contrast enhanced cardiac magnetic resonance parameters**

| | Total Population (n=49) | Endothelial Dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=39) | Yes (n=10) | |
| LVEdV (ml) [a] | 142,8±29.2 | 144.3±31.0 | 137.0±20.8 | 0.49 |
| LVEsV (ml) [a] | 66.3±21.8 | 66.4±22.9 | 65.7±17.7 | 0.92 |
| LVEF (%)[a] | 53.6±8.7 | 53.9±8.7 | 52.4±9.3 | 0.62 |
| Wall motion score index [a] | 1.42±0.29 | 1.41±00.30 | 1.46±0.23 | 0.64 |
| Oedema mass [b] | 19.6 (14.4) | 19.1 (15.6) | 23.6 (14.4) | 0.76 |
| Transmural necrosis [c] | 23 (46.9) | 18 (46.2) | 5 (50.0) | 0.89 [e] |
| Infarct mass | | | | |
| Total [b] | 14.7 (12.6) | 11.7 (9.8) | 19.9 (11.9) | 0.11 |
| Percent [b] | 12.6 (14.4) | 11.6 (12.9) | 20.3 (14.5) | 0.08 |
| Indexed to APPROACH | 3.7 (4.5) | 3.1 (3.9) | 5.6 (5.5) | 0.08 |
| Indexed to BARI | 3.8 (4.3) | 3.0 (3.6) | 5.5 (5.1) | 0.07 |
| Salvage mass [b] | 4.5 (10.4) | 5.4 (11.5) | 3.1 (7.6) | 0.19 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Presented as number (%); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction

**In summary**, the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT was not related to larger infarctions (measured by troponin release or by ceCMR). There was an improvement in echocardiogram parameters, both in patients with and without endothelial dysfunction, between the initial and the follow-up exams.

## 5.2. Impact of the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT on microvascular reperfusion

Microvascular reperfusion was evaluated by:

- ST elevation and deviation resolution on the ECGs performed immediately after and 90 and 180 minutes after the coronary revascularization.
- Angiographic indicators, including corrected TIMI frame count and TIMI myocardial perfusion grade.
- Microvascular obstruction, evaluated by contrast enhanced cardiac magnetic resonance.

### 5.2.1. Endothelial dysfunction and ST resolution on the ECG

The residual total ST elevation and deviation and the percentage of resolution of these ST changes are presented in Table 60 and in Figure 23 and Figure 24, according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation.

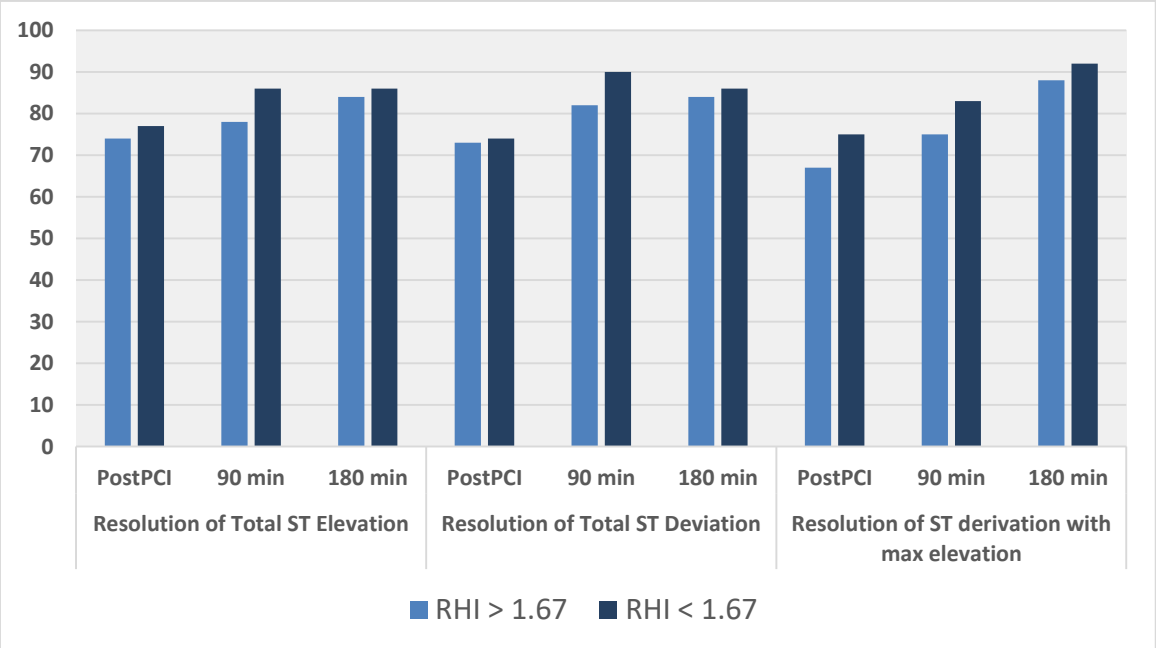


**Figure 23 – ST elevation and deviation resolution (median values) according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

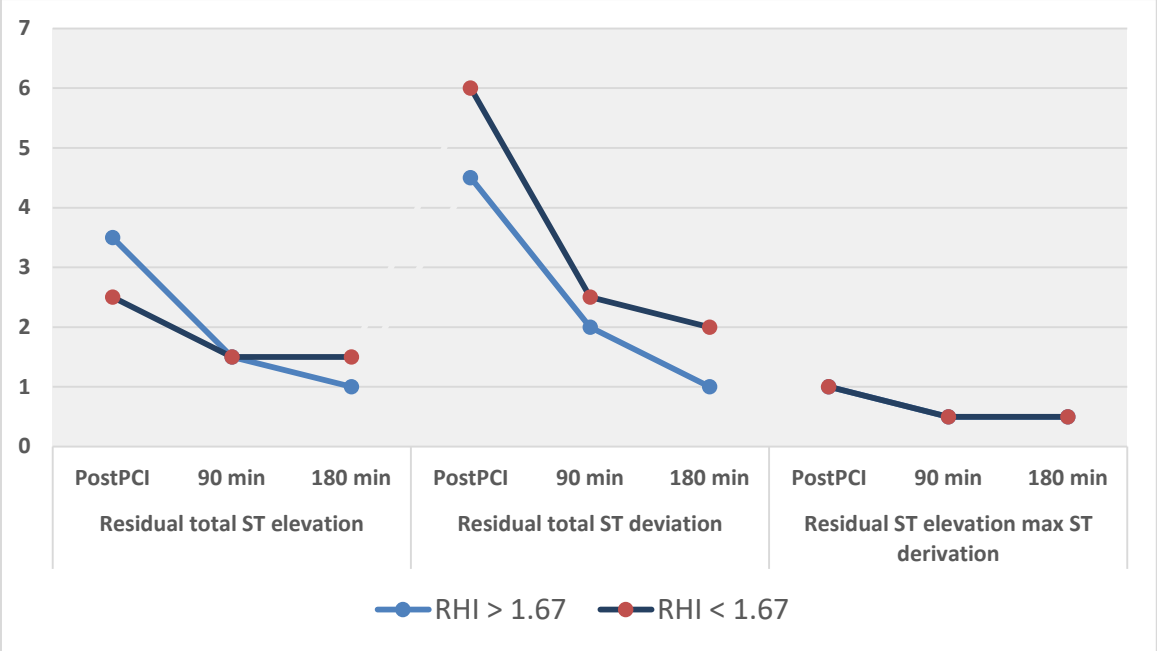


**Figure 24 – ST residual changes (median values) according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

**Table 60 – ECG ST resolution and residual changes according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| **Immediately post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 3.3 (6.0) | 3.5 (6.0) | 2.5 (6.0) | 0.96 |
| % Resolution | 74.5 (39.0) | 74.0 (42.0) | 77.0 (37.0) | 0.83 |
| Total ST deviation [b] | 4.8 (6.0) | 4.5 (6.0) | 6.0 (7.0) | 0.44 |
| % Resolution | 73.5 (44) | 73.0 (48.0) | 74.0 (34.0) | 0.92 |
| ST at derivation with max elevation) [b] | 1.0 (2.0) | 1.0 (2.0) | 1.0 (2.0) | 0.88 |
| % Resolution | 68.0 (47.0) | 67.0 (51.0) | 75.0 (25.0) | 0.95 |
| **90 minutes post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.5 (4.0) | 1.5 (5.0) | 1.5 (4.0) | 0.77 |
| % Resolution | 79.5 (32.0) | 78.0 (34.0) | 86.0 (25.0) | 0.70 |
| Total ST deviation [b] | 2.0 (4.0) | 2.0 (5.0) | 2.5 (3.0) | 0.65 |
| % Resolution | 82.5 (33.0) | 82.0 (36.0) | 90.0 (26.0) | 0.89 |
| ST at derivation with max elevation [b] | 0.5 (2.0) | 0.5 (2.0) | 0.5 (1.0) | 0.55 |
| % Resolution | 77.5 (34.0) | 75.0 (37.0) | 83.0 (31.0) | 0.64 |
| **180 minutes post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.5 (3.0) | 1.0 (4.0) | 1.5 (3.0) | 0.70 |
| % Resolution | 84.5 (23.0) | 84.0 (26.0) | 86.0 (12.0) | 0.78 |
| Total ST deviation [b] | 1.5 (4.0) | 1.0 (5.0) | 2.0 (4.0) | 0.31 |
| % Resolution | 88.0 (26.0) | 88.0 (28.0) | 92.0 (28.0) | 0.60 |
| ST at derivation with max elevation [b] | 0.5 (2.0) | 0.5 (2.0) | 0.5 (1.0) | 0.80 |
| % Resolution | 83.0 (33.0) | 83.0 (33.0) | 85.0 (31.0) | 0.61 |
| QS waves present | 37 (61.7) | 30 (61.2) | 7 (63.6) | 0.85 [e] |

[a] Values expressed as median (interquartile range); [b] Values expressed in mV; [c] values expressed as n(%); [d] Mann-Whitney test for continuous variables with a non-normal distribution; [e] Chi-Square test, with Yates correction.

There were no significant differences in any of the ECG parameters evaluated, immediately after the P-PCI, at 90 minutes or at 180 minutes. The presence of Q waves in the final ECG was also similar in the two groups (Table 60).

### 5.2.2. Endothelial dysfunction and angiographic indicators of microvascular reperfusion

Corrected TIMI frame count and TIMI myocardial perfusion grade results according to the presence of endothelial dysfunction on the first EndoPAT evaluation are presented in Table 61. There were no significant differences on either parameters.

**Table 61 – Angiographic indicators of microvascular reperfusion according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| Variable | Total population (n=60) | Endothelial dysfunction (RHI<1.67) | | *p* value |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| Corrected TIMI frame count [a] | 17.0 (7.0) | 18.0 (8.0) | 14.0 (8.0) | 0.27 [c] |
| TMPG 2-3 [b] | 49 (81.7) | 38 (77.6) | 11 (100.0) | 0.24 [d] |

[a] data presented as median (interquartile range); [b] data presented as n(%); [c] Mann-Whitney test; [d] Chi-Square test, with Yates correction.

### 5.2.3. Endothelial dysfunction and microvascular obstruction on the ceCMR

There were no significant differences in the number of patients with microvascular obstruction and in the mass of microvascular obstruction between patients with and without RHI<1.67 (Table 62).

**Table 62 – Microvascular obstruction on the ceCMR according to the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT evaluation**

| | Total population (n=49) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=39) | Yes (n=10) | |
| Microvascular obstruction | | | | |
| MVO present [a] | 13 (26.5) | 10 (25.6) | 3 (30.0) | 0.90 [e] |
| Mass of MVO [b] | 5.7 (4.0) | 6.1 (8.5) | 5.4 (-) | 0.57 |

[a] Presented as number (%); [b] Presented as median (interquartile range); [c] Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [d] Yates correction

**In summary**, the presence of endothelial dysfunction (RHI<1.67) on the first EndoPAT was not related to non-invasive (ST resolution) and invasive (cTFC and TMPG) indicators of microcirculatory reperfusion. Likewise, endothelial dysfunction was not related with microvascular obstruction on the ceCMR.

# 6. Secondary outcome – Extent of myocardial infarction and microvascular reperfusion according to RHI on the second EndoPAT

## 6.1. Impact of the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT on the extent of myocardial infarction

### 6.1.1. Endothelial dysfunction and Troponin release

The peak values and the area under the curve of troponin I, according to the presence of endothelial dysfunction on the second EndoPAT evaluation are presented in Table 63. Both total values and values indexed to the area-at-risk scores (APPROACH and BARI) are presented, in patients with and without endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation. Patients with endothelial dysfunction had significantly higher values of both peak TnI and AUC of TnI, both total and indexed to the area-at-risk scores.

**Table 63 – Impact of endothelial dysfunction (RHI<1.67, measured on second EndoPAT) on Troponin I release**

| Variable [a, b] | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| $TnI_{peak}$ | 95 (96) | 67 (81) | 118 (186) | 0.024 |
| $TnI_{peak}$ (APPROACH) | 25 (32) | 17 (20) | 34 (56) | 0.009 |
| $TnI_{peak}$ (BARI) | 24 (31) | 17 (22) | 33 (47) | 0.008 |
| $TnI_{peak}$ (2 scores) | 24 (31) | 17 (21) | 33 (55) | 0.009 |
| $TnI_{AUC}$ | 1293 (1580) | 1076 (1042) | 2305 (2486) | 0.012 |
| $TnI_{AUC}$ (APPROACH) | 403 (522) | 315 (303) | 664 (1080) | 0.008 |
| $TnI_{AUC}$ (BARI) | 383 (448) | 314 (326) | 618 (799) | 0.007 |
| $TnI_{AUC}$ (2 scores) | 393 (482) | 314 (300) | 641 (984) | 0.007 |

[a] Presented as median (IQR); [b] Peak value and area under the curve (AUC) of 7 troponin I (TnI, in mg/dL) measurements performed in the first 48 hours after the primary angioplasty; total values and values indexed to the APPROACH, BARI or both are presented; [c] Mann-Whitney Test.

### 6.1.2. Endothelial dysfunction and echocardiography parameters

Patients with endothelial dysfunction had significantly higher end-systolic volumes, lower LVEF and worse wall motion score index in the first echocardiogram. Accordingly with the difference

in WSMI, they also had higher values of global longitudinal strain (Table 64). These differences were no longer visible in the echocardiogram performed at 3 month (Table 65).

**Table 64 – Impact of endothelial dysfunction (RHI<1.67, measured on second EndoPAT) on the first echocardiogram parameters**

| Variable | Total population | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=26)** | **(n=15)** | **(n=11)** | |
| LVEdV (ml) [a] | 109.8±23.4 | 106.5±30.2 | 113.8±11.4 | 0.48 |
| LVEsV (ml) [a] | 55.9±12.6 | 51.1±12.3 | 61.7±10.9 | 0.047 |
| LVEF (%)[a] | 48.6±7.1 | 51.4±4.7 | 45.3±8.3 | 0.045 |
| Wall motion score index [b] | 1.44 (0.41) | 1.35 (0.47) | 1.77 (0.47) | 0.006 |
| Left atria volume (ml/m$^2$) [a] | 36.1±11.0 | 36.7±12.1 | 35.3±10.0 | 0.78 |
| **Doppler measurements** | **(n=21)** | **(n=11)** | **(n=10)** | |
| E/A ratio [a] | 1.10±0.40 | 1.17±0.40 | 0.97±0.0.39 | 0.30 |
| E/e' ratio [a] | 8.91±3.30 | 8.83±3.91 | 9.05±2.18 | 0.89 |
| **2D speckle tracking imaging** | **(n=21)** | **(n=11)** | **(n=10)** | |
| Global longitudinal strain [a] | -13.16±2.35 | -14.32±1.72 | -11.89±2.35 | 0.014 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

**Table 65 – Impact of endothelial dysfunction (RHI<1.67, measured on second EndoPAT) on the 3 month Echocardiogram parameters**

| Variable | Total Population | Endothelial Dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=30)** | **(n=19)** | **(n=11)** | |
| LVEdV (ml) [a] | 109.7±28.1 | 103.9±29.8 | 119.7±22.4 | 0.14 |
| LVEsV (ml) [a] | 53.8±21.4 | 49.8±21.0 | 60.7±21.2 | 0.18 |
| LVEF (%)[a] | 52.2±7.9 | 53.2±7.6 | 50.5±8.3 | 0.37 |
| Wall motion score index [b] | 1.24 (0.40) | 1.21 (0.35) | 1.29 (0.66) | 0.33 |
| Left atria volume (ml/m$^2$) [a] | 40.6±15.4 | 42.2±16.9 | 38.0±12.7 | 0.46 |
| **Doppler measurements** | **(n=29)** | **(n=19)** | **(n=10)** | |
| E/A ratio [a] | 1.31±0.68 | 1.30±0.62 | 1.34±0.82 | 0.87 |
| E/e' ratio [a] | 8.96±3.93 | 9.13±4.19 | 8.59±3.51 | 0.74 |
| **2D speckle tracking imaging** | **(n=29)** | **(n=19)** | **(n=10)** | |
| Global longitudinal strain [a] | -15.43±3.55 | -15.95±3.01 | -14.4±4.39 | 0.28 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

When the 2 echocardiograms were compared, significant improvements in the wall motion score index were found in both patients with and without endothelial dysfunction. There was also a trend for an improvement in the global longitudinal strain in the group without

endothelial dysfunction. There were no other significant differences, independently of the presence of endothelial dysfunction.

**Table 66 – Baseline and 3 months echocardiographic parameters according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Echo parameters | RHI > 1.67 | | | RHI < 1.67 | | |
|---|---|---|---|---|---|---|
| | Echo1 | Echo2 | P value[c] | Echo1 | Echo2 | P value[c] |
| **2D measurements** | | **n=15** | | | **n=11** | |
| LVEdV (ml) [a] | 106.5±3..2 | 101.0±33.0 | 0.33 | 113.8±11.8 | 119.7±22.4 | 0.61 |
| LVEsV (ml) [a] | 51.1±12.3 | 48.3±22.3 | 0.24 | 61.7±10.9 | 60.7±21.1 | 1.00 |
| LVEF (%)[a] | 51.4±4.8 | 53.2±5.1 | 0.24 | 45.3±8.3 | 50.5±8.3 | 0.24 |
| WMSI [b] | 1.35 (0.47) | 1.18 (0.35) | 0.012 | 1.65 (0.47) | 1.24 (0.74) | 0.049 |
| Left atria (ml/m2) [a] | 36.7±12.1 | 43.8±18.8 | 0.16 | 35.3±10.0 | 38.0±12.7 | 0.16 |
| **Doppler measurements** | | **n=12** | | | **n=11** | |
| E/A' ratio [a] | 1.14±0.40 | 1.24±0.43 | 0.61 | 0.97±0.39 | 1.34±0.82 | 0.08 |
| E/e' ratio [a] | 9.0±4.1 | 8.2±4.7 | 0.37 | 9.1±2.2 | 8.6±3.5 | 0.72 |
| **2D speckle tracking imaging** | | **n=10** | | | **n=10** | |
| Global longitudinal strain [a] | -14.3±1.8 | -16.1±2.6 | 0.07 | -11.9±2.3 | -14.4±4.4 | 0.53 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [C] p-value for the comparison between the first and the second Echo only in patients with 2 evaluations; paired samples T-Test for variables with normal distribution and Wilcoxon test for variables with non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

### 6.1.3. Endothelial dysfunction and contrast enhanced cardiac magnetic resonance

Contrast enhanced CMR was performed in 29 of the 38 patients with a second EndoPAT evaluation. Of these, 11 (37.9%) had endothelial dysfunction (RHI<1.67) (Table 67).

Patients with endothelial dysfunction (RHI<1.67) had significantly lower left ventricular ejection fraction and higher wall motion score index, as compared to patients with RHI>1.67.

There was a trend for more transmural necrosis (22.2% vs. 63.6%, p=0.06) and higher infarct mass (median value 10.1 vs. 17.5, p=0.08) in patients with RHI<1.67. The percent mass of infarct indexed to area-at-risk scores (APPROACH and BARI) also tended to be higher in patients with endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation.

**Table 67 – Impact of endothelial dysfunction (RHI<1.67) measured on second EndoPAT on the contrast enhanced cardiac magnetic resonance parameters**

| | Total population (n=29) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=18) | Yes (n=11) | |
| LVEdV (ml) [a] | 138.6±26.9 | 139.2±26.5 | 137.5±28.9 | 0.87 |
| LVEsV (ml) [a] | 63.5±21.0 | 59.3±19.4 | 70.4±22.6 | 0.17 |
| LVEF (%)[a] | 53.9±8.4 | 56.6±8.1 | 49.5±7.2 | 0.025 |
| Wall motion score index [a] | 1.37±0.33 | 1.28±0.31 | 1.53±0.32 | 0.05 |
| Oedema mass [b] | 19.1 (19.0) | 17.2 (14.1) | 21.2 (28.6) | 0.28 |
| Transmural necrosis [c] | 12 (38.7) | 4 (22.2) | 7 (63.6) | 0.06 [e] |
| Infarct mass | | | | |
| Total [b] | 11.6 (9.3) | 10.1 (10.3) | 17.5 (15.4) | 0.08 |
| Percent [b] | 11.5 (13.7) | 10.2 (7.6) | 17.5 (21.8) | 0.10 |
| Indexed to APPROACH | 3.2 (7.0) | 2.7 (2.6) | 4.9 (11.5) | 0.10 |
| Indexed to BARI | 3.4 (5.8) | 2.3 (2.7) | 5.1 (11.5) | 0.09 |
| Salvage mass [b] | 5.0 (14.0) | 5.0 (8.8) | 4.7 (27.6) | 0.87 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Presented as number (%); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction

**In summary**, the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT was related with larger infarcts, as assessed by troponin I release and ceCMR. These patients also had lower LVEF and worse wall motion score index and GLS in the acute echocardiogram and in the ceCMR, although these differences were not present in the follow-up exam echo.

## 6.2. Impact of the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT on microvascular reperfusion

### 6.2.1. Endothelial dysfunction and ST resolution on the ECG

The residual total ST elevation and deviation and the percentage of resolution of these ST changes are presented in Table 68 , in Figure 25 and in Figure 26, according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation.

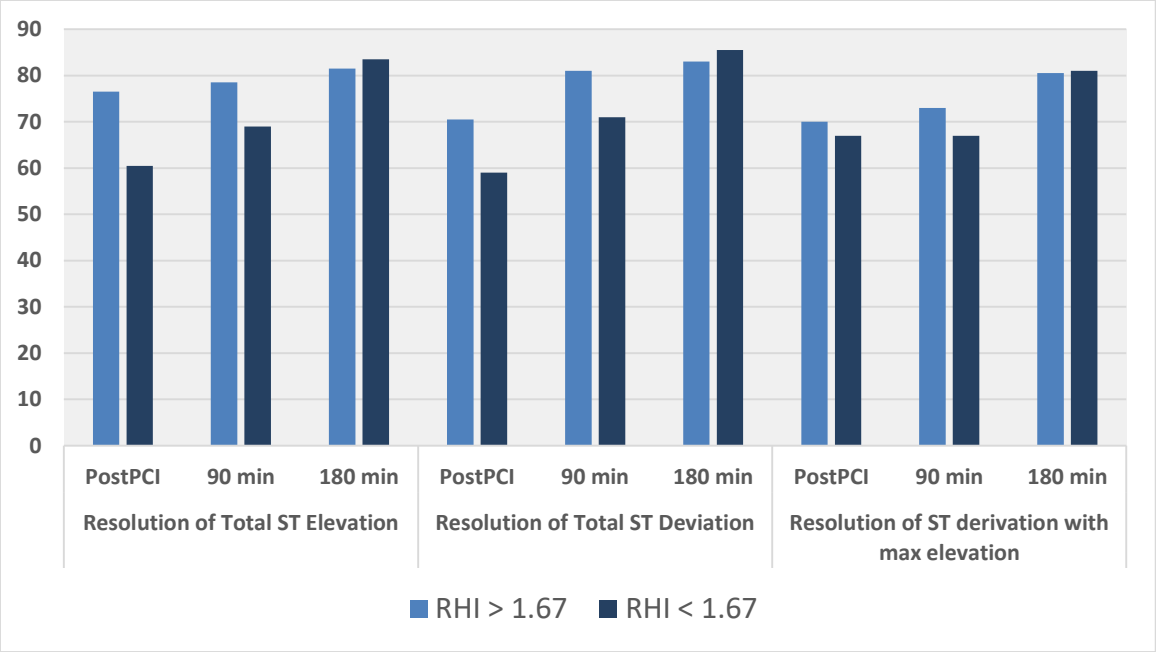


**Figure 25 – ST elevation and deviation resolution (median values) according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

Although there was a tendency for higher resolution of ST elevation and deviation (particularly in post-PCI and 90 minutes ECGs) in patients without endothelial dysfunction, this difference was not statistically significant.

Residual total ST elevation immediately after PCI and at 90 minutes was higher in patients with endothelial dysfunction and there was a trend for similarly worse results in residual ST deviation and residual ST elevation in these patients.

The presence of Q waves was similar in both groups.

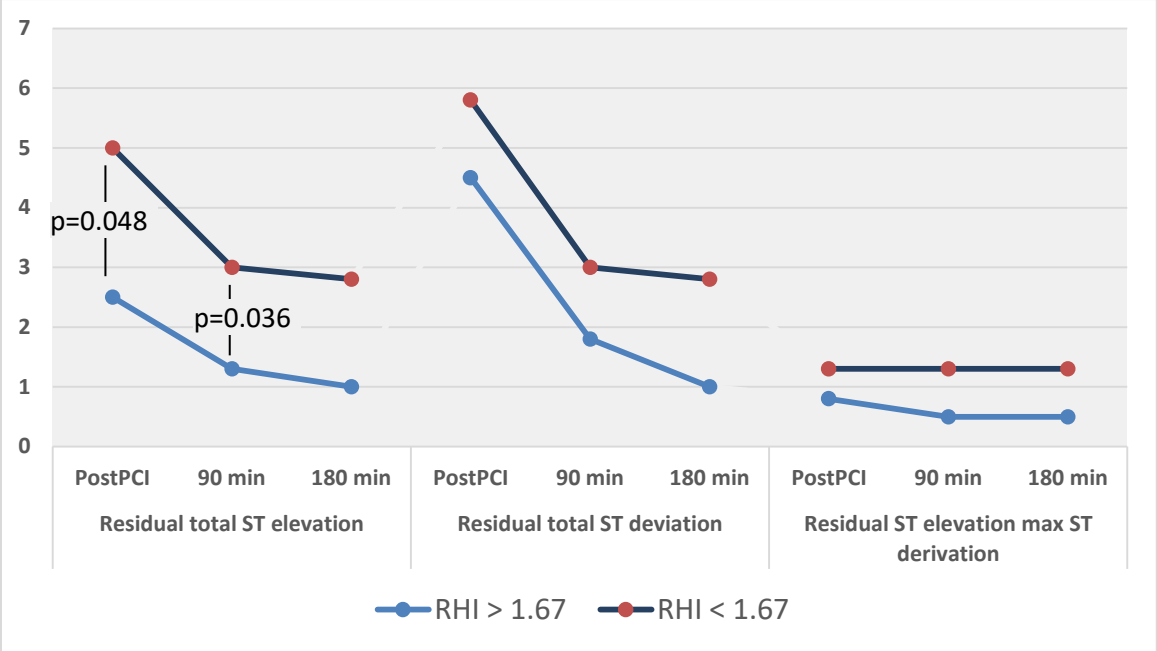


**Figure 26 – ST residual changes (median values) according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

**Table 68 – ECG ST resolution and residual changes according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=49) | Yes (n=11) | |
| **Immediately post-Angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 3.0 (6.0) | 2.5 (5.0) | 5.0 (12.0) | 0.048 |
| % Resolution | 74.5 (39.0) | 76.5 (41.0) | 60.5 (72.0) | 0.23 |
| Total ST deviation [b] | 4.8 (6.0) | 4.5 (5.0) | 5.8 (14.0) | 0.22 |
| % Resolution | 67.8 (39.0) | 70.5 (39.0) | 59.0 (78.0) | 0.39 |
| ST at derivation with max elevation) [b] | 1.0 (2.0) | 0.8 (2.0) | 1.3 (3.0) | 0.06 |
| % Resolution | 67.0 (33.0) | 70.0 (40.0) | 67.0 (52.0) | 0.15 |
| **90 minutes post-Angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.8 (4.0) | 1.3 (4.0) | 3.0 (7.0) | 0.036 |
| % Resolution | 77.0 (29.0) | 78.5 (28.0) | 69.0 (53.0) | 0.07 |
| Total ST deviation [b] | 2.0 (5.0) | 1.8 (5.0) | 3.0 (7.0) | 0.191 |
| % Resolution | 78.0 (30.0) | 81.0 (31.0) | 71.0 (59.0) | 0.181 |
| ST at derivation with max elevation [b] | 1.0 (2.0) | 0.5 (1.0) | 1.3 (2.0) | 0.07 |
| % Resolution | 69.0 (31.0) | 73.0 (37.0) | 67.0 (44.0) | 0.19 |
| **180 minutes post-Angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.3 (4.0) | 1.0 (3.0) | 2.8 (5.0) | 0.30 |
| % Resolution | 85.5 (26.0) | 81.5 (22.0) | 83.5 (52.0) | 0.67 |
| Total ST deviation [b] | 1.5 (5.0) | 1.3 (4.0) | 2.8 (5.0) | 0.74 |
| % Resolution | 84.5 (32.0) | 83.0 (32.0) | 85.5 (54.0) | 0.80 |
| ST at derivation with max elevation [b] | 0.5 (1.0) | 0.5 (1.0) | 1.3 (2.0) | 0.20 |
| % Resolution | 81.0 (30.0) | 80.5 (29.0) | 81.0 (40.0) | 0.65 |
| QS waves present | 24 (63.2) | 13 (59.1) | 11 (68.8) | 0.54 |

[a] Values expressed as median (interquartile range); [b] Values expressed in mV; [c] values expressed as n(%); [d] Mann-Whitney test for continuous variables with a non-normal distribution; [e] Chi-Square test

### 6.2.2. Endothelial dysfunction and angiographic indicators of microvascular reperfusion

Corrected TIMI frame count and TIMI myocardial perfusion grade results according to the presence of endothelial dysfunction on the second EndoPAT evaluation are presented in Table 69. There was a trend toward higher values of cTFC and worse TMPG in patients with endothelial dysfunction, although it did not reach statistical significance.

**Table 69 – Angiographic indicators of microvascular reperfusion according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| Variable | Total population (n=38) | Endothelial dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| Corrected TIMI frame count [a] | 17.0 (7.0) | 16.4 (7.0) | 19.5 (12.0) | 0.07 |
| TMPG 2-3 [b] | 28 (73.7) | 19 (86.4) | 9 (56.3) | 0.09 [d] |

[a] data presented as median (interquartile range); [b] data presented as n(%); [c] Mann-Whitney test; [d] Chi-Square test, with Yates correction.

### 6.2.3. Endothelial dysfunction and microvascular obstruction on the ceCMR

Only 8 patients with the second EndoPAT evaluation had microvascular obstruction on the ceCMR: 2 (11.1%) in the group with RHI<1.67 and 6 (54.5%) in the group with RHI>1.67 (p=0.03). Likewise, the microvascular obstruction mass was higher in the group with RHI<1.67 (median value 5.3 vs. 6.8), although the difference was not significant (Table 70).

**Table 70 - Microvascular obstruction on the ceCMR according to the presence of endothelial dysfunction (RHI<1.67) on the second EndoPAT evaluation**

| | Total Population (n=29) | Endothelial Dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=18) | Yes (n=11) | |
| Microvascular obstruction | | | | |
| MVO present [a] | 8 (27.6) | 2 (11.1) | 6 (54.5) | 0.03 [d] |
| MVO mass [b] | 6.1 (15.5) | 5.3 (-) | 6.8 (20.8) | 0.29 |

[a] Presented as number (%); [b] Presented as median (interquartile range); [c] Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [d] Yates correction

**In summary**, there was a clear trend for worse angiographic and electrocardiographic indictors of microvascular reperfusion in patients with endothelial dysfunction on the second EndoPAT. The proportion of patients with MVO on the ceCMR was higher in patients with RHI<1.67 and there was a trend for higher MVO mass in these patients.

# 7. Secondary outcome – Extent of myocardial infarction and microvascular reperfusion according to IMR values

In order to evaluate the impact of IMR on the extent of myocardial necrosis and its relation to invasive and non-invasive indicators of coronary microvascular reperfusion, patients were divided into two groups, according to the median value of IMR measured (24). To simplify the presentation of results and its discussion, patients with an IMR above the median 24 value will be classified as having coronary microvascular damage, as opposed to those with IMR values below 24. Where indicated, IMR as a continuous variable was also evaluated. In the following pages, the main patient characteristics according to IMR values will be shortly described, followed by the analysis of the impact of coronary microvascular damage.

## 7.1. IMR and baseline characteristics

### 7.1.1. IMR and patient's main characteristics

Patients with coronary microvascular dysfunction (IMR>24) were older (Table 71). In fact, there was a weak, but significant correlation between IMR and age (r=0.28, p=0.03).

**Table 71 – Main characteristics of patients according to median IMR**

| Variable | Total population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| **Physical characteristics** | | | | |
| Age (years) [a] | 59.6±12.7 | 54.7±11.3 | 64.4±12.2 | 0.002 |
| Male gender [b] | 48 (80.0) | 26 (86.7) | 22 (73.3) | 0.20 |
| BMI [a] | 27.5 ±4.0 | 27.4±4.4 | 27.6±3.7 | 0.79 |
| Waist circumference [a] | 99.2±12.2 | 96.5±12.0 | 102.3±12.0 | 0.11 |
| **Risk Factors and previous coronary disease** [b] | | | | |
| Hypertension | 43 (71.7) | 19 (63.3) | 24 (80.0) | 0.15 |
| Diabetes | 15 (25.0) | 7 (23.3) | 8 (26.7) | 0.77 |
| Dyslipidaemia | 30 (50.0) | 16 (53.3) | 14 (46.7) | 0.61 |
| Active smoking | 26 (43.3) | 16 (53.3) | 10 (33.3) | 0.12 |
| Previous angina | 9 (15.0) | 4 (13.3) | 5 (16.7) | 1.00 [d] |
| Previous revascularization | 2 (3.3) | 1 (3.3) | 1 (3.3) | 0.49 [d] |
| **Previous medication** [b] | | | | |
| Aspirin | 5 (8.3) | 3 (10.0) | 2 (6.7) | 0.96 |
| ACEi/ARBs | 23 (38.3) | 12 (40.0) | 11 (36.7) | 0.87 |
| Beta-blockers | 4 (6.7) | 3 (10.0) | 1 (3.33) | 0.63 [d] |
| Statins | 9 (15.0) | 3 (10.0) | 6 (20.0) | 0.44 [d] |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Independent t-test for continuous variables, Chi-Square for categorical variables; [d] Yates correction

There were no significant differences in gender, other physical characteristics or risk factors.

NT-pro-BNP levels tended to be higher in patients with an IMR>24 and there was a weak but significant correlation between these 2 variables (r=0.33, p=0.015). Likewise, glucose and HbA1c levels on admission were higher in patients with IMR>24, and significant correlations were found between these laboratory tests and IMR (r=0.34, p=0.009 and r=0.67, p<0.001, respectively). Finally, there was a trend for higher values of hs-CRP in patients with coronary microvascular damage (Table 72).

**Table 72 – Laboratory result on admission according to median IMR**

| Variable | Total population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| Creatinine (mg/dL) [b] | 0.90 (0.31) | 0.89 (0.17) | 0.94 (0.44) | 0.14 |
| NT-pro-BNP (pg/mL) [b] | 137.5 (255.5) | 93 (165.5) | 167.5 (222.8) | 0.055 |
| hs-CRP (mg(dL) [b] | 0.44 (0.59) | 0.33 (0.36) | 0.49 (0.84) | 0.09 |
| Glucose (mg/dL) [b] | 133.5 (61.0) | 122.0 (48.8) | 141.5 (60.0) | 0.046 |
| HbA1c (%)[b] | 5.7 (1.1) | 5.5 (0.6) | 5.9 (3.5) | 0.047 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution.

### 7.1.2. IMR and angiography/angioplasty variables

There were no significant differences in ischemic (pain-to-balloon) or hospital-to-balloon times between patients with and without microvascular coronary damage (IMR>24). Main angiographic characteristics, including culprit artery, presence of multivessel disease and Syntax score were also similar in both groups (Table 73).

Likewise, the area-at-risk, measured both by APPROACH and BARI scores, was similar. However, patients with an IMR>24 had a significantly higher prevalence of initial TIMI flow 0-1 (93.3% vs. 73.3%, p=0.038).

Treatment options, including the use of mechanical aspiration and stent implantation technique were similar in patients with and without IMR>24 (Table 74). However, patients with lower IMR values were more likely to have received abciximab during the P-PCI procedure (36.7% vs 10.0%, p=0.015).

**Table 73 – Ischemic times and angiographic characteristics according to median IMR**

| Variable | Total Population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value[d] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| Pain-to-balloon time (min)[c] | 209 (148) | 206 (107) | 225 (163) | 0.19 |
| Door-to-balloon time (min) [c] | 78 (45) | 78 (34) | 79 (59) | 0.89 |
| Culprit artery [b] | | | | |
| Left anterior descending | 28 (46.7) | 14 (46.7) | 14 (46.7) | |
| Left circumflex | 13 (21.7) | 6 (20.6) | 7 (23.3) | 0.94 |
| Right coronary artery | 19 (31.7) | 10 (33.3) | 9 (30.0) | |
| Multivessel disease [b] | 25 (41.7) | 15 (50.0) | 20 (66.7) | 0.19 |
| Syntax score [c] | 15.5 (10.0) | 16.9 (10.0) | 14.8 (8.0) | 0.21 |
| Area at risk scores [a] | | | | |
| APPROACH score | 27.8 (2.0) | 27.8 (3.0) | 27.8 (3.0) | 0.48 |
| BARI score | 28.0±6.3 | 27.2±6.5 | 28.0±6.3 | 0.95 |
| Initial TIMI flow 0-1 [b] | 50 (83.3) | 22 (73.3) | 28 (93.3) | 0.038 |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [E] Yates correction

**Table 74 – Angioplasty treatment options according to median IMR value**

| Variable [a] | Total Population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value[b] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| Mechanical aspiration | 26 (43.3) | 14 (46.7) | 12 (40.0) | 0.60 |
| Balloon pre-dilatation | 35 (58.3) | 15 (50.0) | 20 (66.7) | 0.19 |
| Stent implantation | 57 (95.0) | 28 (93.3) | 29 (96.7) | 1.00 |
| Balloon post-dilatation | 23 (38.3) | 12 (40.0) | 11 (36.7) | 0.79 |
| Abciximab treatment | 14 (23.3) | 11 (36.7) | 3 (10.0) | 0.015 |

[a] Presented as number (%); [b] Chi-Square test

### 7.1.3. Multivariable analysis of IMR predictors

Since there were several relevant differences in the populations with and without microvascular coronary damage and in order to clarify the independent predictors of IMR, multivariable analysis was performed, using IMR as a continuous variable. Variables statistically significant on the univariate analysis were included in the regression model: age, initial TIMI flow, admission glucose, admission HbA1c and abciximab treatment. Since glucose levels and HbA1c are interdependent variables, 2 different models were used, with these 2 blood tests. Results are shown in Table 75.

**Table 75 – Multivariable analysis of IMR predictors**

| Variable | Hazard Ratio (95% CI) | P value |
|---|---|---|
| **Model 1 (with admission glucose levels)** | | |
| Glucose (mg/dL) | 0.32 (0.03-0.19) | 0.012 |
| Age (years) | 0.28 (0.07-1.10) | 0.026 |
| Initial TIMI flow 0-1 | 0.17 (-6.9-32.5) | 0.20 |
| Abciximab treatment | -0.15 (-25.2-6.3) | 0.23 |
| **Model 2 (with admission HbA1c levels)** | | |
| HbA1c (%) | 0.55 (0.47-11.4) | <0.001 |
| Age (years) | 0.32 (0.21-1.19) | 0.006 |
| Initial TIMI flow 0-1 | 0.18 (-4.0-32.0) | 0.13 |
| Abciximab treatment | -0.05 (-19.5-12.3) | 0.65 |

In Model 1, age and glucose levels at admission were the only independent predictors of IMR. When other clinically relevant variables (gender, previous diabetes, mechanical aspiration, culprit artery and ischemic time) where added to the model in a stepwise approach, age (HR=0.31, p=0.022) and glucose levels (HR=0.34, p=0.022) kept their independent relation with IMR. Importantly, this was true for glucose levels even after adding previous diabetes history to the model.

In Model 2, age and HbA1c were the only independent predictors of IMR. Again, when other clinically relevant variables (gender, previous diabetes, mechanical aspiration, culprit artery and ischemic time) where added to the model in a stepwise approach, age (HR=0.35, p=0.004) and HbA1c levels (HR=0.61, p<0.001) kept their independent relation with IMR.

**In summary**, IMR values were associated with age and with admission glucose and HbA1c levels. Higher IMR values were also observed in patients with initial TIMI flow 0-1 and in patients who were not treated with abciximab, but these associations were not significant in the multivariable analysis. Finally, patients with higher IMR also had higher values of NT-pro-BNP.

## 7.2. Relation between IMR and the extent of myocardial infarction

### 7.2.1. IMR and Troponin release

The peak values and the area under the curve of troponin I, according to the presence of microvascular coronary damage (IMR>24) are presented in Table 76.

Patients with an IMR>24 had significantly higher values of both peak TnI and AUC of TnI, either total or indexed to the area-at-risk scores. IMR significantly correlated with peak TnI and AUC of TnI (r=0.508, and r=0.490, respectively; p<0.001 for both), and those correlations were even stronger when TnI release was indexed to area-at-risk scores (r=0.551 and r=0.530, for APPROACH score; r=0.523 and r=0.503, for BARI score; p<0.001 for all analysis).

**Table 76 –Troponin I values according to median IMR**

| Variable [a, b] | Total Population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| $TnI_{peak}$ | 117±82 | 91±59 | 142±93 | 0.013 |
| $TnI_{peak}$ (APPROACH) | 34±27 | 25±19 | 42±32 | 0.014 |
| $TnI_{peak}$ (BARI) | 33±25 | 25±18 | 40±29 | 0.025 |
| $TnI_{peak}$ (2 scores) | 33±26 | 25±18 | 41±30 | 0.018 |
| $TnI_{AUC}$ | 1938±1283 | 1459±898 | 2418±1438 | 0.003 |
| $TnI_{AUC}$ (APPROACH) | 565±441 | 407±274 | 724±517 | 0.004 |
| $TnI_{AUC}$ (BARI) | 548±405 | 410±275 | 687±468 | 0.004 |
| $TnI_{AUC}$ (2 scores) | 557±421 | 408±274 | 706±490 | 0.005 |

[a] Presented as mean±standard deviation; [b] Peak value and area under the curve (AUC) of 7 troponin I (TnI, in mg/dL) measurements performed in the first 48 hours after the primary angioplasty; total values and values indexed to the APPROACH, BARI or both are presented; [c] Independent t-test.

### 7.2.2. IMR and echocardiography parameters

There were no significant differences in 2D and Doppler measurements in patients with coronary microvascular dysfunction (IMR>24), compared to patients with lower IMR. However, global longitudinal strain was lower in these patients (-14.60±1.37, vs. –12.80±2.54, p=0.013, Table 77).

In the 3 month follow-up echocardiogram, patients with IMR<24 showed significant reductions in end-systolic left ventricular volumes and significant improvements in left ventricular ejection fraction, Doppler measurements (E/A ratio and E/e' ratio), wall motion score index and global longitudinal strain, when compared to patients with IMR>24 (Table 78).

**Table 77 – First (acute) echocardiogram parameters according to median IMR**

| Variable [a, b] | Total Population | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=47)** | **(n=22)** | **(n=25)** | |
| LVEdV (ml) [a] | 105.8±24.2 | 102.5±23.9 | 108.6±24.5 | 0.43 |
| LVEsV (ml) [a] | 54.7±12.7 | 51.5±12.0 | 57.5±12.9 | 0.13 |
| LVEF (%)[a] | 47.9±6.7 | 49.4±6.6 | 46.5±6.6 | 0.17 |
| Wall motion score index [b] | 1.41 (0.35) | 1.49±0.23 | 1.54±0.23 | 0.49 |
| Left atria volume (ml/m$^2$) [a] | 34.8±12.2 | 34.7±13.6 | 35.0±11.3 | 0.95 |
| **Doppler measurements** | **(n=40)** | **(n=17)** | **(n=23)** | |
| E/A ratio [a] | 1.00±0.34 | 1.03±0.35 | 0.97±0.34 | 0.60 |
| E/e' ratio [a] | 9.00±2.71 | 9.01±3.34 | 8.99±2.12 | 0.98 |
| **2D speckle tracking imaging** | **(n=40)** | **(n=17)** | **(n=23)** | |
| Global longitudinal strain [a] | -13.54±2.28 | -14.60±1.37 | -12.80±2.54 | 0.013 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

**Table 78 – Second (3 month) echocardiogram parameters according to median IMR**

| Variable [a, b] | Total Population | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=54)** | **(n=29)** | **(n=25)** | |
| LVEdV (ml) [a] | 109.7±26.4 | 104.2±20.5 | 116.3±31.3 | 0.10 |
| LVEsV (ml) [a] | 52.9±18.8 | 47.7±13.2 | 59.4±22.6 | 0.025 |
| LVEF (%)[a] | 52.6±7.1 | 54.6±6.5 | 50.1±7.1 | 0.024 |
| Wall motion score index [b] | 1.24 (0.35) | 1.21±0.18 | 1.43±0.25 | <0.001 |
| Left atria volume (ml/m$^2$) [a] | 39.1±15.4 | 38.1±15.3 | 40.3±15.8 | 0.60 |
| **Doppler measurements** | **(n=52)** | **(n=28** | **(n=24)** | |
| E/A ratio [a] | 1.20±0.56 | 1.25±0.51 | 1.14±0.63 | 0.05 |
| E/e' ratio [a] | ±3.83 | 7.81±2.58 | 10.34±3.59 | 0.006 |
| **2D speckle tracking imaging** | **(n=51)** | **(n=28)** | **(n=23)** | |
| Global longitudinal strain [a] | -15.77±3.11 | -16.81±1.86 | -14.50±3.83 | 0.007 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

In the comparison of the two echocardiogram examinations according to the presence of coronary microvascular damage (Table 79), IMR clearly selected patients with different evolutions:

- Patients with IMR<24 evolved with significantly reductions in end-systolic left ventricular volumes, improvement in left ventricular ejection fraction and in E/e' ratio. Additionally, they showed important and very significant improvements in wall motion score index and global longitudinal strain. Finally, they tended to show lower left atria volumes and better E/A ratio.

- On the contrary, patients with IMR>24 significantly increased their end-diastolic left ventricular volumes, with no significant improvement in LVEF. They also had a significant increase in left atria volumes and a significant worsening in E/e' ratio. Their wall motion score index improved, although the magnitude and the statistical significance of this improvement was much lower than the one observed in patients with IMR<24. Additionally, they showed no improvement in global longitudinal strain.

**Table 79 – Baseline and 3 months echocardiographic parameters according to median IMR**

| | IMR < 24 | | | IMR > 24 | | |
|---|---|---|---|---|---|---|
| Echo parameters | Echo1 | Echo2 | P value[c] | Echo1 | Echo2 | P value[c] |
| **2D measurements** | **n=22** | | | **n=23** | | |
| LVEdV (ml) [a] | 102.5±23.9 | 101.2±20.3 | 0.78 | 108.3±26.0 | 118.3±32.1 | 0.043 |
| LVEsV (ml) [a] | 51.5±12.0 | 45.3±11.5 | 0.01 | 56.8±12.8 | 60.7±23.0 | 0.29 |
| LVEF (%)[a] | 49.4±6.6 | 55.4±5.1 | 0.001 | 47.3±5.2 | 49.7±6.8 | 0.13 |
| WMSI [b] | 1.41 (0.32) | 1.12 (0.12) | <0.001 | 1.41 (0.35) | 1.35 (0.41) | 0.010 |
| Left atria (ml/m2) [a] | 34.7±13.6 | 37.9±16.8 | 0.08 | 34.0±10.3 | 41.4±16.6 | 0.026 |
| **Doppler measurements** | **n=18** | | | **n=19** | | |
| E/A' ratio [a] | 1.01±0.34 | 1.14±0.33 | 0.08 | 0.97±0.35 | 1.14±0.64 | 0.13 |
| E/e' ratio [a] | 9.0±3.3 | 7.3±2.7 | 0.005 | 9.0±2.3 | 10.6±3.7 | 0.03 |
| **2D speckle tracking imaging** | **n=16** | | | **n=19** | | |
| Global longitudinal strain [a] | -14.6±1.4 | -17.2±1.3 | <0.001 | -13.3±2.4 | -14.4±3.6 | 0.10 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] p-value for the comparison between the first and the second Echo only in patients with 2 evaluations; Paired samples T-Test for variables with normal distribution and Wilcoxon test for variables with non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

### 7.2.3. IMR and contrast enhanced cardiac magnetic resonance

Of the 49 patients that did the ceCMR, 20 (40.8%) had an IMR above the median value of 24, and 15 (30.6%) above the reported prognostic value of 40.

IMR correlated with infarction mass (r=0.70, p<0.001) and percent infarct mass (r=0.54, p<0.001). Additionally, IMR also correlated with wall motion score index (r=0.42, p=0.003) and salvage mass (r=0.35, p=0.014).

When patients were stratified according to the mean IMR value for the global population, the ones with lower IMR (<24) had also significant lower wall motion score index (1.34±0.31 vs. 1.53±0.23, p=0.026). Their ejection fraction was slightly higher, but the difference was not statistically significant (Table 80).

**Table 80 – Contrast enhanced cardiac magnetic resonance parameters according to median IMR**

| | Total Population (n=49) | Coronary microvascular damage (IMR>24) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=29) | Yes (n=20) | |
| LVEdV (ml) [a] | 142.8±29.2 | 141.3±23.2 | 144.9±36.7 | 0.68 |
| LVEsV (ml) [a] | 66.3±21.8 | 65.0±18.3 | 68.2±26.5 | 0.62 |
| LVEF (%)[a] | 53.6±8.7 | 54.2±9.0 | 52.8±9.0 | 0.59 |
| Wall motion score index [a] | 1.42±0.29 | 1.34±0.31 | 1.53±0.23 | 0.026 |
| Oedema mass [b] | 19.6 (14.4) | 18.4 (13.6) | 22.5 (26.9) | 0.25 |
| Transmural necrosis [c] | 23 (46.9) | 8 (27.6) | 15 (75.0) | 0.001 |
| Infarct mass | | | | |
| Total [b] | 14.7 (12.6) | 11.4 (10.9) | 17.6 (15.0) | 0.031 |
| Percent [b] | 12.6 (14.4) | 11.6 (12.1) | 17.0 (15.4) | 0.035 |
| Indexed to APPROACH | 3.7 (4.5) | 3.2 (3.2) | 4.9 (6.8) | 0.050 |
| Indexed to BARI | 3.8 (4.3) | 3.3 (3.1) | 5.1 (5.2) | 0.044 |
| Salvage mass [b] | 4.5 (10.4) | 5.4 (9.7) | 3.9 (11.7) | 0.59 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Presented as number (%); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yates correction.

On the contrary, patients with higher IMR (>24) had more frequently transmural infarctions and higher total and percent infarct masses, as compared with patients to lower IMR.

**In summary**, the presence of coronary microvascular damage (IMR>24) was associated with larger infarcts, as measured by troponin I release and ceCMR. Patients with lower IMR values had a significantly better evolution in all echocardiogram parameters, with an improvement in left ventricular dimensions, function and dynamics, which was not seen in patients with an IMR>24.

## 7.3. Relation between IMR and microvascular reperfusion

### 7.3.1. IMR and ST resolution on the ECG

The percentage of resolution of ST elevation and deviation resolution is presented in Table 81 and in Figure 27, according to the presence of coronary microvascular damage (IMR>24). The ST residual changes are presented in Figure 28.

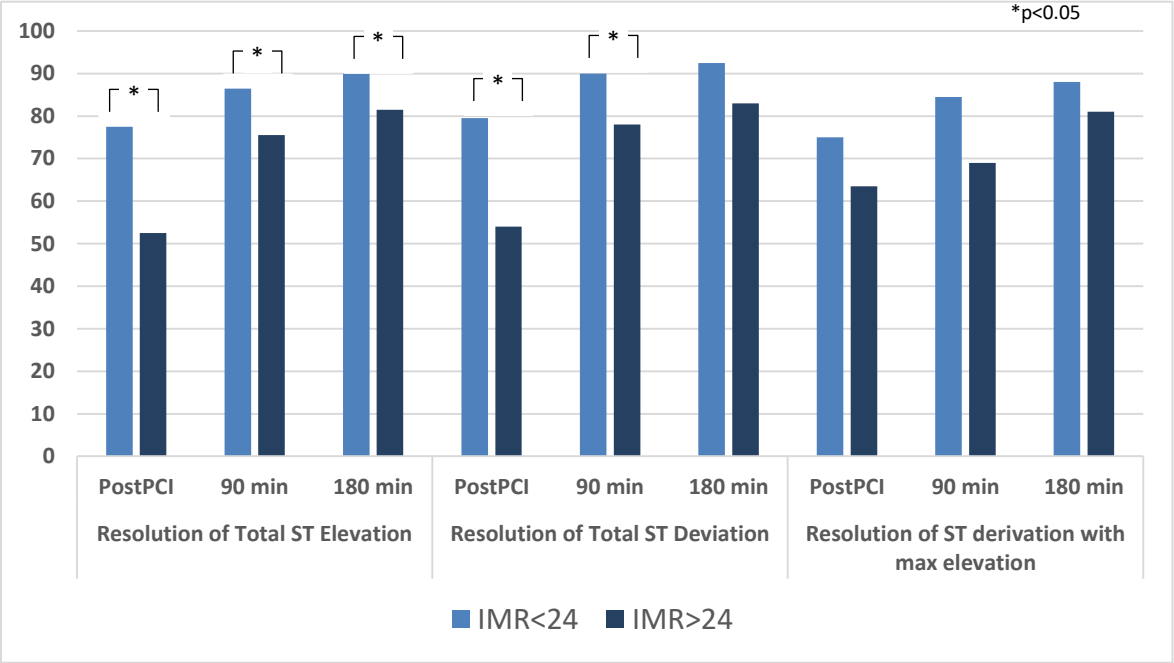


**Figure 27 – ST elevation and deviation resolution (median values) according to the presence of coronary microvascular dysfunction (IMR>24)**

Patients with IMR<24 had significantly better ST resolution of both total ST elevation and deviation and significantly less ST residual elevation and deviation in ECGs performed immediately after the PCI and at 90 and 180 minutes. They also showed a trend for better results in ST elevation resolution and residual changes in the lead with maximum ST elevation before the P-PCI.

The presence of Q waves was numerically higher in patients with IMR>24, but the difference was not statistically significant (Table 81).

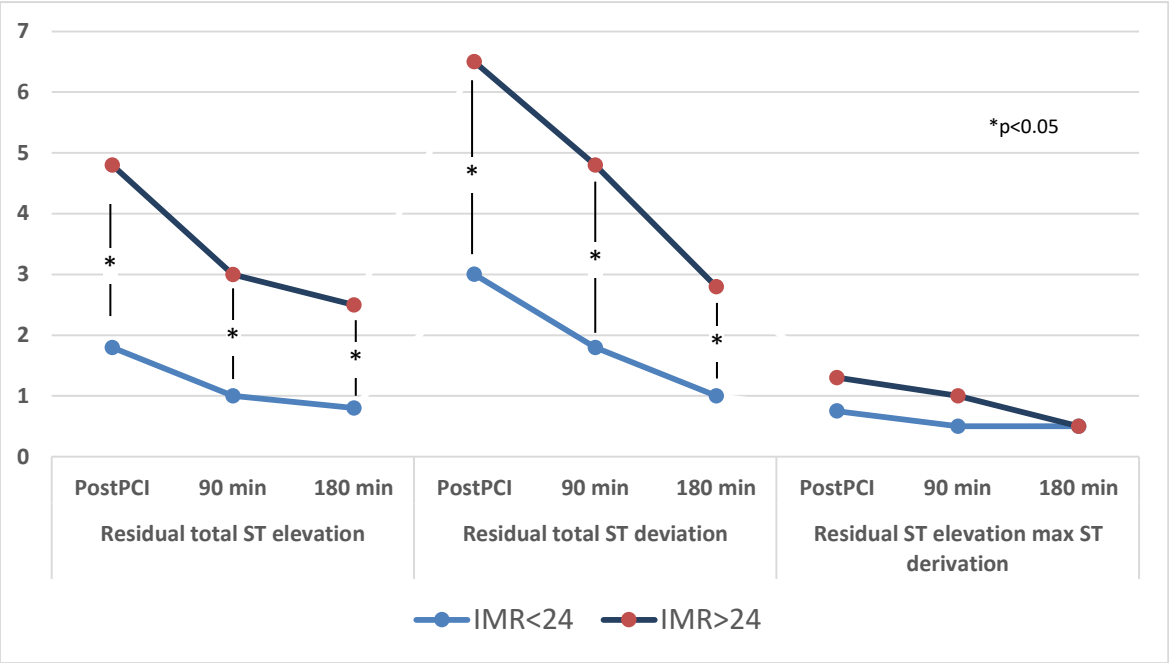


**Figure 28 – ST residual changes (median values) according to the presence of coronary microvascular dysfunction (IMR>24)**

**Table 81 – ECG ST resolution and residual changes according to the presence of coronary microvascular dysfunction (IMR>24)**

| Variable | Total population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value[d] |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| **Immediately post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 3.3 (6.0) | 1.8 (4.0) | 4.8 (11.0) | 0.016 |
| % Resolution | 74.5 (39.0) | 79.0 (33.0) | 52.5 (79.0) | 0.02 |
| Total ST deviation [b] | 4.8 (6.0) | 3.0 (5.0) | 6.5 (11.0) | 0.035 |
| % Resolution | 73.5 (44.0) | 79.5 (34.0) | 54.0 (53.0) | 0.046 |
| ST at derivation with max elevation) [b] | 1.0 (2.0) | 0.75 (2.0) | 1.3 (3.0) | 0.037 |
| % Resolution | 68.0 (47.0) | 75.0 (40.0) | 63.5 (56.0) | 0.051 |
| **90 minutes post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.5 (4.0) | 1.0 (3.0) | 3.0 (7.0) | 0.008 |
| % Resolution | 79.5 (32.0) | 86.5 (29.0) | 75.5 (36.0) | 0.014 |
| Total ST deviation [b] | 2.0 (4.0) | 1.3 (3.0) | 4.8 (8.0) | 0.015 |
| % Resolution | 82.5 (33.0) | 90.0 (26.0) | 78.0 (46.0) | 0.042 |
| ST at derivation with max elevation [b] | 0.5 (2.0) | 0.50 (1.0) | 1.0 (2.0) | 0.047 |
| % Resolution | 77.5 (34.0) | 84.5 (37.0) | 69.0 (28.0) | 0.077 |
| **180 minutes post-angioplasty ECG** [a] | | | | |
| Total ST elevation [b] | 1.5 (3.0) | 0.8 (3.0) | 2.5 (5.0) | 0.022 |
| % Resolution | 84.5 (23.0) | 90.0 (23.0) | 81.5 (40.0) | 0.048 |
| Total ST deviation [b] | 1.5 (4.0) | 1.0 (3.0) | 2.8 (5.0) | 0.049 |
| % Resolution | 88.0 (26.0) | 92.5 (24.0) | 83.0 (37.0) | 0.086 |
| ST at derivation with max elevation [b] | 0.5 (2.0) | 0.5 (1.0) | 0.5 (1.0) | 0.117 |
| % Resolution | 83.0 (33.0) | 88.0 (33.0) | 81.0 (30.0) | 0.162 |
| QS waves present | 37 (61.7) | 16 (53.3) | 21 (70.0) | 0.18 |

[a] Values expressed as median (interquartile range); [b] Values expressed in mV; [c] values expressed as n(%); [d] Mann-Whitney test for continuous variables with a non-normal distribution; [e] Chi-Square test.

### 7.3.2. IMR and angiographic indicators of microvascular reperfusion

Corrected TIMI frame count and TIMI myocardial perfusion grade results according to the presence coronary microvascular damage are presented in Table 82. Patients with IMR>24 had higher cTFC values (Table 82) and IMR significantly correlated with cTFC (r=0.64, p<0.001).

**Table 82 – Angiographic indicators of microvascular reperfusion according to the presence of coronary microvascular dysfunction (IMR>24)**

| Variable | Total Population (n=60) | Coronary microvascular damage (IMR>24) | | *p* value |
|---|---|---|---|---|
| | | No (n=30) | Yes (n=30) | |
| Corrected TIMI frame count [a] | 17.0 (7.0) | 14.0 (7.0) | 20.0 (10.0) | <0.001 [c] |
| TMPG 2-3 [b] | 49 (81.7) | 28 (93.3) | 21 (70.0) | 0.019 [d] |

[a] data presented as median (interquartile range); [b] data presented as n(%); [c] Mann-Whitney test; [d] Chi-Square test.

The presence of TIMI myocardial perfusion grade 2-3 was also significantly higher in patients with IMR<24.

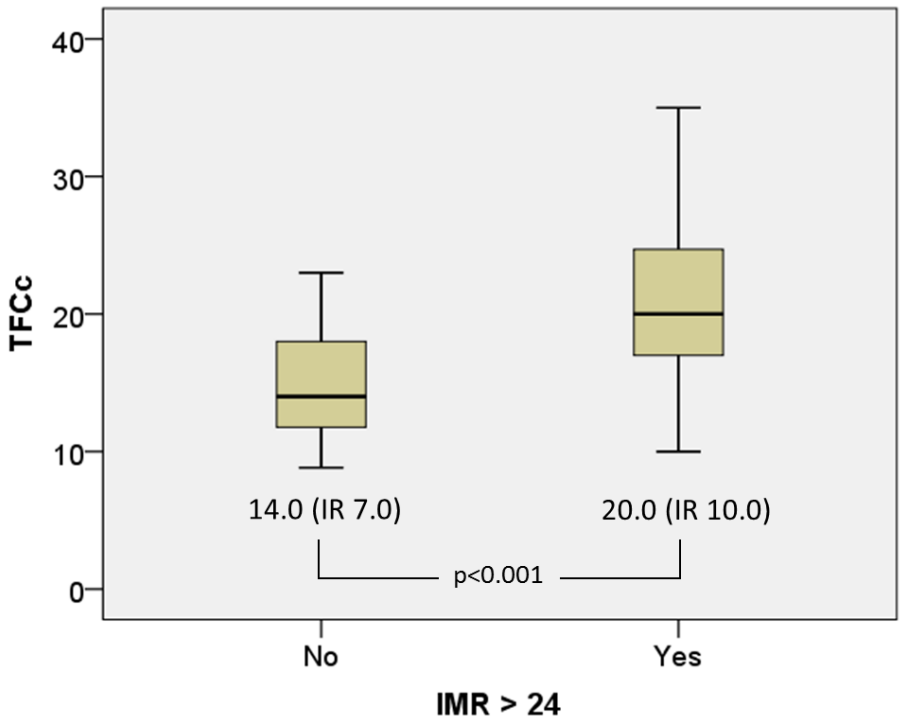


**Figure 29 – Corrected TIMI frame count, according to the presence of coronary microvascular dysfunction (IMR>24)**

### 7.3.3. IMR and microvascular obstruction on the ceCMR

IMR strongly correlated with microvascular obstruction as measured on the ceCMR (r=0.91, p<0.0001, Figure 30),

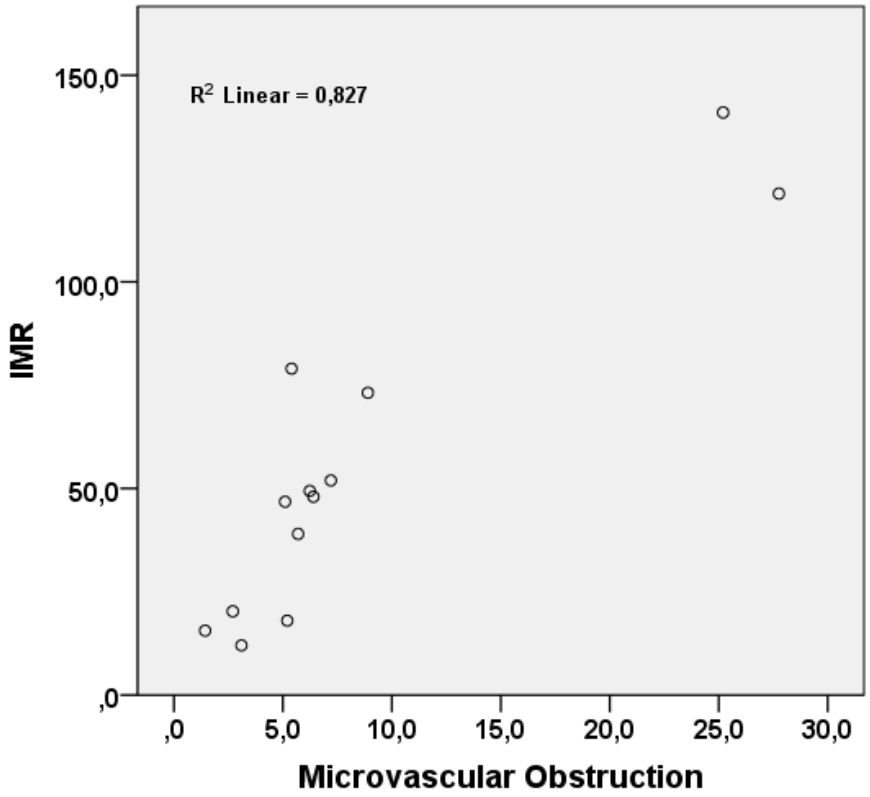


**Figure 30 – Correlation between IMR and microvascular obstruction on the ceCMR**

Patients with IMR>24 were more likely to have microvascular obstruction on the ceCMR (45.0% vs. 13.8%, p=0.015) and their microvascular obstruction mass was significantly larger (Table 83).

**Table 83 – Microvascular obstruction on the ceCMR according to median IMR**

| | Total Population (n=49) | Coronary microvascular damage (IMR>24) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=29) | Yes (n=20) | |
| Microvascular obstruction | | | | |
| MVO present [a] | 13 (26.5) | 4 (13.8) | 9 (45.0) | 0.015 |
| Mass of MVO [b] | 5.7 (4.0) | 2.9 (2.9) | 6.4 (11.5) | 0.006 |

[a] Presented as number (%); [b] Presented as median (interquartile range); [c] Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [d] Yates correction.

The receiver operating characteristics (ROC) analysis of IMR to predict microvascular obstruction (Figure 31) showed an AUC of 0.723 (CI 95% 0.500-0.896, p=0.018). The optimal cutoff values of IMR for predicting microvascular obstruction was 33 (sensitivity of 69.2% and specificity of 80.6%).

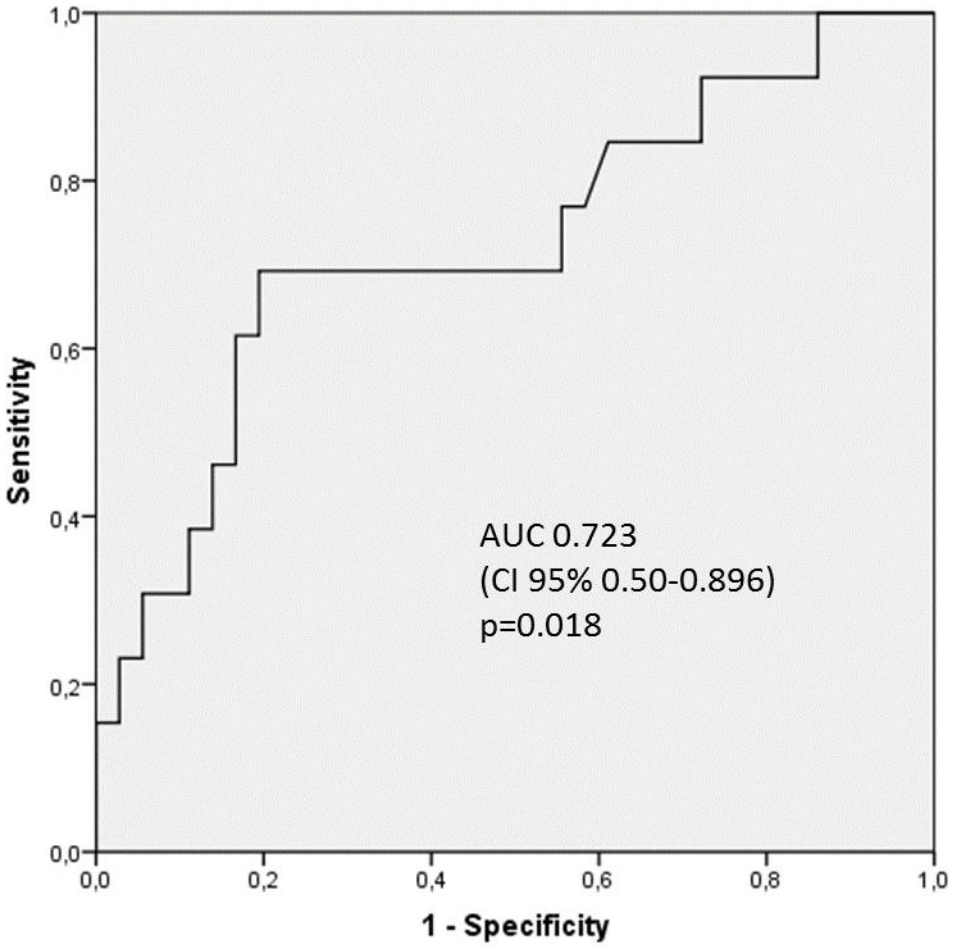


**Figure 31 – Receiver operating characteristics (ROC) curve of IMR to predict microvascular obstruction**

**In summary**, patients with IMR<24 showed significantly better ST resolution and significantly better angiographic indicators of microcirculatory reperfusion (cTFC and TMPG). Likewise, they had significantly lower microvascular obstruction in the ceCMR. IMR and microvascular obstruction were strongly correlated.

# 8. Summary of the results

## 1.1. Primary endpoint: IMR values and first EndoPAT

### 1.1.1. IMR values in patients with and without RHI<1.67

- There were no significant differences in IMR values in patients with and without RHI<1.67 on the first EndoPAT evaluation: median 16.0 (IQR 37.3) vs. median 24.0 (IQR 31.2), p=0.17.
- RHI values in the first EndoPAT evaluation were higher than expected (2.15±0.58) and only 11 patients (18.3%) had endothelial dysfunction as defined by an RHI<1.67.
- With the exception of active smoking and previous beta-blocker, therapy RHI values <1.67 were not related to any baseline patient characteristics, including age, risk factors, blood tests on admission, coronary anatomy or procedural aspects of the P-PCI.

### 1.1.2. IMR values according to RHI tertiles

- There were no significant differences in IMR values according to RHI tertiles: median 19.4 (IQR 35.0), median 40.5 (IQR 31.2) and median 23.3 (IQR 30.3), respectively for tertiles 1, 2 and 3.
- RHI tertiles did not relate either to any baseline patient characteristics, including age, risk factors, blood tests on admission, coronary anatomy or procedural aspects of the P-PCI.

### 1.1.3. Additional analysis: IMR values according to RHI as a continuous variable

- There was no significant relation between IMR values and RHI values measured on the first EndoPAT, evaluated as a continuous variable.
- RHI as a continuous variable did not relate either to any baseline patient characteristics, including age, risk factors, blood tests on admission, coronary anatomy or procedural aspects of the P-PCI.

## 1.2. Primary endpoint: IMR values and second EndoPAT

### 1.2.1. IMR values in patients with and without RHI<1.67

- RHI values in the second EndoPAT were lower (1.87±0.60) and more patients had RHI values <1.67 (n=16, 42%)
- There was a clear trend towards higher values of IMR in patients with RHI<1.67: median 40.5 (IQR 54.4) vs. median 22.0 (IQR 26.0), p=0.09. The prevalence of microvascular coronary damage, either defined as an IMR >24 (median value) or an IMR >40 (value published in the literature as prognostic marker in STEMI patients) was almost 2 times higher in patients with RHI<1.67, although differences did not reach statistical significance.
- Patients with RHI<1.67 also tended to have more severe coronary artery disease (multivessel disease, higher areas-at-risk and worse initial TIMI flow. Likewise, their values hs-PCR on admission tended to be higher.
- There were no other significant differences between groups in baseline patient's characteristic, other blood tests on admission or procedural aspects of the P-PCI.

### 1.2.2. IMR values according to RHI tertiles

- There was a trend for lower IMR values in the third tertile of RHI: 19.5 (IQR 30.6), compared to median 39.0 (IQR 43.4) and median 23.8 (IQR 42.5), respectively in tertiles 1 and 2 (p=0.64). The proportion of patients with microvascular damage (defined as an IMR above the median value of 24) also tended to increase with RHI tertiles: 38.5% vs. 53.8% vs. 66.7% (p=0.37).
- Patients in the higher tertile of RHI tended to have less severe coronary artery disease (less multivessel disease and lower SYNTAX scores) and had lower areas-at-risk (significant difference for the BARI score).
- There were no other significant differences between groups in baseline patient's characteristic, blood tests on admission or procedural aspects of the P-PCI.

### 1.2.3. Additional analysis: IMR values according to RHI as a continuous variable

- There was a tendency for lower RHI values in patients with higher IMR results
- RHI values were higher in male patients and in patients with previous treatment with ACEi/ARBs;
- RHI values tended to be lower in diabetics and previously revascularized patients.

- Additionally, RHI values on the second EndoPAT correlated with the area-at-risk and tended to be lower in patients with more complex coronary artery disease.

## 1.3. Secondary endpoint: Extent of myocardial infarction and microvascular reperfusion according to RHI values on the first EndoPAT

### 1.3.1. Extent of microvascular infarction

- The presence of RHI<1.67 on the first EndoPAT was not related to larger infarctions (measured by troponin release or by ceCMR).
- There was an improvement in echocardiogram parameters, both in patients with and without endothelial dysfunction, between the first and the follow-up exams.

### 1.3.2. Microvascular reperfusion

- The presence of RHI<1.67 on the first EndoPAT was not related to non-invasive (ST resolution) or invasive (cTFC and TMPG) indicators of microcirculatory reperfusion.
- Likewise, endothelial dysfunction was not related to microvascular obstruction on the ceCMR

## 1.4. Secondary endpoint: Extent of myocardial infarction and microvascular reperfusion according to RHI values on the second EndoPAT

### 1.4.1. Extent of microvascular infarction

- The presence of RHI<1.67 on the second EndoPAT was related to larger infarcts, as evaluated by the troponin I release and ceCMR.
- These patients also had lower LVEF and worse wall motion score index and GLS in the acute echocardiogram and in the ceCMR, although these differences were not present in the follow-up echo.

### 1.4.2. Microvascular reperfusion

- The presence of RHI<1.67 on the second EndoPAT was related to higher residual ST elevation on the post-PCI and 90 minutes ECGs; there was also a trend for lower ST resolution in these patients.
- There was a clear trend for worse cTFC and TMPG values in patients with RHI<1.67 on the second EndoPAT evaluation.
- The proportion of patients with MVO on the ceCMR was higher in patients with RHI<1.67 and there was a trend for higher MVO mass in these patients.

## 1.5. Secondary endpoint: Extent of myocardial infarction and microvascular reperfusion according to IMR values

### 1.5.1. Extent of microvascular infarction

- IMR values were associated with age and with admission glucose and HbA1c levels. Higher IMR values were also observed in patients with initial TIMI flow 0-1 and in patients who were not treated with abciximab, but these associations were not significant in the multivariate analysis. Finally, patients with higher IMR also had higher values of NT-pro-BNP.
- The presence of coronary microvascular damage (IMR>24) was associated with larger infarcts, as measured by troponin I release.
- IMR correlated with infarction mass, percent infarct mass, wall motion score index and salvage mass as measured in the ceCMR. Patients with higher IMR (>24) had more frequently transmural infarctions and higher total and percent infarct masses.
- Patients with lower IMR values had a significantly better evolution in all echocardiogram parameters, with an improvement in left ventricular dimensions, function and dynamics, which was not seen in patients with an IMR>24.

### 1.5.2. Microvascular reperfusion

- Patients with IMR<24 showed significantly better ST resolution and significantly better angiographic indicators of microcirculatory reperfusion (cTFC and TMPG).
- Likewise, they had significantly lower microvascular obstruction in the ceCMR. IMR and microvascular obstruction were strongly correlated.

# DISCUSSION

# 1. Main findings of the study

The main findings of this study were:

1. **First EndoPAT**:
    - RHI values measured acutely after primary PCI in patients with STEMI were higher than expected.
    - RHI was not related to IMR values or any other variables.
    - An RHI<1.67 was not related to larger infarcts.
    - An RHI<1.67 was not related to microcirculatory reperfusion/obstruction.

2. **Second EndoPAT**:
    - RHI values tended to relate to IMR values. Higher RHI values also tended to relate to larger myocardial area-at-risk, more severe coronary artery disease, worse initial TIMI flow and higher baseline hs-PCR values.
    - An RHI<1.67 was related to larger infarcts, lower LVEF and worse WMSI.
    - An RHI<1.67 was associated with more MVO and worse indirect indicators of microvascular reperfusion.

3. **Index of microcirculatory resistance**:
    - IMR was independently associated with age and admission glucose metabolism blood tests (glucose and HbA1).
    - IMR values were strongly related to microvascular obstruction. Patients with higher IMR values had significantly better indirect indicators of microvascular reperfusion.
    - IMR values were related to larger infarcts.
    - Patients with an IMR<24 had significantly less left ventricular remodelling in the follow-up echocardiogram.

The discussion of these results will be organized in the following sections:

- **Section 2:** Patient inclusion and validity of the sample.
- **Section 3**: First EndoPAT results
- **Section 4:** Second EndoPAT results
- **Section 5**: IMR results

## 2. Patient inclusion and validity of the sample

Our study included 60 patients, approximately 11% of the total population of STEMI patients admitted to our hospital during the study period.

Although this seems like a very low inclusion rate, it is important to compare it to other similar published studies. Unfortunately, of all published studies using IMR in STEMI patients (Table 84), only one presents the inclusion flow diagram according to the CONSORT recommendations for transparent reporting of trials[246] and the STROBE statement for observational studies[247], as we did. In the study mentioned, by Ahn and colleagues[248], the inclusion rate over 30 months was even lower (7.6%). Three other studies reported the rate of inclusion (ranging from 15.6% to 58.7%), but they did not follow the abovementioned recommendations for data reporting (Table 84).

**Table 84 – Main studies performed with IMR in STEMI patients**

| Author (Year) | Nº of patients | Period of inclusion | Patients screened (% included) | Mean age | Ischemic time (min) [c] | IMR values |
|---|---|---|---|---|---|---|
| Fearon (2008)[238] | 28 | NR | NR | 62.2 | 287±138 | Mean 39± 26<br>Median 32 |
| Lim (2009)[249] | 40 | NR | NR | 54±12 | 346±274 | Mean 34±24 |
| Sezer (2010)[250] | 35 | NR | NR | 58.4±9.3 | 210±145 | Mean 28.7±10.3 |
| McGeoch (2010) [251] | 53 | NR | NR | 58 (32-83) | 258 (132-420) | Median 35<br>(IQR 24-63) |
| Yoo (2012)[252] | 34 | NR | NR | 57±4 | 194±123 | NR [b] |
| Payne (2012)[253] | 108 | 10 months | NR | 57.8±10.2 | 186 (137-331) | Median 26<br>(IQR 17-41) |
| Fearon (2013)[64] | 253 [a] | NR | NR | 56.8±10.6 | NR | Median 31<br>(IQR 21-49) |
| Fukunaga (2014)[254] | 88 | 29 months | 150 (58.7%) | 67±13 | 345±193 | Median 33<br>(IQR 8-170) |
| Anh (2014)[255] | 40 | 15 months | 256 (15.6%) | NR [b] | NR [b] | NR [b] |
| Cuculi (2014)[256] | 82 | 12 months | NR | 62±12 | 209±163 | Mean 42.8±30.0 |
| Patel (2015)[173] | 34 | NR | 131 (23.1%) | 61.0±10.6 | 174 (114-276) | Median 28.6<br>(IQR 20.0-43.5 |
| Park (2016)[257] | 89 | 47 months | NR | 54±10 | NR [b] | Mean 26.5±16.7 |
| Palmer (2016)[258] | 31 | NR | NR | 60±10 | 138±161 | Median 24<br>(IQR 23-45) |
| Ahn (2016)[248] | 40 | 30 months | 529 (7.6%) | 56±9 | 276±116 | Mean 33±19 |
| **Baptista** | **60** | **36 months** | **543 (11.0%)** | **59.6±12.7** | **209 (IQR 148)** | **Median 24 (IQR 33).** |

[a] multicentric registry; [b] Reported only for subgroups, not for the total population; NR: not reported; [c] Ischemic time: time between symptom onset and the first balloon dilation ("pain-to-balloon" time)

Regarding this issue, there are some important points that deserve mention:

- First of all, including acute STEMI patients in trials (particularly observational trials, in which additionally procedures will not bring any gains to the patient) is difficult. Patients are often under extreme stress and the process of informed consent is severely constrained. In this setting, pushing for an equivocal informed consent should not be done.[259–262]
- Secondly, even though measuring IMR is a safe procedure to perform immediately after P-PCI[180], it still implies introducing a new guide-wire into the culprit coronary artery and administrating adenosine. As such, all patients with hemodynamic, rhythm or conduction disturbances need to be excluded. In our cohort, in addition, all patients had TIMI 3 flow at the end of the procedure. Although the later was not necessary (a TIMI flow <3 was not an exclusion criteria), usually patient with final compromised flow have ongoing pain and often some hemodynamic and rhythm instability and it is understandable that operators chose not to include these patients.
- Finally, most research studies performed with IMR are relatively complex and require at least one more diagnostic test to be performed (MPE, ceCMR, PET, etc.). More complex trials imply more contra-indications and limitations for patient inclusion.

Still, it has to be acknowledged that this is a selected population, younger and probably healthier than the general population of patients admitted with acute STEMI. Mean age in our study (59.6±12.7 years), for instance, although numerically superior to most other published studies (Table 84), was significantly lower than the mean age of the total population of patients admitted to our hospital in the same period, but not included in the study (N=484; 63.6±13.4, p=0.029).

While it is plausible to argue that results in such selected cohorts should not be extrapolated to the global population of STEMI patients, it is also fair to acknowledge that the consistent results observed in IMR studies were obtained even though more severe patients were excluded. As such, results would eventually have been even more meaningful if those more severe patient had been included. Despite these selection issues, the mean age and the median ischemic time in our cohort are within the intervals of values reported in the previous trials (Table 84).

Additionally, the main epidemiological characteristics of the included cohort of patients is similar to other recent STEMI studies, both performed in single centres, and reported in the Portuguese National Interventional Cardiology Registry. Likewise, there are apparently no significant differences, when comparing these results to a recent large international registry (Table 85). Therefore, the sample collected for this study seems to be a valid one.

**Table 85 – Comparison of patient's main characteristics in contemporary national and international registries in STEMI**

| | Current study | Sousa (2012)[263] | Timóteo (2013)[264] | Portuguese Nat. Registry (2013)[265] | CathPCI Registry (2011)[266] |
|---|---|---|---|---|---|
| No. of patients | 60 | 223 | 607 | 3.524 | 24.449 |
| Inclusion | Selected | Consecutive | Consecutive | Consecutive | Consecutive |
| Age (years) | 60±13 | 60±12 | 62±13 | 65±12 | 61±13 |
| Male Gender | 80% | 76% | 76% | 74% | 78% |
| BMI | 27.5±4.0 | NR | NR | NR | 29.1±6.1 |
| **Risk Factors** | | | | | |
| Diabetes | 25.0% | 17.0% | 21.9% | 32.5% | 20.7% |
| Hypertension | 71.7% | 56.0% | 62.0% | NR | 65.3% |
| Dyslipidaemia | 50.0% | 50.0% | 51.1% | NR | 65.0% |
| Current smoker | 43.3% | 56.0% | 45.0% | NR | 42.7% |
| **Primary PCI** | | | | | |
| Ischemic time [a] | 209 (IQR 148) | 270 (-) | NR | NR | NR |
| Stent used | 95.0% | NR | NR | 92.2% | 90.6% |
| Mechanical thrombectomy | 43.3% | NR | NR | 46.7% | 39.2% |
| IIb/IIIa inhibitors | 23.3% | NR | NR | 23.6% | 42.8% |

[a] Ischemic time: time between symptom onset and the first balloon dilation ("pain-to-balloon" time); NR: not reported

# 3. First EndoPAT results

## 3.1. Implications of the RHI results in the first EndoPAT evaluation

This was the first study ever to measure peripheral endothelial function in the acute phase (immediately after primary PCI) of ST elevation myocardial infarction patients.

Reactive hyperemia index (RHI) results on this first EndoPAT evaluation were clearly higher than expected, even if outlier results (5 extreme high and 1 extreme low) are not considered.

In fact, when comparing these results to other published studies using EndoPAT in patients with coronary artery disease, the values we reported in the first EndoPAT evaluation (2.15±0.58) are the highest and the number of patients with endothelial dysfunction (18.3%, defined as an RHI<1.67) is the lowest (Table 86), even though the other studies were performed either in stable angina or in stabilized ACS patients.

**Table 86 – Values of RHI reported in studies performed with EndoPAT in patients with coronary artery disease**

| Author (year) | No. of patients | Clinical setting | Time of procedure | RHI values |
|---|---|---|---|---|
| Schoenenberger (2012)[267] | 362 | PCI | 24 hours | RHI 1.82±0.48<br>45.0% patients had an RHI<1.67 |
| Baptista (2013)[19] | 231 | Diagnostic angiography | NR (not acute) | RHI 2.10+/-0.63 |
| Yamamoto (2014)[268] | 86 | STEMI, P-PCI | 3 weeks | RHI 1.70 (1.46-2.14) in patients with restenosis vs.<br>RHI 1.87 (1.65-2.19) in patients without restenosis |
| | | | 8 months | RHI 1.75 (1.46-2.06) in patients with restenosis vs.<br>RHI 2.12 (1.61-2.45) in patients without restenosis |
| Kandhai-Ragunath (2014)[269] | 71 | STEMI. P-PCI | 4-6 weeks | RHI 1.90±0.58<br>47.9% patients had an RHI<1.67 |
| Baptista (2016)[18] | 58 | STEMI, P-PCI | 5th-7th day | RHI median 1.78 (IQR 0.74)<br>43.1% patients had an RHI<1.67 |
| Levi (2016)[270] | 30 | STEMI, P-PCI | 48-72h | RHI 1.91±0.3 in patients with no reflow<br>RHI 2.09±0.11 in patients with normal flow |
| **Current study** | | | | |
| **First EndoPAT** | **60** | **STEMI, P-PCI** | **1h** | **RHI 2.15±0.58 (2.08±0.42 after exclusion of outliers)**<br>**18.3% patients had RHI<1.67** |
| **Second EndoPAT** | **38** | **STEMI, P-PCI** | **24h** | **RHI 1.87±0.60**<br>**42.1% patients had an RHI<1.67** |

NR: not reported; RHI: reactive hyperemia index; STEMI: ST elevation myocardial infarction: P-PCI: primary percutaneous coronary intervention.

The presence of endothelial dysfunction in patients with acute coronary syndromes has been repeatedly demonstrated (Table 87):

- Elbaz and colleagues evaluated invasively the endothelial function in non-culprit arteries in patients with NST-ACS using intracoronary infusion of acetylcholine and found that 81% of patients had a blunted response to this endothelium-dependent vasodilator.[271]
- Spiro and colleagues used flow-mediated dilation and low flow-mediated constriction to demonstrate that endothelial function is significantly more compromised in patients with ACS than in patients with stable coronary artery disease, both before and after angioplasty.[272]
- More recently, Careri evaluated endothelial function in the first 12 hours both in stable and NST-ACS patients, and showed that flow-mediated dilation is significantly lower in acute patients.[132]
- Finally, we also demonstrated that patients with STEMI treated with P-PCI have a high prevalence of endothelial dysfunction evaluated with PAT.[18]

This *endothelial dysfunction* was associated with a worse prognosis, using different techniques and at different times after the symptoms onset (from 12 hours to several days or weeks). Importantly, the absence of late (2 to 3 months) recovery of this *endothelial dysfunction* was also associated with a dire outcome (Table 87).

In the face of all this evidence, how do we explain the results obtained in our first endothelial function evaluation?

As previously stated, endothelial function testing is particularly challenging in acute patients, for several reasons: in addition to the unpredictable nature of the acute event onset, the unstable condition and the pain, anxiety and fear patients usually experience, most of the times it is not possible to comply with several recommendations for endothelial function evaluation (including fasting state, morning exam, avoidance of caffeine or smoking, etc.).[199]. Additionally, patients were evaluated after an arterial puncture (for the urgent angiography) and several venous punctures (both for blood tests and for drugs administration).

All these factor might have influenced the results. However, another important phenomenon may have occurred: as explained in the Introduction (section 7.1, page 66) and in the Methods (section 3.9, page 95), the RHI is calculated as the ratio between hyperaemic and baseline pulse volume analysis (PAT ratio) *normalized* for the same ratio in the contralateral arm. If the presence of peripheral vasoconstriction (likely to occur in acute patients[273,274]), the normalization using a constricted control arm may have led to an *increase* in the ratio of reactive hyperemia, thus explaining the unexpected high values of RHI found in our study. Importantly, this initial vasoconstriction usually normalizes in the first 24 hours in stable patients.[273]

The low prevalence of *endothelial dysfunction* (defined as an RHI<1.67) in our cohort could eventually also be explained by the fact that this threshold (defined for stable populations) does not apply in acute patients. That was the reason why we initially planned an analysis based on tertiles of RHI and the motive that led us to perform the unplanned analysis using RHI as a continuous variable.

However, independently of the analysis performed (RHI as dichotomous variable using the 1.67 threshold; RHI divided in tertiles; or, RHI as a continuous variable), RHI values in the first EndoPAT evaluation did not relate to any baseline patient characteristics, including age, risk factors (except a trend for lower values in active smokers) or previous pharmacological treatment. Likewise, we did not find any significant relations between the first and the second EndoPAT evaluations.

All together, these results argue *against the validity* of this initial EndoPAT test.

**Table 87 – Studies evaluating endothelial function in the acute/subacute phase of acute coronary syndromes**

| Author (year) | No. of patients | Clinical presentation | Technique | Time of evaluation | Results |
|---|---|---|---|---|---|
| **Prevalence of endothelial dysfunction** | | | | | |
| Elbaz (2005)[271] | 43 | NST-ACS | Ach | Acute and 6 months | 81% of patients had impaired response to Ach in non-culprit arteries in the acute evaluation, of which 77% recovered in the 6 months evaluation |
| Spiro (2011)[272] | 96 | Stable angina and NSTEMI | FMD<br>L-FMC | Before/after PCI, and 2.5 months | L-FMC significantly higher in NSTEMI patients<br>FMD and L-FMC significantly lower after PCI; FMD and L-FMC improved in FUP |
| Careri (2013)[132] | 100 | Stable angina and NST-ACS | FMD | 12 hours | FMD on admission significantly lower in patients with NST-ACS compared to those with stable CAD (2.1±1.2% vs 4.8±1.9%, p<0.001) |
| Baptista (2016)[18] | 58 | STEMI, P-PCI | PAT | 5th-7th day | 43.1% patients had endothelial dysfunction (RHI<1.67) |
| **Prognosis of endothelial dysfunction** | | | | | |
| Fichtlscherer (2004)[131] | 198 | NST-ACS | VOP [a] | 5th day | Blunted forearm blood flow responses to Ach associated with worse prognosis (HR 0.54, CI95% 0.33-0.89, p<0.002) |
| | | | | 8 weeks | Recovery of blood flow response associated with better prognosis (ROC AUC 0.77; 95% CI 0.664-0.874, p=0.003) |
| Karatzis (2006)[130] | 98 | NST-ACS | FMD | 24 h | FMD<1.9% (lower tertile) associated with shorter event-free survival (HR 3.035, 95% CI 1.148 -8.023, p=0.025) |
| Guazi (2009)[329] | 179 | 20% STEMI<br>80% NSTEMI | FMD | 5th day | Arterial diameter change <4.5% related with event-free survival (38.5% vs. 78.4% in diabetics and 67.7% vs. 88.7% in non-diabetics) |
| Wang (2009)[330] | 101 | STEMI, P-PCI | FMD | 5th day | FMD associated with events (HR: 0.705, 95% CI:0.573 – 0.868, P= 0.0010); optimal FMD threshold was 5.5% (ROC area 0.689, p=0.0012) |
| Careri (2013)[132] | 100 | Stable angina and NST-ACS | FMD | 12 hours<br>3 months | FMD significantly recovered between acute and 3 months evaluations;<br>Absence of recovery in FMD was associated with more evets |
| **Left ventricular remodelling and infarction extent** | | | | | |
| Bissinger (2011)[133] | 93 | STEMI, P-PCI | FMD | 3rd day and 6 months | FMD lower in diabetics<br>Improvement in FMD inversely correlated with increase in LDEDV |
| Baptista (2016)[18] | 58 | STEMI, P-PCI | PAT | 5th-7th day | RHI<1.67 associated with higher peak TnI values in STEMI patients |

[a] VOP: venous occlusion plethysmography; L-FMC: low-flow-mediated constriction; FMD: flow-mediated dilation; PAT: peripheral arterial tonometry; STEMI: ST elevation myocardial infarction: NST-ACS: acute coronary syndromes without ST elevation; NSTEMI: non-ST elevation myocardial infarction; LVEDV: left ventricle end-diastolic volume

## 3.2. IMR, microvascular obstruction and extent of infarction according to RHI on the first EndoPAT

We found no relation between RHI on the first EndoPAT (analysed as a dichotomous variable, in tertiles or as a continuous variable) and IMR. Likewise, we found no relation of RHI and any of the measures of necrosis extension or any of the indicators of microvascular obstruction.

Three explanations can justify these findings:

- The first one would be that endothelial function is not involved in the acute mechanisms that occur in STEMI patients. If this was the case, we could hypothesize that microvascular coronary dysfunction and the consequent myocardial infarction extent are only related to non-endothelial dependent factors (including distal embolization of thrombi and debris, and/or other unknown factors). A large amount of evidence argues against this hypothesis: i) even though peripheral endothelial function was never evaluated acutely in STEMI patients, coronary *endothelial dysfunction* has been clearly demonstrated in NST-ACS patients in the acute angiography[271] and peripheral *endothelial dysfunction* was also demonstrated in ACS patients before PCI;[272] ii) patients with *endothelial dysfunction* measured with EndoPAT have an higher risk for events[129,275–278], so it would be at least expectable to find a high percentage of STEMI patients with *endothelial dysfunction*, even if it did not relate to IMR or other indicators of coronary microvascular dysfunction; iii) if non-endothelial dependent factors, including distal embolization during P-PCI, prevailed as the main contributors for coronary microvascular dysfunction, it would be expectable that strategies targeted to that complication (like thrombectomy and distal protection devices) would improve indicators of coronary microvascular damage, but that is not the case[73–75], iv) thromboembolic distal embolization by itself results in the release of vasoactive factors[279], implying that some degree of *endothelial dysfunction* would be expectable in these STEMI patients.
- The second explanation could be that peripheral endothelial function evaluated with EndoPAT does not reflect coronary endothelial function in STEMI patients. This is also unlikely, since a good correlation between coronary and peripheral endothelial function was shown with several different techniques[195,206,208], including EndoPAT[207], in stable patients and there is no reason to suppose that this would be different in STEMI patients. Our results with the second EndoPAT, although performed 24 hours after the first one, also argue against this hypothesis (see below).
- Finally, the third possible explanation is that EndoPAT measurements in the acute phase are not valid. For all the reasons described in the previous section, this seems to be the more plausible explanation for the observed results. It implies, therefore, that EndoPAT cannot be used to evaluate peripheral endothelial function in the acute phase of STEMI, after P-PCI.

# 4. Second EndoPAT results

## 4.1. RHI results in the second EndoPAT evaluation

On the second EndoPAT evaluation (38 patients), values were significantly lower (1.87±0.60, p=0.006; Table 15) as compared to the first EndoPAT evaluation and, importantly, they were similar to the values reported by Schoenenberger and colleagues (1.82±0.48) in the only study published that also measured RHI 24 hours after P-PCI in STEMI patients.[267]

Accordingly, the percentage of patients with an RHI<1.67 in this second evaluation (42.1%) is very consistent with the results observed in all studies that reported this information in the acute/subacute phase of ACS: 47.9% in the study by Yamomoto *et al*[268], 43.1% in our pilot study[18] and 45.0% in the study by Schoenenberger *et al*[267] (Table 87).

It is also important to underscore that the values of RHI on the second EndoPAT evaluation did not correlate with the values on the first EndoPAT (r=0.260, p=0.131). Among patients with *endothelial dysfunction* (RHI<1.67) on the first EndoPAT, half evolved to normal endothelial function on the second one. On the other hand, 44.8% patients with a normal endothelial function on the first EndoPAT ended up with *endothelial dysfunction* (RHI<1.67) on the second evaluation.

The second EndoPAT evaluation was performed in a much calmer situation, still in the Coronary Care Unit, but with much less stress involved. Medication known to affect endothelial function (like statins and anti-platelets) was given to all patients studied. Importantly, the time of the day was not the same for all measurements, since it depended on the hour of the primary angioplasty. Accordingly, the patients could be or not on a fasting state.

Still, the fact that this second EndoPAT results are apparently not related to the first ones and the consistency of the results when compared to other similar studies suggests that the problems of validity discussed for the first evaluation are not confirmed in the second one.

This is further reinforced by the observation that patients with previous (before the current event) ACEi/ARBs treatment had higher RHI values, with a similar trend observed in patients previously treated with statins and beta-blockers. Similarly, there was a trend for lower RHI values in patients with known diabetes and previous coronary revascularization. All these observations are in accordance to previous publications on endothelial function.[121,199,280]

## 4.2. IMR, microvascular obstruction and extent of infarction according to RHI on the second EndoPAT

### 4.2.1. IMR, microvascular obstruction and extent of infarction

The prevalence of microvascular coronary damage, either defined as an IMR >24 (median value) or an IMR >40 (value published in the literature as prognostic marker in STEMI patients) was almost 2 times higher in patients with *endothelial dysfunction* (RHI<1.67), although differences did not reach statistical significance. The number of patients with IMR>24 or IMR>40 also decreased from the first to the third tertile of RHI. Likewise, there was a clear trend towards higher values of IMR in patients with *endothelial dysfunction*: median values (IQR) 40.5 (54.4) vs. 22.0 (26.0) in patients without *endothelial dysfunction* (p=0.09).

In accordance to the IMR results, the presence of *endothelial dysfunction* (RHI<1.67) also tended to relate with electrocardiographic (particularly residual ST elevation after P-PCI) and angiographic (cTFC and TMPG) indicators of microvascular reperfusion.

Altogether, these data suggest a higher damage of coronary microvasculature in patients with *endothelial dysfunction* evaluated on the second EndoPAT, which is confirmed by the results of the ceCMR: patients with an RHI<1.67 had an almost 5 times higher probability (11.1% vs. 54.5%, p=0.03) of having microvascular obstruction on the ceCMR, and tended to have higher obstruction masses.

Endothelial dysfunction was also clearly related to the extension of the infarct: patients with an RHI<1.67 had significantly higher values of both peak TnI and AUC of TnI, either total or indexed to the area-at-risk scores. Correspondingly, they had a 3 times higher risk of transmural necrosis (22.2% vs. 63.6%, p=0.06) and a clear tendency to higher total and percent infarct masses in the ceCMR. In line with these results, they also had lower left ventricular ejection fractions and higher (worse) wall motion score indexes, both in the initial echocardiogram and in the ceCMR.

To our knowledge, this is the first study reporting the association of early (24 hours) measured peripheral endothelial dysfunction with microvascular coronary damage and with myocardial infarction extension in patients with STEMI.

### 4.2.2. Endothelial dysfunction: cause, consequence or both?

The fact that patients with endothelial dysfunction had a tendency for more severe coronary artery disease (the prevalence of multivessel disease was almost two times higher in patients with endothelial dysfunction [68.8% vs. 36.4%] and SYNTAX scores also tended to be higher

in these patients) is in accordance with our previous work[19] and suggests that at least the severity of *previous* coronary artery disease is related to endothelial dysfunction. We also found a trend for lower initial TIMI flow in patients with endothelial dysfunction, which his in agreement with a recent study by Kandhai-Ragunat and colleagues. These authors documented lower RHI values (although measured 4 to 6 weeks after the STEMI) in patients with an occluded artery before the P-PCI, as compared to patients with a patent artery (2.08±0.34 vs. 1.75±0.35; p<0.007),[269] suggesting that endothelial dysfunction may be involved in microvascular reperfusion in STEMI patients.

However, we also found a significant negative correlation between both area-at-risk scores and RHI. The area-at-at risk evaluated by the BARI score was significantly lower in patients in the third tertile (higher values) of RHI and there was a trend for lower RHI in patients that had the LAD as culprit artery. Both larger areas-at-risk and the LAD as culprit artery are associated with larger infarctions, implying that the documented lower RHI values may indirectly measure the extension of the infarction, as opposed to being part of the pathophysiological process that caused it. Indeed our finding that patients with *endothelial dysfunction* in the first 24 hours after STEMI have larger infarcts and lower ejection fraction are in line with the evidence of neuro-humoral activation in patients with heart failure. In fact, it was already demonstrated that the reduction in stroke volume produces a lower endothelial shear stress, which causes a dysregulation in NO synthase isoforms gene expression[281,282], eventually leading to a reduced NO bioavailability.[283] Furthermore, there is an additional reduction in NO bioavailability caused by direct NO destruction by reactive oxygen species, mainly driven by an increase in angiotensin II and aldosterone activity, and purine metabolism.[284]

While we cannot infer from our results whether the endothelial dysfunction was a *cause* or a *consequence* of the severity of the myocardial infarction, it is reasonable to conclude that peripheral endothelial dysfunction in these patients may be a marker of the severity of the myocardial disease and, therefore, of its systemic consequences, which makes it useful to assess the effectiveness of therapy and predict events, independently of its exact pathophysiological role.

In fact, in patients with acute myocardial infarction, early treatment with statins, currently known to reduce events, was initially evaluated through its beneficial effect in the endothelial function.[285,286] Likewise, in heart failure patients, peripheral endothelial function has been described as a tool to predict the response to cardiac resynchronization therapy, and endothelial dysfunction improvement after CRT correlated with functional improvement[287], being related to an increase in cardiac output[288], likely mediated by shear-stress.[289]

In summary, albeit demonstrating the exact role of endothelial dysfunction in STEMI patients is not possible at this point, we demonstrated that its measurement early after primary PCI is feasible and related both with the extent of infarct and microvascular obstruction, which makes it a valuable tool both for identifying high risk patients and for testing new pharmacological or device-based strategies.

# 5. IMR results

## 5.1. IMR and microvascular obstruction

IMR strongly correlated with all other indirect indicators of microvascular reperfusion. Regarding ECG, patients with IMR<24 had significantly better ST resolution of both total ST elevation and deviation and significantly less ST residual elevation and deviation in ECGs performed immediately after the PCI and at 90 and 180 minutes. They also showed better results in ST elevation resolution and residual changes in the lead with maximum ST elevation before the P-PCI.

Most studies performed with IMR did not report this and some of the ones that did it[248,250,290] could not find significant differences in ECG parameters between patients with and without microvascular obstruction. However, it must be acknowledge that the classic thresholds of 50 or 70% in ST resolution were derived from thrombolytic trials in which, in fact, a significant percentage of patients did not reperfuse the culprit artery. In the primary-PCI era, a normal flow (TIMI 3) is almost always obtained and therefore the vast majority of patients evolves with significant ST resolution in the first hours after the procedure – in our cohort, 68.3% and 81.7% had an ST resolution higher than 70% respectively at 90 and 180 min after the P-PCI. While we must accept that a consensus is still lacking about which leads to analyse, the optimal timing of electrocardiogram analysis, and whether standard ECG or continuous ECG monitoring is preferable, ECG may still have his role in identifying patients with microvascular obstruction, being an easy and inexpensive exam to perform.

Concerning the angiographic indicators, corrected TIMI frame count was significantly higher in patients with IMR>24 and there was a significant correlation between IMR and cTFC (r=0.64, p<0.001), Likewise, a TIMI myocardial perfusion grade 2-3 was significantly less frequent in patients with IMR>24. Strangely, of the 6 studies that evaluated the role of IMR in predicting MVO, only 1 reported these data – Carrick *et al* found significantly higher cTFC in lower tertiles of IMR and a significantly higher percentage of patients with TMPG 2/3 in the lower tertiles[290], in line with our results. Likewise, in the largest cohort with IMR published to date, there were significant associations with both variables.[64]

These ECG and angiographic results suggest that IMR is a good predictor of microvascular obstruction, which was confirmed in the ceCMR: IMR strongly correlated with microvascular obstruction (r=0.91, p<0.0001), patients with an IMR>24 were much more likely to have microvascular obstruction on the ceCMR (45.0% vs. 13.8%, p=0.015) and their microvascular obstruction mass was significantly higher.

These results confirm those previously published by others (Table 4) and support the feasibility of IMR as a potentially useful tool for early stratification of patients with acute STEMI following coronary reperfusion.

## 5.2. IMR and infarct extension

Patients with an IMR>24 had significantly higher values of both peak TnI and AUC of TnI, and IMR was significantly correlated with both, particularly when those measurements were indexed to the area-at-risk scores.

Previous studies reported similar results, but only with peak CK (Fearon et al[238]; r=0.61, p=0.005) or peak TnI (Mcgeoch et al; r=0.52, p=0.01[251]). Others have shown that when patients are divided into groups according to IMR values, those in the higher IMR groups also have higher mean CK[249,252,257] or TnI[257] values.

However, evidence suggests that peak CK and peak TnI are weak predictors of the extension of myocardial necrosis, particularly when these measurements are performed only in the first 24 hours after admissio[291]. Among the studies that evaluated IMR, our study is the first to systematically measure TnI values in the first 48 hours after primary PCI, establishing a curve of TnI release and calculating both its area under the curve and peak values. We obtained results similar to the ones previously published, reinforcing the consistency of IMR measurements in STEMI patients.

The cardiac biomarker results were confirmed by the ceCMR evaluation: IMR correlated with infarction mass (r=0.70, p<0.001) and percent infarct mass (r=0.54, p<0.001). Additionally, IMR also correlated with wall motion score index (r=0.42, p=0.003) and salvage mass (r=0.35, p=0.014). Again, these results confirm those in three recently published studies, also performed with ceCMR[251,253,290] and a fourth that used SPECT.[250]

Taken together, these results confirm IMR as a valuable tool for predicting myocardial infarction extension in STEMI patients treated with P-PCI.

## 5.3. IMR and left ventricular remodelling

We confirmed the results of others (Table 3), showing that patients with IMR<24 have less left ventricular remodelling in the follow up echocardiogram (with significant reductions in end-systolic left ventricular volumes and significant improvements in left ventricular ejection fraction, Doppler measurements [E/A ratio and E/e' ratio] and wall motion score index), when compared to patients with IMR>24.

Additionally, we showed for the first time that baseline (in the acute echocardiogram) global longitudinal strain (GLS) was lower in patients with lower IMR (-14.60±1.37, vs. –12.80±2.54, p=0.013). We recently published this analysis in a subset of the final cohort of patients[56] and the results were confirmed in the final cohort. In our study, IMR evaluated immediately after P-PCI correlated with GLS evaluated in the first 24 hours after infarction. However, there was no relation between IMR and the conventional echocardiographic parameters, evaluated at the same time, suggesting the higher diagnostic accuracy of strain techniques. The additional value of strain for

myocardium evaluation after infarction has been extensively reported: Sjøli *et al*. evaluated a population of patients with STEMI by echocardiography and CMR and concluded that LV global strain is a more precise and reproducible diagnostic predictor of large infarcts when compared to LVEF.[292] Global strain after STEMI has been reported to be well associated with echocardiographic WMSI, CMR-determined infarct size, and LVEF measured by CMR, SPECT, standard echocardiography and contrast echocardiography; additionally, it was found to be the best predictor of low LVEF, measured by the gold standard CMR[293]. To the best of our knowledge, this study is the first to report the association between IMR and strain after STEMI. In stable coronary artery disease, IMR was already correlated with strain in evaluation of LV contractile reserve on dobutamine echocardiography.[294] In STEMI patients, Park *et al*. found myocardial strain to be well correlated with coronary flow reserve (CFR), as determined by intracoronary measurement after P-PCI in anterior STEMI, and suggested its utility in predicting myocardial functional recovery, non-invasively.[295] Laøgstrup *et al*. on the other hand, reported that the magnitude of myocardial deformation correlates with the severity of alterations in microcirculation, detected by CFR, assessed by transthoracic echocardiography. They also demonstrated longitudinal global and regional strain to be a significantly better tool than WMSI to investigate LV dysfunction after acute myocardial infarction.[296] In the present study, the IMR was able to independently predict myocardial deformation, evaluated immediately after infarction by GLS, which is considered to be a very sensitive and informative parameter of infarction extension and functional recovery. This association between IMR and GLS was not only present initially, but persisted at 3 months, when the relationship of IMR with LVEF and WMSI also manifested.

Importantly, the association between IMR and GLS was independent of the area-at-risk and the NT-pro-BNP values on admission, suggesting that IMR has an additional value in predicting left ventricular recovery in these patients[56].

## 5.4. Predictors of IMR

Patients with coronary microvascular dysfunction (defined as having an IMR higher than the mean value 24) were older and there was a weak, but significant correlation between IMR and age (r=0.28, p=0.03). This result is similar in three recent studies (Table 88), but discordant from the results from the largest multicentric study, published by Fearon *et al*[64]. However, in this last dataset, mean age was significantly lower, which may indicate a more selected population (Table 84). Similarly to other studies published, there were no significant differences in IMR according to gender and other physical characteristics or risk factors, including diabetes.

We found no significant differences in ischemic (pain-to-balloon) or hospital-to-balloon times between patients with and without microvascular coronary damage (IMR>24). Once again, this result is similar to that reported by most studies that evaluated this aspect (Table 88).

Patients with an IMR>24 had a significantly higher prevalence of initial TIMI flow 0-1 (93.3% vs. 73.3%, p=0.038), which is an expected result and in agreement with all other published studies.

Importantly, treatment options, including the use of aspiration thrombectomy catheters and IIb/IIIa inhibitors, were similar in patients with or without IMR>24 (IIb/IIIa inhibitors were more often used in patients with lower IMR, but that difference was not significant on the multivariable analysis), in accordance with the majority of studies that reported on this question (Table 88)

**Table 88 – Predictors of IMR in STEMI patients**

| Author (Year) | Nº of patients | Age | Ischemic time (min) | IIb/IIIa inhibitors | Aspiration thrombectomy | Admission glucose/HbA1c |
|---|---|---|---|---|---|---|
| Bonello (2010)[297] | 45 | + | - | + [a] | + [a] | NR |
| Sezer (2010)[250] | 35 | - | - | NR | NR | NR |
| Fearon (2013)[64] | 253 | - | - | - | NR | NR |
| Baek (2015)[298] | 113 | + | + | - | - | - |
| Carrick (2016) | 259 | Trend | - | - | - | NR |
| Park (2016)[257] | 89 | + | - | - | - | NR |
| Ahn (2016)[248] | 40 | NR | NR | + | Trend | NR |
| **Baptista** | **60** | **+** | **-** | **-** | **-** | **++** |

[a] Univariate analysis, [b] p=0.057

Glucose and HbA1c levels on admission were higher in patients with IMR>24, and significant correlations were found between these laboratory tests and IMR (r=0.34, p=0.009 and r=0.67, p<0.001, respectively). The impact of both admission glucose and HbA1c was still significant after adjusting for other variables, including history of diabetes.

Admission glucose levels were clearly confirmed as an independent prognostic marker of both in-hospital and long-term outcome, regardless of diabetic status.[299,300] The exact deleterious mechanisms of hyperglycaemia are not completely understood: hyperglycaemia during STEMI is probably caused by an inflammatory and adrenergic response to ischemic stress, when catecholamines are released and glycogenolysis induced. Although the pathophysiological mechanisms involved have not yet been fully elucidated, it is believed that hyperglycaemia is associated with an increase in free fatty acids (which induce cardiac arrhythmias), insulin resistance, chemical inactivation of nitric oxide and the production of oxygen reactive species (with consequent microvascular and endothelial dysfunction), a prothrombotic state, and vascular inflammation. It is also related to myocardial metabolic disorders, leading to thrombosis, extension of the damaged area, reduced collateral circulation, and ischemic preconditioning[301].

Several studies have demonstrated that high admission glucose levels in STEMI patients treated with P-PCI are related with different indicators of microvascular coronary dysfunction, including ECG ST resolution[302], angiographic markers (like cTFC and TMPG)[303] and CFR measured by echocardiography.[304,305] Admission glucose levels are also significantly higher in patients with microvascular obstruction in ceCMR.[306,307]

Likewise, the prognostic impact of admission HbA1c was also demonstrated in STEMI patients.[308–310] Glycosylated haemoglobin (HbA1c) is an established marker of long-term glycaemic control in patients with diabetes mellitus, and elevated HbA1c levels in such patients are associated with an increased risk for future microvascular and macrovascular disease.[310] Additionally, elevated HbA1c

appears to be also predictive for cardiovascular disease and mortality in patients *without* diabetes mellitus, regardless of fasting glucose levels, indicating that long-term glycometabolic derangement also poses a risk for cardiovascular events.[311] However, both HbA1c and glucose levels probably reflect different patient populations, and their association with outcome is probably due to different mechanisms.

To the best of our knowledge, this is the first study to identify a significant relation between glucose levels on admission and microvascular coronary dysfunction as evaluated by IMR. Regarding HbA1c, only one other study reported its relation with IMR. In this study, as opposed to our findings, there was no difference in HbA1c between tertiles of IMR[298] . Our results, however, show an unequivocal and significant relation between these 2 variables and IMR.

Although not surprising, this was not a planned endpoint of our study. However, we plan to investigate it further.

# STUDY LIMITATIONS AND STRENGTHS

This study has several limitations, which should be taken into account when interpreting the results.

- Firstly, the inclusion rate was very low and the cohort included represents only 11% of the population of patients with STEMI in the period in which recruitment occurred. Although the majority of patients was excluded due to predefined exclusion criteria, a significant number (n=99) of patients was not included due to unknown reasons or to the operator's decision. Although this limitation was also described in the only study that reported inclusion rate as extensively as we did, it must still be acknowledged that this is a highly selected population. However, as noted in the discussion, the patients selected appear to be the ones with *less* risk. Patients with more severe clinical presentation, more prolonged ischemic times and hemodynamic instability probably would have larger infarcts and more severe microvascular obstruction, which would likely increase the diagnostic ability of IMR and EndoPAT.
- Secondly, the EndoPAT exams were not performed according to the recommendations for endothelial function evaluation: patients were not fasting, coffee or tea consumption was not excluded and the morning hour of the exam was not respected. However, measuring endothelial function in the acute phase was exactly the purpose of the study and we did acknowledge that the acute measurement probably is not valid exactly because of these technical constraints. The second EndoPAT evaluation, on the other hand, was performed in a much less stressful situation and the results are in accordance with previous published studies, suggesting it is feasible, even though recommendations for performing the exam were not followed strictly. Importantly, all the operators performing the EndoPAT evaluation were highly trained in this procedure, having participated in several previous studies.
- Thirdly, the number of patients with the second EndoPAT evaluation is small. In fact, this evaluation was not initially planned and it was only decided when we realized that the first EndoPAT values were probably not valid. Probably some of the tendencies described would be statistically significant if the cohort was larger.
- Fourthly, imaging exams (both ceCMR and echocardiograms) are not available for all patients. This is a limitation that is difficult to overcome. In studies which reported inclusion rate according to recent recommendations, authors faced the same problem, for several reasons: some patients refuse to perform follow-up exams, while others do the exams but technical issues prevent its interpretation. However, in spite of this limitation, results with bot echocardiogram and ceCMR were very consistent.

Our study also has some important strengths:

- We have one of the largest single-centre databases in IMR evaluation in STEMI patient in the world, systematically evaluating all indirect indicators of microvascular reperfusion: ECGs were performed before and after reperfusion (including at 90 and 180 minutes) for all patients and cTFC and TMPG were also registered in all cases. Most of the studies performed in this area are very inconsistent in reporting these data, as highlighted in the discussion.

- The evaluation of myocardial infarction by troponin release was also much more comprehensive: blood samples were collected at predetermined times in all patients for the first 48 hours allowing for a precise evaluation of both peak and area under the curve values for the estimation of infarction extent. None of the studies already published performed such a thorough evaluation.

# CONCLUSIONS AND FUTURE PERSPECTIVES

Coronary microvascular circulation remains a largely unknown territory in patients with ST elevation myocardial infarction, and this is an area where almost no scientific advances were made in the last years.

The recent availability of new techniques for non-invasive measurement of endothelial dysfunction and for invasive assessment of the coronary microcirculation in this clinical setting offered new research opportunities in this field and led to the study presented on this thesis, which was designed with the intention of improving our knowledge on the role of endothelial dysfunction in patients with STEMI. Based on the data presented, the main conclusions of this study are:

1. **Reactive hyperemia index measurement with EndoPAT immediately after primary PCI in ST elevation myocardial infarction patients probably cannot be performed, due to the serious unavoidable technical pitfalls in the acute phase.**

   Although we found no relation between this test and the index of microcirculatory resistance, other measures of microvascular coronary dysfunction or the extent of the myocardial infarction, the validity of these results is questionable.

   Other non-invasive tests available, like flow-mediated dilation, should also have the same pitfalls we found with EndoPAT, since they too depend on the baseline vasoconstrictor status and on all the factors that influence it in the acute phase of a coronary event. Invasive methods for testing coronary endothelial function, on the other hand, remain dependent on pharmacological stimuli and are not an option in acute patients. Therefore, evaluation of the endothelial function in the acute phase apparently is not feasible with the current technology.

2. **Reactive hyperemia index measurement with EndoPAT 24 hours after primary PCI in ST elevation myocardial infarction patients is feasible and related both to the extension of the infarct and to microvascular obstruction (including a tendency for lower IMR values in patients with higher RHI values).**

   Despite the fact that measurement of RHI in this subacute phase is not able to differentiate if the endothelial dysfunction found is a cause or a consequence (or both!) of the myocardial infarction, these results are quite important, for several reasons: firstly, they prove that the coronary endothelial dysfunction previously documented in ACS patients with invasive methods is also measurable by an non-invasive peripheral technique in a subacute phase. Secondly, they confirm endothelial function having a central role in the pathophysiological processes occurring in STEMI patients. Thirdly, they suggest that RHI could eventually be used as a surrogate for coronary endothelial function in this setting, allowing research on the efficacy of new pharmacological or device-based strategies with a simple and reproducible method.

However, there is still a lot to be learned regarding the evolution of endothelial function in the first days, weeks and months in STEMI patients. It is particularly important to understand how the recovery of endothelial dysfunction impacts positively on left ventricular remodelling and on future events, since this may be an important target for present or future treatment options. This is a line of research that we would like to follow in the near future.

3. **The index of microcirculatory resistance measured immediately after primary PCI in ST elevation myocardial infarction patients predicts microvascular obstruction in the ceCMR and correlates both with infarct extension and myocardial salvage. It is also closely related to infarct extension measured by Troponin I release and to indirect indicators of microvascular coronary obstruction.**

Although this was not new, it was important to confirm the feasibility, safety and utility of measuring IMR in the acute phase of STEMI patients. Unfortunately, since this is an expensive technique, it will be difficult to implement it routinely in this population. However, measuring IMR in patients with clinical indication for a pressure-wire (i.e., patients with intermediate lesions in which the pressure-wire is to be used for fractional flow reserve evaluation), including patients with STEMI, may allow us to further increase our knowledge of this technique. Indeed, there are several ongoing trials trying to assess complete vs. differed revascularization of non-culprit arteries in STEMI patients, using pressure-wire to guide the decisions and I am currently involved in the design of a new one, using a non-hyperaemic technology.

Likewise, measuring IMR in different clinical settings may be useful to the understanding of the role of microvascular coronary circulation in coronary disease. An example, is the currently ongoing SAVE-IT trial (which is evaluating the role of fractional flow reserve in orienting revascularization decisions in surgical valve patients with intermediate coronary lesions), which I helped to design and implement, and which includes a substudy with IMR evaluation in severe aortic stenosis patients.

4. **Age and glucose metabolic status (both acute, measured by glucose on admission, and previous, measured by HbA1c levels on adission) are independent predictors of IMR**

The impact of glucose metabolism on IMR was not an unexpected finding, considering all that is known about the impact of glucose levels (and, to a lesser extent, of HbA1 levels) in STEMI patients.

However, despite the significant relation we found between these glucose metabolism indicators and IMR, the fact that all pharmacological approaches that tried to improve the outcomes of these patients failed is just another crude evidence of an irrefutable fact in coronary artery disease patients: we are still far from understanding what happens in the microcirculation. In order to further improve the outcomes of our patients, this is a limitation we need to overcome.

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# APPENDIX – PAPERS

## 1 – Endothelial dysfunction evaluated by peripheral arterial tonometry is related with peak TnI values in patients with ST elevation myocardial infarction treated with primary angioplasty

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# Endothelial dysfunction evaluated by peripheral arterial tonometry is related with peak TnI values in patients with ST elevation myocardial infarction treated with primary angioplasty

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ABSTRACT

*Purpose:* The role of endothelial-dependent function in patients with acute ST elevation myocardial infarction (STEMI) is not clear. Endothelial dysfunction may contribute to the pathophysiological processes occurring after STEMI and influence the extension of myocardial necrosis. Endothelial-dependent dysfunction evaluated by peripheral arterial tonometry (PAT) has already showed to be correlated with microvascular coronary endothelial dysfunction. Our purpose was to evaluate the impact of endothelial dysfunction on peak Troponin I (TnI) values, as a surrogate for the extension of myocardial infarction, in patients with STEMI treated with primary angioplasty (P-PCI).

*Methods:* 58 patients with STEMI treated with P-PCI (mean age 59.0 ± 14.0 years, 46 males) were included. Endothelial function was assessed by reactive hyperaemia index (RHI) determined by PAT. Patients were divided in two groups according to the previously reported RHI threshold for high risk (1.67). The extension of myocardial necrosis was evaluated by peak TnI levels.

*Results:* RHI median value was 1.78 (IQR 0.74); 25 patients had endothelial dysfunction (RHI < 1.67). The two groups had no significant differences in age, gender, main risk factors and pain-to-balloon time. Patients with an RHI < 1.67 had significant larger infarcts: TnI 73.5 ng/mL (IQR 114.42 ng/mL) versus TnI 33.2 ng/mL (IQR 65.2 ng/mL); $p = 0.028$. On multivariate analysis, the presence of an RHI < 1.67 kept significant impact on TnI peak values ($p = 0.02$).

*Conclusions:* The presence of endothelial-dependent dysfunction, assessed by PAT, is related with higher peak TnI values in STEMI patients treated with P-PCI. These results strength the possibility that endothelial-dependent dysfunction may be a marker of poor prognosis and eventually a therapeutic target in patients with STEMI.



## 1. Introduction

Primary percutaneous coronary intervention (P-PCI) is an established reperfusion strategy in the treatment of acute myocardial infarction with ST-segment elevation (STEMI) (Steg et al., 2012; PT et al., 2013). Despite achieving normal epicardial coronary artery flow after P-PCI, a significant proportion of patients (ranging between 20% and 40%) have a poor outcome because of microvascular coronary damage (Bekkers et al., 2010). The extent of microvascular dysfunction has been shown to be an important and independent contributor to subsequent changes in left ventricular geometry and performance (Bolognese et al., 2004). Patients with impaired microvascular perfusion have larger infarcts, as evaluated by CK and troponin release, less electrocardiographic ST elevation resolution, larger long-term left ventricular wall motion abnormalities and lower left ventricular ejection fraction (Van't Hof et al., 1997; Amaya et al., 2011; Desmet et al., 2004; Ito et al., 1996). As a consequence of these, myocardial malperfusion is associated with higher event rates, risk of progression to heart failure and mortality (Schröder et al., 2001; Jaffe et al., 2010).

The precise mechanisms underlying myocardial malperfusion after the restoration of epicardial blood flow are not known and probably are multifactorial: endothelium-independent microvascular dysfunction (caused by thrombi, debris, embolization, ventricular hypertrophy, myocardial, and vascular oedema and smooth muscle dysfunction) is one of the mechanisms involved (Luscher and LA, 2012; Lerman et al., 2007). The contribution of endothelial-dependent dysfunction, on the other hand, is less well established, even knowing that it plays a crucial role in vascular tone and blood flow regulation (Gutiérrez et al., 2013), which are main determinants of myocardial infarction extension. Several techniques were recently developed that allow the evaluation of endothelial-dependent dysfunction. Peripheral arterial tonometry

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(PAT), a simple and reproducible technique (CE et al., 2012) based on the change in finger pulse wave amplitude in response to reactive hyperaemia, is closely correlated with coronary artery endothelial function (Bonetti et al., 2004), allowing a non-invasive evaluation of endothelial-dependent dysfunction.

We hypothesized that endothelial-dependent dysfunction (evaluated with PAT), as a determinant of coronary microvascular flow, could have an impact on the extension of myocardial infarction, measured by peak Troponin I (TnI) values.

## 2. Methods

### 2.1. Study population

A total of 92 consecutive patients with acute ST elevation myocardial infarction submitted to primary angioplasty admitted in a single center between March and October 2011 were prospectively screened for inclusion in the study. After excluding patients that died (n = 6), patients with residual ischemia (including patients still needing nitrates for pain relief) and hemodynamic or rhythm instability in the first 24 h (n = 6), patients admitted during weekend days (in which performing the endothelial evaluation was not possible, due to logistical reasons; n = 10) and patients not willing to participate or not able to consent (n = 8), 62 patients were considered for endothelial function evaluation. Of these, 4 were excluded, due to low quality or technical problems in the endothelial function evaluation (Fig. 1). Fifty eight patients were finally included in the study protocol (68% of the screened patients). The institutional ethical committee approved the study, which was conducted in compliance with the Declaration of Helsinki.

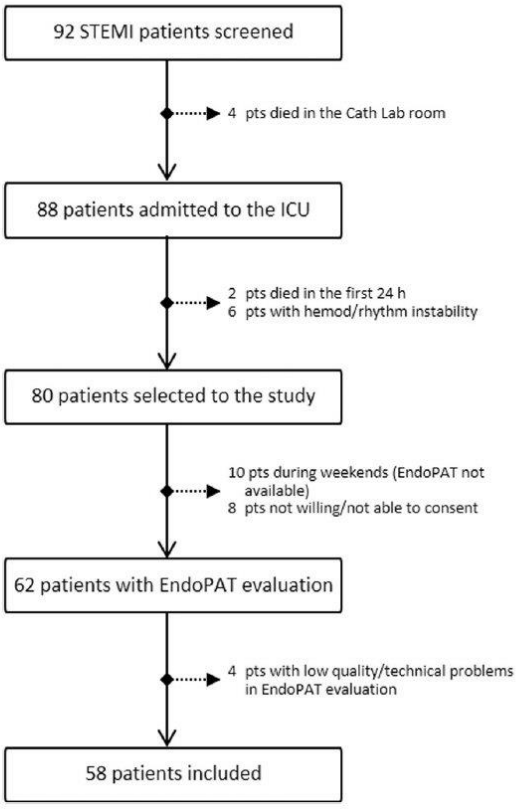


**Fig. 1.** Flow chart of patients included. (STEMI: ST elevation acute myocardial infarction; ICU: Intensive Care Unit; pts: patients.)

Clinical characteristics (age, gender, cardiovascular risk factors and clinical evolution), left ventricular ejection fraction (measured by echocardiography) and main angiographic characteristics (culprit coronary artery, number of vessels with disease) were assessed in all patients.

### 2.2. Endothelial function evaluation

Peripheral endothelial function was measured in the next morning after the acute event by digital pulse amplitude with the Endothelial Peripheral Arterial Tonometry (Endo-PAT2000, Itamar Medical, Caesarea, Israel), as previously described (Bonetti et al., 2004; Bonetti et al., 2003). Briefly, a blood pressure cuff was placed on 1 upper arm, while the contralateral arm served as a control. The peripheral arterial tonometric finger probe was placed on each index finger, and after a baseline period of 5 min, total occlusion was performed with a blood pressure cuff on the right arm for 5 min. The cuff was inflated to suprasystolic pressures to obtain complete occlusion. Thereafter, the cuff was deflated and the hyperemic response of the occluded right arm was recorded and compared to the nonoccluded left arm (control arm). The data were digitally analyzed (EndoPAT2000 software version 3.4.4) to obtain the reactive hyperemia index (RHI). RHI values below 1.67 were considered as endothelial dysfunction (ED), as previously reported (Bonetti et al., 2004).

### 2.3. Laboratory analysis

Blood samples were collected at the time of the primary angioplasty and 6, 12, 18, 24 and 48 h after the procedure, according to the usual Intensive Care Unit (ICU) protocol for acute myocardial infarction patients. Troponin I was measured by using sandwich chemiluminescent immunoassay based LOCI™ technology, with a Dimension Vista™ Intelligent Lab System (Siemens Healthcare Diagnostics™). The peak value for each patient was recorded. Creatin Kinase (CK) and its subfraction Mb (CK-Mb) were also measured. High sensitivity CRP, glucose, lipid profile and NT-pro-BNP levels were determined as part of the standard admission evaluation of these patients in the ICU.

### 2.4. Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 20.0. Data are presented as mean ± SD or median (interquartile range) for continuous variables and as number and frequencies for categorical variables. Distribution was tested with the Kolmogorove–Smirnov test. Continuous variables were compared using the Student t if they had a normal distribution and with the Mann–Whitney test if they didn't. Categorical variables were tested using the $\chi^2$ test.

To evaluate the impact of different variables in troponin I values, a univariate statistical analysis was done. Variables that showed a significant impact on troponin I, as well as variables considered clinically relevant, were include in a linear multivariate regression model, in order to identify independent predictors of troponin I values. A 2-sided p value of $<0.05$ was considered statistically significant.

## 3. Results

### 3.1. Population characteristics and RHI values

Baseline characteristics are presented in Table 1. Forty-six patients (79.3%) were male and the mean age was 59.0 ± 14.0 years. The median value of RHI was 1.78 (IQR 0.74). Twenty-five (43%) patients had endothelial dysfunction (defined as a RHI below 1.67). Fig. 2 shows endoPAT evaluations in patients with (2B) and without (2A) endothelial dysfunction.

There were no significant differences between the groups of patients with and without endothelial dysfunction concerning age, gender, and traditional risk factors (although there was a trend for more patients

**Table 1**
Baseline characteristics according to the presence of endothelial dysfunction.

| | Total population (n = 58) | RHI < 1.67 (n = 25) | RHI ≥ 1.67 (n = 33) | p Value |
|---|---|---|---|---|
| Age (years)[d] | 59.0 ± 14.0 | 60.8 ± 14.3 | 57.6 ± 13.8 | 0.40[a] |
| Male gender n(%) | 46 (79.3%) | 18 (72.0%) | 28 (84.8%) | 0.19[b] |
| BMI[e] | 26.3 (4.6) | 27.4 (3.7) | 25.4 (5.0) | 0.49[c] |
| SBP (mmHg)[d] | 115 ± 20.7 | 114 ± 22.2 | 116.7 ± 20.0 | 0.24[a] |
| DBP (mmHg)[d] | 71.0 ± 12.0 | 69.2 ± 12.3 | 72.2 ± 11.9 | 0.15[a] |
| Heart rate (bpm)[d] | 69.1 ± 11.4 | 71.1 ± 12.6 | 67.7 ± 10.5 | 0.33[a] |
| Ankle-brachial index[d] | 1.08 (0.19) | 1.07 (0.12) | 1.08 (0.22) | 0.49[c] |
| Previously known risk factors n(%) | | | | |
| Hypertension | 37 (63.8%) | 16 (64.0%) | 21 (63.6%) | 0.60[b] |
| Diabetes | 12 (20.7%) | 8 (32.0%) | 4 (12.1%) | 0.33[b] |
| Dyslipidemia | 24 (41.4%) | 10 (40.0%) | 14 (42.4%) | 0.64[b] |
| Smoking habits | 27 (46.4%) | 11 (44.0%) | 16 (48.5%) | 0.47[b] |
| LVEF (%)[d] | 53.0 (16.0) | 47.5 (24.0) | 54.0 (13.0) | 0.69[c] |
| LVEF ≤40% n(%) | 9 (15.5%) | 6 (24.0%) | 5 (15.2%) | 0.30[b] |
| Killip–Kimbal class > 1 n(%) | 15 (25.9%) | 7 (28.5) | 8 (24.2%) | 0.49[b] |
| Culprit coronary artery n(%) | | | | |
| Left anterior descending | 24 (41.4%) | 14 (56.0%) | 10 (30.3%) | |
| Left circumflex artery | 8 (13.8%) | 4 (16.0%) | 4 (12.1%) | 0.074[b] |
| Right coronary artery | 26 (44.8%) | 7 (28.0%) | 19 (57.6%) | |
| Culprit artery = LAD n(%) | 24 (41.4%) | 14 (56.0%) | 10 (30.3%) | 0.045[b] |
| Pain-to-balloon (min)[e] | 163 (195) | 152 (218) | 165 (131) | 0.89[c] |
| Admission lab measurements | | | | |
| NT-pro-BNP (pg/mL)[e] | 311 (724) | 282 (724) | 344 (790) | 0.98[c] |
| hsCRP (mg/dL)[e] | 0.73 (0.50) | 0.68 (0.45) | 0.81 (0.66) | 0.35[c] |
| HbA1c (IQR) (%)[e] | 5.80 (1.35) | 5.60 (.90) | 5.85 (1.58) | 0.99[c] |
| LDL cholesterol (mg/dL)[e] | 133 ± 45 | 141 ± 46 | 127 ± 46 | 0.27[a] |
| HDL cholesterol (mg/dL)[e] | 38 (13) | 37 (13) | 38 (13) | 0.50[c] |

BMI: body mass index; DBP: diastolic blood pressure; bpm: beat per minute; hsCRP: high sensitivity protein C reactive; IQR: interquartile range; LVEF: left ventricular ejection fraction; SBP: systolic blood pressure; TnI: Troponin I.
[a] Student-t.
[b] $\chi^2$.
[c] Mann–Whitney.;
[d] Data presented as mean ± SD.
[e] Data presented as median (interquartile range).

with diabetes with endothelial dysfunction: 32.0% vs. 12.0%, p = 0.33). BMI, blood pressure, heart rate and ankle-brachial index were similar, as well as mean left ventricular ejection fraction and Killip–Kimbal class at presentation. The left anterior descending artery was more frequently the culprit artery in patients with endothelial dysfunction (56.0% vs. 30.3%, p = 0.045). Time between the onset of pain and the first mechanical intervention in the cath lab (pain-to-balloon time) was similar in both groups (around 3.5 h). TIMI 3 flow was obtained in all patients at the end of the procedure. All patients were acutely treated with aspirin and Clopidogrel and, according to the ICU protocol, all patients were also treated with statins.

### 3.2. Myocardial infarction extension — peak TnI values

Peak Troponin I, presented as median and interquartile range, was significantly higher in patients with endothelial dysfunction: 73.5(107.1) vs. 35.2 (64.3) ng/mL, p = 0.028. CK and CK-Mb were also higher in patients with endothelial dysfunction (Fig. 3 and Table 2).

To identify the predictors of myocardial necrosis extensions, evaluated by peak TnI values, a univariate analysis was performed. On this analysis, RHI values showed a significant inverse relation with TnI peak values (β = −0.333, p = 0.011). There was a trend to higher values of TnI in relation to heart rate (β = 0.28, p = 0.059) and a trend for an inverse relation with left ventricular ejection fraction (β = −0.29, p = 0.058). Other continuous variables, including age (β = 0.07, p = 0.60), BMI (β = 0.05, p = 0.69), time-to-balloon (β = 0.03, p = 0.83), NT-pro-BNP (β = 0.12, p = 0.40), hsCRP (β = 0.24, p = 0.86) and HbA1c (β = 0.03, p = 0.85) didn´t show a significant relation with peak TnI.

The presence of endothelial dysfunction (RHI < 1.67) was significantly associated with higher peak TnI values (median/IQR 73.5/107.1 vs. 35.2/64.3, p = 0.028), as described previously. Left ventricular ejection fraction <40% was also associated with higher TnI values (98.6/85.9 vs. 45.8/61.7, p = 0.046). Importantly, having the left anterior descending as the culprit artery didn't related significantly with TnI values (65.6/73.2 vs. 40.6/66.3, p = 0.13). Other categorical variables, including male gender (47.6/64.2 vs. 78.8/73.0 ng/mL, p = 0.47), Killip class >1 (88.3/80.1 vs. 49.4/59.6, p = 0.23), diabetes mellitus (52.7/71.0 vs. 39.1/72.9, p = 0.35), dyslipidemia (35.5/72.2 vs. 54.2/69.7, p = 0.57), hypertension (35.5/63.4 vs. 77.8/104.4 ng/mL, p = 0.09) and smoking habits (49.4/63.4 vs. 51.2/70.1, p = 0.80) were also not related with peak TnI values.

Based on the univariate analysis, two multivariate models were tested: the first model included only variables statistically significant or with a strong trend on the univariate analysis: RHI < 1.67, LVEF < 40% and heart rate; the second model also included other variables considered clinically relevant: age, diabetes, time-to-balloon, Killip class >1 and LAD as culprit artery (Table 3). In both models tested, RHI kept and independent relation with peak TnI values.

## 4. Discussion

We showed that peak troponin I values are higher in acute ST elevation myocardial infarction patients with endothelial dysfunction (RHI < 1.67). To our knowledge, this is the first study evaluating endothelial function with PAT soon (first 24 h) after reperfusion in patients with acute ST elevation myocardial infarction.

Coronary endothelial dysfunction seems to be present in most patients with acute coronary syndromes, and is reversible in a matter of months (Elbaz et al., 2005; Careri et al., 2013). In patients with non-ST elevation acute coronary syndromes, peripheral endothelial function

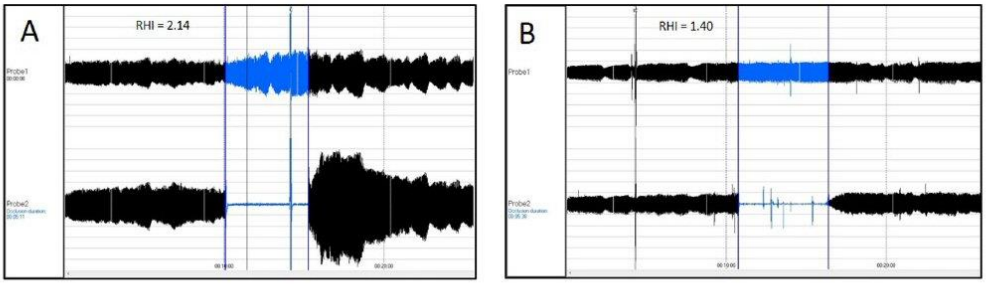


**Fig. 2.** Two examples of peripheral endothelial function measured by digital pulse amplitude with endothelial peripheral arterial tonometry (Endo-PAT2000). A. Patient with normal reactive hyperemic response (RHI 2.14), with increase of signal amplitude after unilateral cuff occlusion (at bottom), corrected with contralateral finger signal (at top). This patient had a peak TnI of 22.4 ng/mL. B. Abnormal response (RHI 1.40) with a blunted increase in signal amplitude after unilateral cuff occlusion. Peak TnI was 271.0 ng/mL.

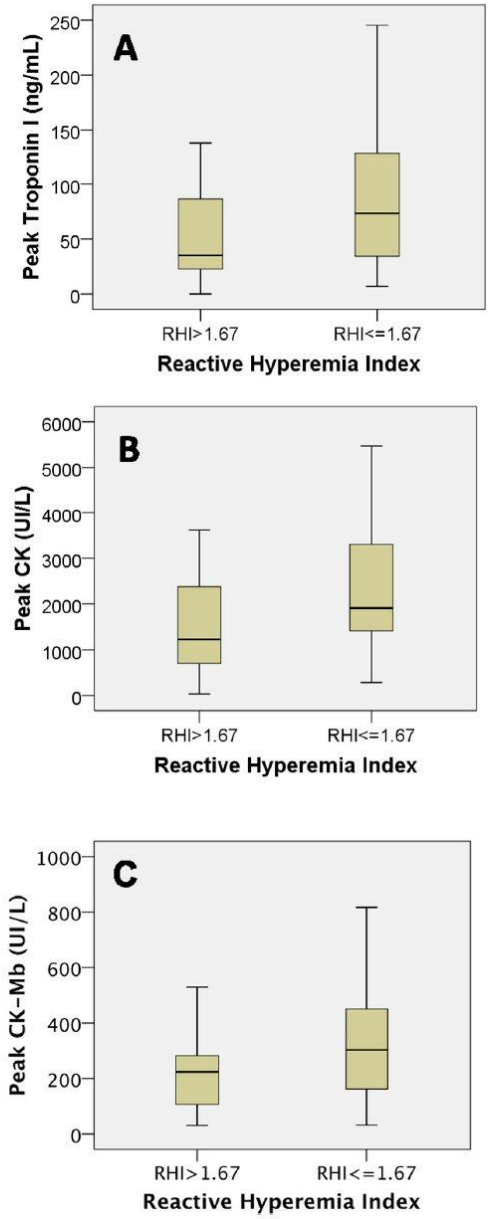


**Fig. 3.** Troponin I (A), creatine kinase (B) and creatine kinase Mb (C) values according to the presence of endothelial dysfunction (RHI ≤ 1.67).

has been shown as an independent predictor of major events, and subsequent normalization of endothelial function in these patients predicts a lower risk of events (Fichtlscherer et al., 2004; Karatzis et al., 2006; Chen et al., 2011).

**Table 2**
Cardiac enzymes peak values, according to the presence of endothelial dysfunction.

| | Total population (n = 58) | RHI < 1.67 (n = 25) | RHI ≥ 1.67 (n = 33) | p Value |
|---|---|---|---|---|
| Peak TnI (ng/mL) | 50.3 (68.1) | 73.5 (107.1) | 35.2 (64.3) | 0.028[a] |
| Peak CK (UI/L) | 1.586 (1.938) | 1.909 (2.181) | 1.227 (1.758) | 0.045[a] |
| Peak CK-Mb (UI/L) | 252 (240) | 303 (312) | 224 (186) | 0.032[a] |

Data presented as median (interquartile range).
[a] Mann–Whitney.

**Table 3**
Multivariate models for peak TnI values.

| Model | Variable | Multivariate analysis | |
|---|---|---|---|
| | | β | p Value |
| Model 1 | Reactive hyperemia index (RHI) < 1.67 | 0.33 | 0.02 |
| | Left ventricular ejection fraction (LVEF) <40% | 0.32 | 0.03 |
| | Heart rate (per bpm) | 0.11 | 0.44 |
| Model 2 | Reactive hyperemia index (RHI) < 1.67 | 0.31 | 0.04 |
| | Left ventricular ejection fraction (LVEF) <40% | 0.22 | 0.18 |
| | Killip class >1 | 0.22 | 0.15 |
| | Culprit artery = left anterior descending | 0.20 | 0.23 |
| | Time pain-to-balloon (per minute) | 0.09 | 0.53 |
| | Diabetes mellitus | 0.08 | 0.59 |
| | Heart rate (per bpm) | 0.08 | 0.62 |
| | Age (per year) | 0.02 | 0.88 |

In patients with ST elevation acute myocardial infarction treated with primary angioplasty (P-PCI), the evidence is scarce. In a group of 93 patients, Bissinger A. and colleagues (Bissinger et al., 2011) measured endothelial-dependent response with flow-mediated dilation (FMD) in brachial artery 3 days after the P-PCI procedure, and again at 6 months. They found that improvement of FMD in both diabetic and non-diabetic patients after a 6-month follow-up inversely correlated with the increase of left ventricular end-diastolic volume. Shyh-Ming Chen and colleagues (Chen et al., 2011) evaluated endothelial function with FMD in 39 young patients with acute STEMI treated with P-PCI, and ound that these patients had severe endothelial dysfunction, as compared to normal controls. In our population, endothelial dysfunction was also present in a significant number of patients (median RHI value 1.78, IQR 1.04–2.52; 43% patients with RHI < 1.67), as compared to values described in patients without coronary artery disease (Matsuzawa et al., 2010; Toggweiler et al., 2010). These results are similar to the ones described by Toggweiler S. and colleagues (Toggweiler et al., 2010), in 85 patients with acute coronary syndromes (mean RHI 1.74 ± 0.48; 46% patients with endothelial dysfunction).

It is uncertain whether the peripheral endothelial dysfunction found in these few studies performed in ACS patients is a contributor or a consequence of the acute event itself. It seems clear that coronary microcirculation dysfunction may be to a large extent endothelial-independent, due to thrombi and debris embolization, myocardial oedema, and other factors (Luscher and LA, 2012; Lerman et al., 2007). However, Careri G and colleagues (Careri et al., 2013) recently showed that in patients with endothelial-dependent dysfunction (measured with FMD) at the time of the ACS, those who improved the value of FMD at three months had a significant better prognosis, as compared to the ones that didn't improved. These results suggest that previous or acquired endothelial-dependent dysfunction probably also plays an important role in ACS patients. The mechanisms responsible for this consistent abnormality, however, remain poorly known. A local inflammatory status, which affects endothelial function and can make atherosclerotic plaques more prone to rupture and platelet adhesion, vasospasm, and stasis, as been shown in patients with ACS (Tousoulis et al., 2006) and several studies have shown that inflammation may depress endothelial function (Clapp et al., 2005; Vlachopoulos et al., 2010). However, contrary to these studies and in accordance to the results of Elbaz M. (Elbaz et al., 2005) and Careri G. (Careri et al., 2013), we didn't find significant differences in hsCRP values in patients with and without endothelial dysfunction. Several other mechanisms have been proposed, but not proven (Laude et al., 2001). Identifying these mechanisms and their role in the acute phase of coronary syndromes will have important clinical and treatment implications.

Peak TnI I values are commonly used in everyday practice as in indicator of the extension of myocardial necrosis, although there are other methods much more reliable for this measurement. However, it should be stressed that peak TnI values are correlated with scintigraphic infarct

size and 1-year mortality in patients with acute ST elevation myocardial infarction treated with primary PCI (Byrne et al., 2010). Our results demonstrate that the presence of endothelial-dependent dysfunction is related with peak TnI values, even when other factors that affect the extension of myocardial infarction (e.g. age, risk factors, pain-to-balloon time, culprit artery and Killip class at admission) are taken into account. Since endothelial-dependent function plays a crucial role in vascular tone and blood flow regulation (Gutiérrez et al., 2013), it is not surprising that endothelial dysfunction is related with larger infarctions. Accordingly, it can be proposed that this is one of the mechanisms throughout endothelial dysfunction affects prognosis in STEMI patients.

### 4.1. Study limitations

The study included a relatively small number of patients. Since endothelial function evaluation with peripheral arterial tonometry is affected by several individual and technical factors, this is an important limitation of the study. Additionally, endothelial function testing may be particularly challenging in acute patients: the unstable condition of the patient make it very difficult to obtain and interpret data concerning vascular reactivity in this context. In our study, we measured endothelial function sooner after the P-PCI, as compared to other studies, in order to try to decrease as much as possible other factors that could interfere with the measurement (the infarction itself, but also drug treatment, e.g. with statins and antiplatelet agents).

## 5. Conclusions and clinical implications

In a population of patients with acute ST elevation myocardial infarction treated with primary angioplasty, the presence of endothelial dysfunction, evaluated with peripheral arterial tonometry, is related with larger myocardial infarctions, as evaluated by peak troponin I values. These results strength the possibility that endothelial-dependent dysfunction may be a marker of poor prognosis and eventually a therapeutic target in patients with acute coronary syndromes.

## Conflict of interest

Authors have no potential conflicts of interest.

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## 2 – The Index of Microcirculatory Resistance as a Predictor of Echocardiographic Left Ventricular Performance Recovery in Patients With ST-Elevation Acute Myocardial Infarction Undergoing Successful Primary Angioplasty

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ACUTE CORONARY SYNDROME

# The Index of Microcirculatory Resistance as a Predictor of Echocardiographic Left Ventricular Performance Recovery in Patients With ST-Elevation Acute Myocardial Infarction Undergoing Successful Primary Angioplasty

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***Background**: This study aims to evaluate the relationship between IMR (Index of Microcirculatory Resistance) and the echocardiographic evolution of left ventricular (LV) systolic and diastolic performance after ST-elevation acute myocardial infarction (STEMI), undergoing primary angioplasty (P-PCI).*
***Methods**: IMR was evaluated immediately after P-PCI. Echocardiograms were performed within the first 24 hours (Echo1) and at 3 months (Echo2): LV volumes, ejection fraction (LVEF), wall motion score index (WMSI), E/e ratio, global longitudinal strain (GLS), and left atrial volume were measured.*
***Results**: Forty STEMI patients were divided in 2 groups according to median IMR: Group 1 (IMR < 26), with less microvascular dysfunction, and Group 2 (IMR >= 26), with more microvascular dysfunction. In Echo1 GLS was significantly better in Group 1 (−14.9 vs. −12.9 in Group 2, P = 0.005). However, there were no significant differences between the two groups in LV systolic volume, LVEF and WMS. Between Echo1 and Echo2, there were significant improvements in LVEF (0.48 ± 0.06 vs. 0.55 ± 0.06, P < 0.0001), GLS (−14.9 ± 1.3 vs. −17.3 ± 7.6, P = 0.001), and E/e ratio (9.3 ± 3.4 vs. 8.2 ± 2.0, P = 0.037) in Group 1, but not in Group 2: LVEF (0.49 ± 0.06 vs. 0.50 ± 0.05, P = 0.47), GLS (−12.9 ± 2.4 vs. −14.4 ± 3.2, P = 0.052), and E/e ratio (8.8 ± 2.4 vs. 10.0 ± 4.7, P = 0.18). WMSI improved significantly more in Group 1 (reduction of −17.1% vs. −6.8% in Group 2, P = 0.015).*
***Conclusion**: Lower IMR was associated with better myocardial GLS acutely after STEMI, and with a significantly higher recovery of the LVEF, WMSI, E/E' ratio and GLS, suggesting that IMR is an early marker of cardiac recovery, after acute myocardial infarction.* (J Interven Cardiol 2016;29:137–145)

## Introduction

Despite successful recanalization of the infarct-related artery in ST-elevation myocardial infarction (STEMI), some patients have impaired microcirculatory reperfusion, implying a poorer prognosis.[1,2]

Limited reperfusion due to injury of coronary microvasculature may lead to myocardium persistent nonviability. The possibility to predict, early during the acute myocardium infarction, whether or not revascularized myocardium is adequately reperfused, and to which extent contractility will recover would be useful.[3] Recently, several invasive and non-invasive techniques have been proposed for direct coronary microcirculation evaluation.[3]

The index of microcirculatory resistance (IMR) is an invasive surrogate of coronary microvascular function.[4,5] It has been reported that IMR predicts residual

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myocardial viability in acute myocardial infarction and left ventricular functional recovery (ejection fraction and wall motion score index), as evaluated by transthoracic echocardiography.[4,6–8]

A few studies reported on the relationship between coronary microvascular function (evaluated by several invasive and non-invasive techniques) and myocardial strain in assessing myocardial function recovery after myocardium infarction.[3,9,10] However the relationship between IMR and global longitudinal strain after STEMI has not been studied.

The purpose of this study was to evaluate the relationship between IMR and left ventricle (LV) functional recovery, evaluated by systolic and diastolic echocardiography parameters in STEMI patients undergoing primary angioplasty (P-PCI).

## Methods

Consecutive patients admitted with a first STEMI and treated with P-PCI, were prospectively included. The inclusion criteria were: first STEMI, defined by chest pain lasting at least 20 minutes and/or ST-segment elevation of at least 1 mm in two or more contiguous leads; pain to balloon time lower than 6 hours (or between 6 and 12 hours if ongoing pain); P-PCI performed with success on the culprit lesion in a native coronary artery; Age over 18 years; informed consent obtained. The exclusion criteria were: previous history of myocardial infarction; patients presenting with left bundle-branch block and patients with implanted pacemaker; cardiogenic shock; known myocardial diseases and severe left ventricular hypertrophy (>15 mm); previous coronary artery bypass surgery; percutaneous revascularization in the last 3 months; contraindication to adenosine; any serious non-cardiac disease associated with a life expectancy of less than 12 months. The institutional ethical committee approved the study, which was conducted in compliance with the Declaration of Helsinki.

Primary PCI and adjuvant treatment were offered according to standard of care for patients with acute STEMI. Non-culprit vessels were never addressed at the time of the index procedure. IMR was measured immediately after successful P-PCI. Importantly, all medical decisions were performed blinded to IMR measurement.

Conventional 2-dimensional Doppler echocardiography and 2-dimensional speckle tracking imaging were performed within the first 24 hours (Echo1) and after 3 months (Echo 2).

At discharge, all patients without contraindication were on therapy with beta-blockers and angiotensin converting enzyme inhibitors (or angiotensin receptor blocker, if they were intolerant to angiotensin converting enzyme inhibitors).

**IMR Measurements.** The index of microvascular resistance was measured immediately after successful P-PCI as follows:[4,5] a coronary pressure wire (*St. Jude Medical*) was calibrated outside the body, equalized to the pressure reading from the guide catheter with the pressure sensor positioned at the ostium of the guiding catheter (after intracoronary administration of 1–2 ml of nitroglycerine), and then advanced to the distal two-thirds of the culprit vessel. Maximal hyperaemia was induced using intravenous adenosine (140 μg/kg per minute) via a central venous catheter. Approximately, 3 millilitres of room-temperature saline were injected down the culprit vessel, and the hyperemic transit times were recorded and automatically averaged, using the Radianalyser™ console. Mean distal coronary pressures were recorded during peak hyperaemia.

The IMR was calculated by multiplying the mean distal coronary pressure by the mean hyperaemic transit time.

**Echocardiographic Study.** Transthoracic echocardiography was performed using a commercially available ultrasound system *(Vivid 7 GE Healthcare)*. Measures were performed offline with *EchoPAC version 113 GE Healthcare* by two observers. To minimize bias, operators performing the measurements were blinded to IMR result. Parameters were measured several times and then averaged.

The echocardiographic quantification of LV volumes and EF was determined from four- and two-chamber views using a semiautomatic border detection based on feature tracking imaging.[11]

LV regional wall-motion analysis was performed with the calculation of the wall-motion score index (WMSI) by 2D echocardiography, according to the European Society of Echocardiography Recommendations, using the 17-segment model on a 1–5 scale: 1-normal, 2-hypokinesia, 3-akinesia, 4-dyskinesia, 5-aneurysmal.[12]

Mitral inflow was obtained by pulsed-wave Doppler-echocardiography with the sample volume between mitral leaflet tips during diastole, and mitral annulus velocities were obtained from the media of septal and lateral annulus by tissue Doppler imaging. The ratio of

early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/é) was calculated. Left atria volumes were obtain by 2D echocardiography and indexed to body area.

Global LV longitudinal strain (GLS) was assessed using speckle-tracking analysis with automated function imaging. Peak longitudinal strain was defined as the change in length of the myocardium from end-diastole to end-systole and expressed as a percentage: longitudinal strain (%) = (L end-systole—L end-diastole)/L end-diastole × 100%, where L is the length of the region of interest. GLS was obtained from 2D gray scale images of the apical four-chamber, two-chamber, and long-axis view with optimized frame rate (50–90 frames/second). Software identified the endocardial border, and myocardial motion was automatically tracked in each imaging view. In segments with poor tracking, the observer readjusted the endocardial trace line until a better tracking was achieved. Mean of the peak systolic longitudinal strain values from the 17 LV segments were calculated to determine LV GLS.

**Statistical Analysis.** Statistical analysis was performed using IBM® SPSS® Statistics 20.0. Data are presented as mean ± SD or median (interquartile range) for continuous variables and as frequencies and proportions for categorical variables. Distribution was tested with the Kolmogorov–Smirnov test. Continuous variables were compared using the Student t-test if they had a normal distribution and with the Mann–Whitney test if they didn't. Categorical variables were tested using the $\chi^2$ test. Relationships between variables were assessed by correlation analysis (Pearson method). A 2-sided P-value of $<0.05$ was considered statistically significant.

## Results

Forty-nine patients were initially included. Of these, 9 patients were excluded, due to poor technical quality of either one or both Echocardiogram exams. Baseline characteristics of the 40 patients analysed are presented in Table 1. Thirty-four patients (85.0%) were male and the mean age was 59.3 ± 12.7 years. All patients were in Killip class I.

Median IMR was 26.0 (interquartile range 32.5). For the purpose of the analysis, patients were divided in 2 groups, according to median IMR: Group 1 (IMR $<26$), with lesser degree of microvascular dysfunction, and Group 2 (IMR $>=26$), with higher degree of microvascular dysfunction. Patients in Group 2 were significantly older and had a trend for higher prevalence of hypertensions, but there were no other significant differences between the 2 groups (including risk factors, previous history and previous medication, ischemia time, culprit artery and coronary artery disease severity). The area at risk, evaluated both by the APPROACH and the BARI scores, was similar between groups. There were also no significant differences in the revascularization procedure, except for the use of abciximab, which was higher in Group 1 patients (Table 1).

IMR correlated with the extent of myocardial infarction measured by peak Troponin I values ($r=0.46$, $P=0.003$) and the area under the curve of TnI values measured every 6 hours, for the first 48 hours ($r=0.54$, $P<0.001$), indexed to the area at risk evaluated by the APPROACH and BARI angiographic scores.[13–15].

**Initial Echocardiographic Evaluation.** In Echo1 (24 hours) there were no significant differences between groups in LV telesystolic volume (TsV), LVEF, WMSI, and E/É ratio (Table 2). GLS (evaluated at baseline in 33 patients) was significantly better in Group 1 patients (−14.9 vs. −12.9 in Group 2, $P=0.005$) (Fig. 1). IMR correlated positively and significantly with the baseline GLS ($r=0.6$, $P=0.001$). On multivariate analysis, including variables found to be significantly related with baseline GLS values on univariate analysis (proBNP values and the area at risk evaluated by the APPROACH score) and other variables considered clinically relevant (age and Abciximab use), the presence of an IMR > 26 still showed to be independently related with baseline GLS ($\beta=0.562$, $P=0.002$).

**Follow-Up Echocardiographic Evaluation.** Results of the second echocardiographic evaluation (3 months) and on the differences between these and the first echocardiographic analysis (24 hours) are depicted in Table 3. In summary, there were significant improvements in LV TsV (53.8 ± 12.3 vs. 48.213.2 ml, $P=0.027$) and LVEF (0.48 ± 0.06 vs. 0.55 ± 0.06, $P<0.0001$) in Group 1, but these improvements were not observed in Group 2 (Fig. 2A). The WMSI improved in both groups (from 1.46 ± 0.24 to 1.19 ± 1.33, $P<0.0001$ in Group 1; and from 1.52 ± 0.22 to 1.42 ± 0.23, $P=0.006$ in Group 2—Fig. 2B), although significantly more in Group 1 (reduction of −17.1% vs. −6.8% in Group 2, $P=0.015$). GLS (evaluated both at baseline and at

**Table 1.** Baseline Characteristics According to the Value of IMR

| | Total (n = 40) | IMR < 26 | IMR > 26 | P |
|---|---|---|---|---|
| Epidemiologic and anthropometric data | | | | |
| Age (years) | 59.3 ± 12.7 | 55.1 ± 12.1 | 63.5 ± 12.1 | 0.036** |
| Male sex n (%) | 34 (85.0) | 18 (90.0) | 16 (80.0) | 0.331*** |
| BMI (Kg/m$^2$) | 27.8 ± 3.6 | 27.7 ± 3.0 | 27.9 ± 4.3 | 0.886** |
| Abdominal circumference (cm) | 99.5 ± 11.9 | 97.1 ± 10.3 | 101.9 ± 13.2 | 0.260** |
| Previously medical history n (%) | | | | |
| Hypertension | 29 (72.5) | 12 (60.0) | 17 (85.0) | 0.078*** |
| Dyslipidaemia | 20 (50.0) | 11 (55.0) | 9 (45.0) | 0.376*** |
| Diabetes | 9 (22.5) | 5 (25.0) | 4 (20.0) | 0.500*** |
| Smoking | 19 (47.5) | 11 (55.0) | 8 (40.0) | 0.264*** |
| Statin | 6 (16.2) | 3 (15.8) | 3 (16.7) | 0.643*** |
| Previous angina | 6 (15.0) | 2 (10.0) | 4 (20.0) | 0.331*** |
| Previous coronary revasc. (PCI) | 1 (2.5) | 1 (5.0) | 0 (0.0) | 0.500*** |
| Medical treatment n (%) | | | | |
| Past | | | | |
| Beta-blockers | 3 (7.5) | 2 (10.0) | 1 (5.0) | 0.500*** |
| ACEi/ARBs | 15 (37.5) | 6 (30.0) | 9 (45.0) | 0.257*** |
| Statins | 8 (20.0) | 4 (20.0) | 4 (20.0) | 0.622*** |
| At discharge/follow-Up | | | | |
| Beta-blockers | 39 (97.5) | 19 (95.0) | 20 (100.0) | 0.500*** |
| ACEi/ARBs | 38 (95) | 19 (95.0) | 19 (95.0) | 0.756*** |
| Primary angioplasty data | | | | |
| Pain-to-balloon time (min) | 202 ± 74 | 187 ± 63 | 217 ± 82 | 0.208** |
| Door-to-balloon time (min) | 73 ± 24 | 70 ± 23 | 76 ± 26 | 0.466** |
| Admission heart rate | 75 ± 15 | 77 ± 17 | 72 ± 13 | 0.303** |
| Admission Systolic BP (mm Hg) | 128 ± 19 | 127 ± 22 | 130 ± 16 | 0.649** |
| Admission Diastolic BP (mm Hg) | 78 ± 11 | 79 ± 12 | 76 ± 10 | 0.641** |
| Culprit artery n (%) | | | | |
| Left anterior descending | 15 (37.5) | 5 (25.0) | 10 (50.0) | 0.264*** |
| Left circumflex | 10 (25.0) | 6 (30.0) | 4 (20.0) | |
| Right coronary artery | 15 (37.5) | 9 (45.0) | 6 (30.0) | |
| Area at risk (percentage of myocardium affected by the culprit lesion) (%) | | | | |
| APPROACH score | 27.8 ± 7.5 | 26.9 ± 6.0 | 28.8 ± 8.9 | 0.430** |
| BARI score | 27.6 ± 6.8 | 27.7 ± 6.2 | 27.5 ± 7.4 | 0.936** |
| TIMI flow before PCI ≥2 n (%) | 6 (15.0%) | 4 (10.0) | 2 (5.0) | 0.331*** |
| Multivessel disease n (%) | 12 (52.5) | 7 (50.0) | 5 (55.0) | 0.500*** |
| Thrombus aspiration n (%) | 21 (52.5) | 10 (50.0) | 11 (55.0) | 0.500*** |
| Lesion pre-dilatation n (%) | 22 (55.0) | 9 (45.0) | 13 (65.0) | 0.170** |
| Abciximab n (%) | 7 (17.5) | 6 (30.0) | 1 (5.0) | 0.046*** |
| TFCc | 18.1 ± 6.6 | 15.6 ± 4.8 | 20.6 ± 7.4 | 0.018** |
| Myocardial blush grade 3 n (%) | 26 (66.7) | 15 (75.0) | 11 (57.9) | 0.214*** |
| Admission proBNP ng/dL* | 109 (132) | 71 (117) | 137 (212) | 0.109**** |

BMI, body mass index; TFCc, corrected TIMI frame count. *Expressed as median (interquartile range). **Student t-test. ***$\chi^2$ test. ****Mann–Whitney test.

**Table 2.** Baseline Echocardiographic Parameters According to the Value of IMR

| | Group 1 (IMR < 26) | Group 2 (IMR > = 26) | P* |
|---|---|---|---|
| LVTdV (ml) | 103.4 ± 3.1 | 105.3 ± 28.8 | 0.835 |
| LVTsV (ml) | 53.8 ± 2.3 | 53.5 ± 13.6 | 0.948 |
| LVEF | 0.48 ± 0.06 | 0.49 ± 0.6 | 0.659 |
| WMSI | 1.46 ± 0.24 | 1.53 ± 0.22 | 0.377 |
| GLS (%) | −14.8 ± 1.3 | −12.7 ± 2.4 | 0.005 |
| Left atria volume (ml/m$^2$) | 30.2 ± 5.1 | 32.5 ± 8.9 | 0.352 |
| Ratio E/E′ | 9.3 ± 3.4 | 8.7 ± 2,3 | 0.587 |

LVTdV, left ventricle telediastolic volume; LVTsV, left ventricle telesystolic volume; LVEF, left ventricle ejection fraction; WMSI, wall motion score index; GLS, global longitudinal strain. *T-test student.

3 months in 28 patients) improved significantly in Group 1 (from −14.9 ± 1.3 to −17.3 ± 7.6, P = 0.001), but not in Group 2 (from −12.9 ± 2.4 to −14.4 ± 3.2, P = 0.052–Fig. 2C). Patients in Group 1 had significantly higher LFEV, lower WMSI and lower GLS values, as compared with patients in Group 2 (Table 3). Additionally, IMR correlated with WMSI (r = 0.6, P < 0.001). On multivariate analysis, the presence of an IMR > 26 was independently associated with 3 months LVEF (β = −0.388, P = 0.024), WMSI (β = 0.441, P = 0.006), and GLS (β = 0.401, P = 0.002).

Concerning diastolic parameters, there was a significant decrease in E/É ratio at 3 months in Group 1 (9.3 ± 3.4 vs. 8.2 ± 2.0, P = 0.037). This improvement was not observed in Group 2, (8.8 ± 2.4 vs. 10.0 ± 4.7, P = 0.18), in which a significant increase in atrial volume was also found (32.5 ± 9.0 vs. 41.6 ± 15.8, P = 0.028).

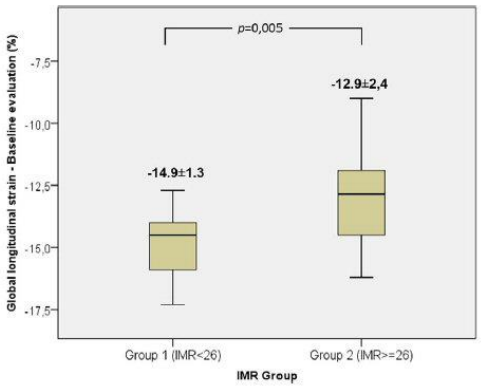


**Figure 1.** GLS in baseline evaluation according to the value of IMR. A comparison of the average GLS in baseline evaluation, in patients with less microvascular dysfunction (Group 1, IMR < 26) with patients with more microvascular dysfunction (Group 2, IMR > = 26).

## Discussion

In this study, lower microvascular dysfunction, as evaluated invasively (IMR < 26) after P-PCI, was associated with a significantly higher recovery of the LV systolic function reflected in better LVEF, WMSI and GLS evaluated after 3 months. Additionally, IMR independently correlated with GLS evaluated in the first 24 hours after infarction and with LVEF, WMSI and GLS evaluated at 3 months. Finally, LV diastolic function also improved in patients with lower microvascular dysfunction, reflected in E/É ratio decrease.

**IMR and Echocardiographic Systolic Parameters.** The extent of microvascular dysfunction has been shown to be an important and independent contributor to subsequent changes in left ventricular geometry and performance. Patients with impaired microvascular perfusion have larger infarcts, with higher necrotic areas, larger long-term left ventricular wall motion abnormalities and lower left ventricular ejection fraction.[16–18] A few studies have been performed showing that IMR, measured after P-PCI in STEMI patients, relates with the extent of myocardial damage and microvascular obstruction, allowing a prediction of late left ventricular recovery.[4,8,17–19] The precise mechanisms underlying myocardial malperfusion after the restoration of epicardial blood flow are likely to be multifactorial: the generation of oxygen free radicals, increased myocardial-cell calcium levels, cellular and interstitial

**Table 3.** Baseline and 3 Months Echocardiographic Parameters According to the Value of IMR

| | Group 1 (IMR < 26) | | | Group 2 (IMR > = 26) | | |
|---|---|---|---|---|---|---|
| Echo parameters | Echo1 | Echo2 | P value* | Echo1 | Echo2 | P value* |
| LVTdV (ml) | 103.4 ± 23.1 | 105.9 ± 22.3 | 0.6 | 104.3 ± 29.4 | 112.4 ± 34.4 | 0.12 |
| LVTsV (ml) | 53.8 ± 12.3 | 48.2 ± 13.2 | 0.027 | 52.9 ± 13.9 | 57.2 ± 23.0 | 0.26 |
| LVEF | 0.48 ± 0.06 | 0.55 ± 0.06 | <0.0001 | 0.49 ± 0.06 | 0.50 ± 0.05 | 0.47 |
| WMSI | 1.46 ± 0.24 | 1.19 ± 1.33 | <0.0001 | 1.52 ± 0.22 | 1.42 ± 0.23 | 0.006 |
| GLS (%) | −14.9 ± 1.3 | −17.3 ± 7.6 | 0.001 | −12.9 ± 2.4 | −14.4 ± 3.2 | 0.052 |
| Left atria (ml/m$^2$) | 30.2 ± 5.1 | 30.9 ± 6.0 | 0.53 | 32.5 ± 9.0 | 41.6 ± 15.8 | 0.028 |
| E/E′ ratio | 9.3 ± 3.4 | 8.2 ± 2.0 | 0.037 | 8.8 ± 2.4 | 10.0 ± 4.7 | 0.18 |

IMR, index of microcirculatory resistance; LVTdV, left ventricle telediastolic volume; LVTsV, left ventricle telesystolic volume; LVEF, left ventricle ejection fraction; WMSI, wall motion score index; GLS, global longitudinal strain. *T-test for paired samples.

edema, endothelial dysfunction, vasoconstriction, and thromboembolism have all been proposed, and it is not clear which of these mechanisms have more impact on the IMR measured values in STEMI patients. Importantly, there is no clear cut-off value for IMR which, as a continuous variable, will be higher in patients with more microvascular dysfunction. Additionally, most published series using this technique in STEMI patients are small (<40 patients) and used median values to separate lesser from higher microvascular dysfunction.

We found that lower IMR values after P-PCI are associated with better recovery of both LVEF and WMSI measured 3 months after the STEMI. These results are in accordance with those reported by Fearon et al. in 29 patients with acute STEMI treated with P-PCI and in which IMR was also measured immediately after the procedure. Indeed, an IMR > 32 was the strongest predictor of 3-month WMSI and was the only significant predictor of recovery of left ventricular function on the basis of the percent change in WMSI.[4] This relation between IMR and late left ventricular recovery after STEMI was again showed by Hong-Seok Lim et al. In their study, including 40 patients with anterior STEMI successfully treated with P-PCI, IMR strongly correlated with myocardial viability (measured by 18F-fluorodeoxyglucose [FDG] positron emission tomography) and with echocardiographic anterior wall motion score change at 6-month.[7] Patients with an IMR < 33 had the best results. Kitabata et al. confirmed that IMR measured after P-PCI for anterior STEMI can predict the transmural extent of infarction defined by contrast-enhanced cardiac magnetic resonance (CMR).[17] Later, *Kitabata* concluded that IMR after P-PCI for anterior STEMI predicts LV remodelling evaluated by CMR.[18] McGeoch et al. also used CMR to show that IMR measured after P-PCI in STEMI patients is a predictor of LVEF and infarct volume on CMR scan 2 days and 3 months after infarction.[6]

In our study, IMR evaluated immediately after P-PCI in STEMI patients correlated with GLS evaluated in the first 24 hours after infarction. However, there was no relationship between IMR and the conventional echocardiographic parameters, evaluated in this same moment, suggesting the higher diagnosis accuracy of strain techniques. The additional value of strain for myocardium evaluation after infarction has been extensively reported. Sjøli et al. evaluated a population of patients with STEMI by echocardiography and magnetic resonance and concluded that LV global strain is a more precise and reproducible diagnostic predictor of large infarcts compared with LVEF.[20] Global strain after STEMI has been reported to be well associated with echocardiographic WMSI, CMR-determined infarct size, and LVEF measured by CMR, Single-photon emission computed tomography, standard echo and contrast echo. It was found to be the best predictor of low LVEF, measured by the gold standard CMR.[21]

To our knowledge, this study is the first to report on the association between IMR and strain after STEMI. In stable coronary artery disease, IMR was already correlated with strain in evaluation of LV contractile reserve on dobutamine echocardiography.[22] In STEMI patients, Park et al. found myocardial strain to be well correlated with coronary flow reserve (CFR), determined by intracoronary measurement after P-PCI in

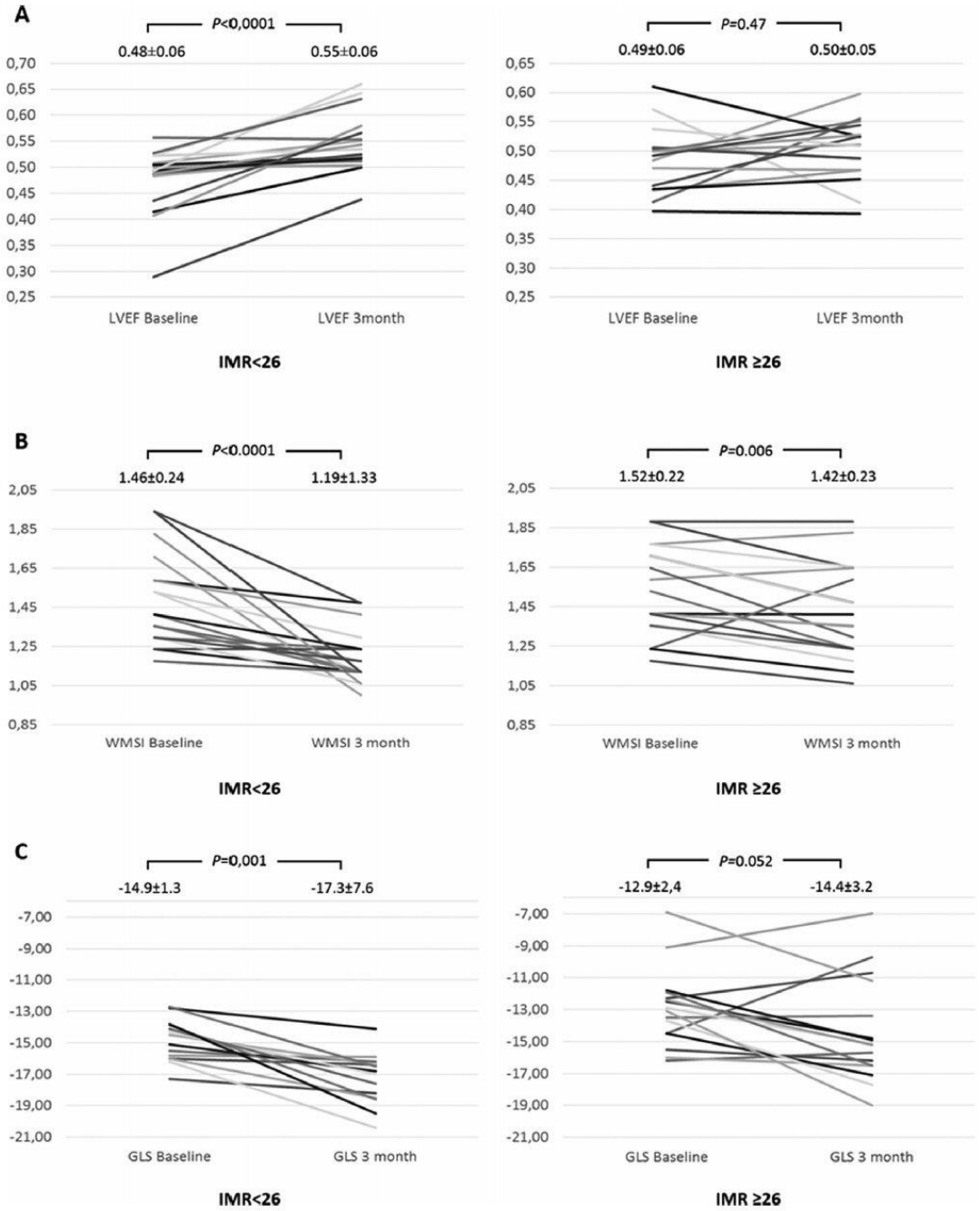


**Figure 2.** Relation between index of microcirculatory resistance and left ventricle functional recovery from baseline to 3 months. Left ventricle functional recovery based on (A) left ventricle ejection fraction (LVEF), (B) wall motion score index (WMSI), and (C) global longitudinal strain (GLS), from baseline to 3 months, in patients with lower microvascular dysfunction (Index of microcirculatory resistance (IMR) <26) in comparison to patients with higher microvascular dysfunction (IMR > 26).

anterior STEMI, and suggested its utility to predict myocardial functional recovery, non-invasively.[3] Logstrup et al. reported that the magnitude of myocardial deformation correlates with the severity of alterations in microcirculation, detected by CFR, assessed by transthoracic echocardiography. They demonstrated longitudinal global and regional strain to be a significantly better tool than WMSI to investigate LV dysfunction after AMI.[10]

In the present study, an IMR > 26 was able to independently predict myocardial deformation, evaluated immediately after infarction by GLS, which is considered as a very sensitive and informative parameter of infarction extension and functional recovery. This association between IMR and GLS was not only present initially, but persisted at 3 months, when the relationship of IMR with LVEF and WMSI also manifest. Importantly, the association between IMR and GLS was independent of the area at risk and the admission proBNP values, suggesting that IMR has an additional value in predicting left ventricular recovery in these patients.

**IMR and Echocardiographic Diastolic Parameters.** We found that lesser microvascular dysfunction, as assessed early post-PCI by IMR, is associated with better LVdiastolic function after 3 months, reflected in a lower ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/é) and a stable left atria volume.

Few reports have been published addressing the relationship between coronary microcirculation and LV diastolic function. Escaned et al. found a correlation between LV diastolic function, evaluated by the ratio E/é, and coronary microcirculation parameters (IMR and instantaneous hyperaemic diastolic velocity pressure slope index—IHDVPS), in a population of diabetic patients with coronary artery desease.[23] Park et al. compared viable and non-viable akinetic myocardium after STEMI and reported that E/é ratio was slightly higher (although not significant, P = 0.09) and the deceleration time of E was significantly shorter in the nonviable group, which also have a lower coronary flow reserve (CFR), than the viable group.[9] The relationship between infarct extension and diastolic dysfunction is not well understood. Since the severity of diastolic dysfunction after STEMI represents an independent prognostic marker, the possibility to early identify patients at a higher risk, may be useful.[24,25]

## Study Limitations

This study is limited by the small number of patients (a problem that all the previous studies performed with this technique also have), and the fact that not all of echocardiographic parameters were suitable for analysis in all patients. Even considering these limitations, our results are in accordance with the previous published studies on LV systolic function recovery. Larger studies will be necessary to confirm these data. Particularly with regard to diastole, more studies are needed to clarify its relationship to the extent of microvascular disease and infarction. The relationship between IMR and strain was limited to longitudinal strain. In spite of being the primary cardiac motion, radial and circumferential strain may provide additional information about functional recovery and strain rate evaluation also could give additional information.

## Conclusions

In STEMI patients, lower microvascular dysfunction as evaluated invasively (IMR < 26) is associated with a significantly higher recovery of the LVEF, WMSI, E/E' ratio and GLS, indicating that IMR is an early marker of cardiac recovery after acute myocardial infarction. IMR was also able to independently predict myocardial deformation evaluated by GLS, acutely after infarction.

## Clinical Implications

Both IMR and strain may be useful to identify patients who benefit from a closer follow-up and intensification of medical therapy to reduce remodelling and LV dysfunction. These results underscore the need of further investigation in order to find additional strategies and procedures, in adjunct to P-PCI, to improve microvascular coronary function after STEMI and to improve prognosis.

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## 3 – Early peripheral endothelial dysfunction predicts myocardial infarct extension and microvascular obstruction in patients with ST elevation myocardial infarction

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# Early peripheral endothelial dysfunction predicts myocardial infarct extension and microvascular obstruction in patients with ST elevation myocardial infarction

# A disfunção endotelial precoce prevê a extensão do enfarte e a presença de obstrução microvascular em doentes com enfarte agudo do miocárdio

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## RESUMO

**Introdução e objectivos**: o papel da disfunção endotelial (DE) em doentes com enfarte agudo do miocárdio com elevação do segmento ST (EAMcST) é mal compreendido. A tonometria arterial periférica (TAP) permite avaliar de forma não invasiva a função endotelial, mas nunca foi usada precocemente após intervenção coronária percutânea primária (ICP-P). O nosso objectivo foi avaliar a relação entre a DE avaliada por TAP, a presença de obstrução microvascular (OMV) e a extensão do enfarte (EE) em doentes com EAMcST.

**Métodos**: a função endotelial foi avaliada pelo índice de hiperemia reactiva (IHR), obtido por TAP. A DE foi definida como um valor de IHR<1,67. A EE foi avaliada pela Troponina I (TnI) e por ressonância magnética cardíaca com contraste (RMCc). A OMV foi avaliada por RMCc e por indicadores indirectos electrocardiográficos e angiográficos. Foi ainda efectuado um ecocardiograma nas primeiras horas.

**Resultados**: foram incluídos 38 doentes (idade média 60,0±13,7 anos, 29 homens). Os valores médios de IHR foram 1,87±0,60 e 16 (42,1%) tinham DE. Os valores máximos (mediana 118/IIQ 186 vs. 67/81, p=0,024) e a área sob a curva de TnI (mediana 2305/IIQ 2486 vs. 1076/1042, p=0,012) foram significativamente superiores nos doentes com DE, que também mostraram uma tendência para mais enfartes transmurais (63,6% vs. 22,2%, p=0,06) e maiores massas de enfarte na RMCc (mediana 17,5/IIQ15,4 vs. 10,1/10,3, p=0.08). Os doentes com RHI<1,67 mostraram valores de fracção de ejecção do ventrículo esquerdo (FEVE) significativamente menores e valores do índice de motilidade segmentar (IMS) significativamente maiores, por ecocardiografia e por RMCc. A presença de OMV na RMCc foi mais frequente nos doentes com DE (54,5% vs. 11,1%, p=0,03), observando-se uma tendência semelhante nos marcadores electrocardiográficos e angiográficos de reperfusão microvascular.

**Conclusões**: A presença de DE avaliada por TAP 24 horas após ICP-P em doentes com EAMcST associa-se a enfartes maiores, menor FEVE, maior IMS e maior prevalência de OMV.



## PALAVRAS CHAVE

Enfarte Agudo do miocárdio; intervenção coronária percutânea primária; função endotelial; tonometria arterial periférica; índice de hiperémia reactiva

## ABSTRACT

**Background and Objectives**: The role of endothelial dysfunction (ED) in patients with ST elevation myocardial infarction (STEMI) is poorly understood. Peripheral arterial tonometry (PAT) allows the non-invasive evaluation of endothelial function, but was never evaluated early after primary percutaneous coronary intervention (P-PCI). Our purpose was to evaluate the relation between ED evaluated by PAT and both the presence of microvascular obstruction (MVO) and infarct extent (IE) in STEMI patients.

**Methods**: Endothelial function was evaluated by the reactive hyperemia index (RHI), measured by PAT. ED was defined as an RHI<1.67. IE was evaluated by troponin I (TnI) release and contrast enhanced cardiac magnetic resonance (ceCMR). MVO was evaluated by ceCMR and by indirect angiographic and ECG indicators. An Echocardiogram was also performed in the first hours.

**Results**: 38 patients were included (mean age 60.0±13.7 years, 29 males). Mean values of RHI were 1.87±0.60 and 16 patients (42.1%) had ED. Peak TnI (median 118/IQR 186 vs. 67/81, p=0.024) and AUC of TnI (median 2305/IQR2486 vs. 1076/1042, p=0.012) were significantly higher in patients with ED, which also had a trend for more transmural infarcts (63.6% vs. 22.2%, p=0.06) and larger infarct mass in the ceCMR (median 17.5/IQR15.4 vs. 10.1/10.3, p=0.08). Left ejection fraction (LVEF) was lower and wall motion score index (WMSI) was higher both in Echo and ceCMR in patients with ED. In the ceCMR, MVO was more frequent in patients with RHI<1.67 (54.5% vs. 11.1%, p=0.03). ECG and angiographic indicators of MVO all showed a trend towards worse results if these patients.

**Conclusions**: The presence of ED evaluated by PAT 24 hours after P-PCI in patients with STEMI is related with larger infarcts, lower LVEF, higher WMSI and higher prevalence of MVO.



## KEYWORDS

Acute myocardial infarction; primary PCI; endothelial function; peripheral arterial tonometry; reactive hyperaemia index

## LIST OF ABREVIATIONS

| | |
|---|---|
| ACS | Acute coronary syndromes |
| AUC | Area under the curve |
| ceCMR | Contrast enhanced cardiac magnetic resonance |
| cTFC | Corrected TIMI frame count |
| ED | Endothelial dysfunction |
| hs-CRP | High sensitive C-reactive protein |
| IE | Infarct extension |
| IMR | Index of microvascular resistance |
| IQR | Interquartile range |
| LVEF | Left ventricle ejection fraction |
| MVO | Microvascular obstruction |
| NO | Nitric oxide |
| NST-ACS | Non-ST elevation acute coronary syndromes |
| PAT | Peripheral arterial tonometry |
| P-PCI | Primary percutaneous coronary intervention |
| RHI | Reactive hyperemia index |
| RHI | Reactive hyperemia index |
| STEMI | ST elevation myocardial infarction |
| TMPG | TIMI myocardial perfusion grade |
| TnI | Troponin I |
| WMSI | Wall motion score index |

## INTRODUCTION

Notwithstanding all the improvements we have seen in primary angioplasty programs, with dramatic reductions in the time between symptom onset and the intervention, and despite a normal epicardial coronary artery flow is almost always achieved in a timely fashion after primary percutaneous coronary intervention (P-PCI) in patients with ST elevation myocardial infarction (STEMI), a significant proportion of patients (from 20% to 60%) has a poor outcome because of microvascular coronary damage.[1,2]

The negative prognostic implications (both on the risk of left ventricle remodelling and on the risk of hard endpoints, including death) associated with coronary microvascular damage has been repeatedly confirmed, with several invasive and non-invasive indicators[2–13]. However, the precise mechanisms underlying coronary microcirculation dysfunction before and after the restoration of epicardial blood flow are largely unknown and likely to be multifactorial. Traditionally, coronary microvascular dysfunction in this setting is seen *as a consequence* of the primary epicardial event and/or of the coronary reperfusion, either pharmacological (thrombolysis) or mechanical (P-PCI). An alternative explanation is that either *pre-existing* or *simultaneous* coronary microvascular dysfunction may have by itself pathophysiological importance and contribute to the extension of the myocardial infarction, left ventricular remodelling and future events.[14] Whatever theory concerning microvascular coronary dysfunction in patients with STEMI is correct (cause, consequence or both), endothelial function seems to be always at the core of the proposed mechanisms. In fact, there is accumulating evidence that endothelial dysfunction (ED) is not just a risk factor and precursor of coronary artery disease, but it also plays a central role leading to acute coronary syndromes and STEMI.[15] In patients with acute coronary syndromes, endothelial function, measured in the peripheral circulation, has been shown to be an independent predictor of events[16], and subsequent normalization of endothelial function in these patients predicts a lower risk.[17,18] Similarly, in patients with STEMI treated with P-PCI, peripheral ED was associated with larger infarctions as measured by troponin peak levels[19] and endothelial function improvement six months after the event also correlated with lower end-diastolic left ventricular volumes.[20]

It is therefore licit to speculate that previous ED or a blunted reaction of the endothelial mechanisms during STEMI may be a central component of coronary microvascular dysfunction in these patients and, as a consequence, a determinant of microvascular reperfusion, infarction extension and left ventricle remodelling. Ideally, this hypothesis would best be proved if endothelial function evaluation had been performed *before* the myocardial infarction, which evidently is not possible. Actually, most studies evaluating endothelial function in acute coronary syndrome patients were performed relatively *late* (several days or weeks) after the onset of the event[19,21–25]. As such, the worse results documented in patients with ED may just be a consequence of larger and more complicated infarcts. Assuming peripheral endothelial function (which is much more easy to measure) as a surrogate of coronary endothelial function, we would expect, if the above theory is true, worse acute ED, worse microvascular reperfusion and worse left ventricle remodelling in patients with larger infarctions.

The purpose of this pilot study was to evaluate the relation between peripheral endothelial function evaluated early (24 hours after reperfusion) by peripheral arterial tonometry, the presence of MVO and the extent of myocardial infarction.

## METHODS

Patients admitted with a first STEMI and treated with P-PCI, were prospectively included. The inclusion criteria were: chest pain lasting at least 20 minutes and ST-segment elevation of at least 1 mm in two or more contiguous leads; pain-to-balloon time lower than 6 hours (or between 6 and 12 hours if ongoing pain); P-PCI performed with success on the culprit lesion in a native coronary artery; age over 18 years; informed consent obtained. The exclusion criteria were: previous history of myocardial infarction; patients presenting with left bundle-branch block and patients with implanted pacemaker; cardiogenic shock; known myocardial diseases and severe left ventricular hypertrophy (> 15 mm); previous coronary artery bypass surgery; percutaneous revascularization in the last 3 months; contraindication to adenosine.; any serious non-cardiac disease associated with a life expectancy of less than 12 months. The institutional ethical committee approved the study, which was conducted in compliance with the Declaration of Helsinki.

Primary PCI and adjuvant treatment were performed according to standard of care for patients with acute STEMI. Endothelial function was evaluated 24 hours after the P-PCI by peripheral arterial tonometry (PAT). Infarct extension was evaluated by contrast enhanced cardiac magnetic resonance (ceCMR) and by the release of troponin I. MVO was evaluated by ceCMR and also by indirect indicators, including ST resolution in the ECG and angiographic indicators (corrected TIMI frame count, TIMI myocardial perfusion grade and the index of microcirculatory resistance). Additionally, an echocardiographic exam was performed in the first hours after P-PCI.

### Evaluation of endothelial function – peripheral arterial tonometry

Endothelial function was evaluated by peripheral arterial tonometry (PAT), using EndoPAT 2000 (Itamar Medical®, Caesarea, Israel). The protocol was the same followed in previous studies.[26] Briefly, a complete digital peripheral arterial tonometry (PAT) endothelial function test includes three phases: baseline, occlusion, and hyperemia. A blood pressure cuff is placed on one upper arm (study arm), while the other arm serves as a control (control arm). A PAT probe is positioned on one finger of each hand and set by the computer to inflate to 10 mm Hg below diastolic pressure or 70 mm Hg (the lower value is selected). Recordings are taken simultaneously from both fingers throughout the study. The response in the control finger not experiencing hyperemia can be used to adjust for systemic effects. After baseline data acquisition, the blood pressure cuff is inflated on one arm to suprasystolic pressures for 5 minutes. During the occlusion period, signals are absent from the hyperaemic finger but continue from the control finger. After cuff release, pulse amplitude increases in the hyperaemic finger. The pulse amplitude recordings are digitized and analysed by an automated, proprietary algorithm. Average pulse amplitude is calculated for each 30-second intervals after cuff occlusion for up to 5 minutes. As a measure of reactive

hyperemia, the pulse amplitude tonometry is then calculated as the ratio of the average amplitude of the PAT signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the PAT signal of a 3.5-min time period before cuff inflation (baseline). Subsequently, PAT index values from the study arm are normalized to the control arm. All these data are analysed by a computer in an operator-independent manner, to get the reactive hyperemia index (RHI). ED was defined as an RHI<1.67, as previously described[26].

## Contrast enhanced cardiac magnetic resonance

Cardiac magnetic resonance was performed in a subset of patients on the 7-8$^{th}$ day post-MI[27], using a 1.5-T magnetic resonance imaging system (Avanto, Siemens Medical System, Erlangen, Germany) equipped with a dedicated cardiac software package and 8 available independent radiofrequency receiver channels, cardiac coil, and vectorcardiogram. After the acquisition of localizing images, long- and short-axis cine images were obtained, using retrospectively ECG-gated breath-hold segmented K-space balanced steady-state free precession pulse sequence (trueFISP) technique. The short-axis cine scans of 6-mm slices were used to determine the left ventricular mass, volume, and function (in-plane resolution 1.6x1.6mm; gap 2mm). STIR technique, a triple-IR black-blood turbo spin echo pulse sequence was used for oedema quantification (area at risk). A bolus of contrast medium (gadopentetate dimeglumine - Magnevist, Schering AG, Berlin, Germany) was injected at a dose of 0.2 mmol/kg. Early enhancement images for MVO assessment were obtained by acquiring an inversion-recovery segmented gradient echo T1-weighted sequence with a high inversion time (approximately 500 ms), 2-4 min after gadolinium injection. Delayed enhancement images were then obtained by acquiring an inversion-recovery segmented gradient echo T1-weighted sequence, 10 to 15 min after the bolus. All post-processing and analyses of the area at risk, myocardial infarct size, and presence of MVO were performed using CVI 42 Version 5 Software (Circle Cardiovascular Imaging Inc, Calgary, Canada) by a cardiologist experienced in CMR and blinded to all clinical and invasive physiological data. Area at risk was manually quantified on short-axis STIR sequences slices, delineating higher intensity areas (no threshold definition) at each slice, with subsequent computation for mass estimation. Infarct size was also assessed manually by planimetry on each short-axis slice, delineating the hyperenhanced area, including areas of hypoenhancement surrounded by the hyperenhanced area, the latter being considered MVO. Infarct size, as a percentage of left ventricular mass, was computed from the sum of hyperenhanced pixels from each of the 10 short-axis images divided by the total number of pixels within the left ventricular myocardium multiplied by 100% (21)[28]. MVO mass was also manually quantified as the sum of hypoenhanced pixels at delayed enhancement sequences as better spacial resolution was found when compared for early enhancement sequences[29].

## Cardiac biomarkers

Troponin I (TnI) values were used to quantify the extent of the myocardial infarction. With that purpose, blood tests for TnI measurement (by sandwich chemiluminescent immunoassay based LOCI™ technology, with a Dimension Vista™ Intelligent Lab System, Siemens Healthcare Diagnostics™) were collected at admission, every 6 hours for the first 24 hours (i.e., 0, 6, 12, 18,

24), and every 12 hours thereafter until 48 hours after admission (i.e., 36 and 48 hours). Peak TnI values and the area under the curve (AUC) of TnI release were calculated, as previously described.[30]. In order to account for lesion location, these values (AUC of TnI and peak TnI values) were indexed to area-at-risk scores (BARI and modified APPROACH scores[31]).

## Angiographic indicators of MVO

At the end of the primary PCI procedure, a final run of images of the culprit artery was registered, at 30 frames per second, in order to measure TIMI frame count and TIMI perfusion grade. If necessary, the view was adjusted, so that the culprit vessel territory was not superimposed. The duration of cine filming was prolonged at least 3 cardiac cycles, to make sure that the entire washout phase was included. These two measures of flow were analysed offline by a blinded operator to other evaluations of the patient.

The corrected TIMI frame count (cTFC) was measured as the number of frames required for epicardial contrast to reach standardized distal landmarks, as previously described.[32] The first frame used for TIMI frame counting was defined as the frame in which a column of dye touched both borders of the coronary artery and moved forward, and the last frame was defined as the frame in which dye begins to enter (but does not necessarily fill) a standard distal landmark in the artery. These frame counts were corrected for the longer length of the left anterior descending coronary artery by dividing the TFC by 1.7, to arrive at the corrected TIMI frame count (cTFC).

TIMI myocardial perfusion grade (TMPG) was classified according to the standard definition[33], in which grade 3 corresponds to normal entry and exit of dye from the microvasculature (with a ground-glass appearance (“blush”) or opacification of the myocardium in the distribution of the culprit lesion that clears normally, and is either gone or mildly or moderately persistent at the end of the washout phase), and grade 0 corresponds to the failure of the dye to enter the microvasculature.

The index of microvascular resistance (IMR) was measured immediately after the P-PCI, as previously described:[34,35] a coronary pressure 0.014 pressure-wire (Certus. St. Jude Medical) was calibrated outside the body, equalized to the pressure reading from the guide catheter with the pressure sensor positioned at the ostium of the guiding catheter (after intracoronary administration of 1-2 ml of nitroglycerin), and then advanced to the distal two-thirds of the culprit vessel. Maximal hyperaemia was induced using intravenous adenosine (140 µg/kg/min) via a central venous catheter. Approximately 3 millilitres of room-temperature saline were injected down the culprit vessel, and the hyperaemic transit times were recorded and automatically averaged, using the RadiAnalyzer™ Xpress (St. Jude Medical) console. Mean distal coronary pressures were recorded during peak hyperaemia. The IMR was calculated by multiplying the mean distal coronary pressure by the mean hyperaemic transit time.

## Echocardiographic evaluation

An echocardiogram was performed in the first hours, by an operator blinded to the EndoPAT, ECG and angiographic results, using a commercially available ultrasound system *(Vivid 7 GE Healthcare*). Measures were performed offline with *EchoPAC version 113 GE Healthcare* by two observers. Parameters were measured several times and then averaged. The echocardiographic quantification left ventricular ejection fraction was determined from four- and two-chamber views using a semiautomatic border detection based on feature tracking imaging.[36] Left ventricular (LV) regional wall-motion analysis was performed with the calculation of the wall-motion score index (WMSI) by 2D echocardiography, according to the European Society of Echocardiography Recommendations, using the 17-segment model on a 1–5 scale: 1-normal, 2-hypokinesia, 3-akinesia, 4-dyskinesia, 5-aneurysmal. [37] Mitral inflow was obtained by pulsed-wave Doppler-echocardiography with the sample volume between mitral leaflet tips during diastole, and mitral annulus velocities were obtained from the media of septal and lateral annulus by tissue Doppler imaging. The ratio of early diastolic mitral inflow velocity to early diastolic mitral annular velocity (E/e´) was calculated. Left atria volumes were obtain by 2D echocardiography and indexed to body area. Global LV longitudinal strain (GLS) was assessed using speckle-tracking analysis with automated function imaging. Peak longitudinal strain was defined as the change in length of the myocardium from end-diastole to end-systole and expressed as a percentage: longitudinal strain (%) = (L end-systole - L end-diastole)/L end-diastole x 100%, where L is the length of the region of interest. GLS was obtained from 2D grey scale images of the apical four-chamber, two-chamber, and long-axis view with optimized frame rate (50–90 frames/sec). Software identified the endocardial border, and myocardial motion was automatically tracked in each imaging view. In segments with poor tracking, the observer readjusted the endocardial trace line until a better tracking was achieved. Mean of the peak systolic longitudinal strain values from the 17 LV segments were calculated to determine LV GLS.

## Statistical Analysis

Continuous variables are presented as mean ± standard deviation (normal distribution) or as median and interquartile range (non-normal distribution); categorical variables are presented as frequencies. All analyses of ED (i.e., RHI<1.67 vs. >1.67) were performed using independent sample T-Test for continuous variables with a normal distribution, Mann-Whitney Test, for continuous variables with a non-normal distribution and Chi-square for categorical variables (with Fisher correction when applicable). Analysis of RHI as continuous variables was performed using a Pearson's correlation for continuous variables with a normal distribution and Spearmans's rho correlation for continuous variables with a non-normal distribution. Statistical tests and corresponding p-values were two-sided and a $p$ value <0.05 was considered as statistically significant. IBM SPSS version 21.0 was used for all statistical analyses.

## RESULTS

Thirty eight patients were included (mean age 60.0±13.7 years, 29 males). Mean values of RHI were 1.87±0.60 and 16 patients (42.1%) had ED (RHI<1.67).

Main characteristics of the population according to the presence of ED are presented in **Table 1**. The flow chart of the exams performed in the study is presented in **Figure 1**.

There were no significant differences in physical characteristics, risk factors or previous medication between the two groups. Admission blood tests were also similar between both populations, except for hs-CRP, which showed a tendency for higher values in patients with ED. There were no significant differences in ischemic (pain-to-balloon) time between patients with and without ED, although there was a trend for lower door-to-balloon times in patients with RHI<1.67. Patients with ED had a prevalence of multivessel disease that was almost twice the one observed in patients without ED (68.8% vs. 36.4%), although this difference was not statistically significant (p=0.10). Syntax score also tended to be higher in patients with ED. There was a clear trend towards higher area-at-risk, both measured by APPROACH and BARI scores (p=0.08 and 0.07, respectively) and these two scores showed a weak, but significant inverse correlation with RHI (r=-0.426, p=0.008 and r=-0.361, p=0.026, respectively). RHI values tended to be lower in patients in which the left anterior descending artery (LAD) was the culprit vessel (1.74±0.65 vs. 2.02±0.51 in other vessels, p=0.15). Patients with ED also showed a trend for worse initial TIMI score (TIMI 0-1: 93.8% vs. 72.7% in patients without ED). Finally, treatment options (use of mechanical aspiration, stent implantation technique and use of abciximab) were similar in patients with or without RHA<1.67 (**Table 1**).

### Extent of the infarction

Both peak values of TnI and the AUC of TnI release were significantly higher in patients with ED (**Figure 2)**. These differences were even more significant when troponin I values were indexed to the area-at-risk, both by the APPROACH and the BARI scores (**Table 2**).

Left ventricular ejection fraction by echocardiography analysis was significantly lower and wall motion score index significantly higher in patients with ED (**Figure 3**). Likewise, global longitudinal strain was significantly higher in patients with an RHI<1.67, although there were no other significant differences in *Doppler* variables (**Table 3**).

In the ceCMR, patients with ED had significantly lower left ventricular ejection fraction and higher wall motion score index, confirming the echocardiography results. There was a trend for more transmural necrosis (22.2% vs. 63.6%, p=0.06) and higher infarct mass (median value 10.1 vs. 17.5, p=0.08) in patients with RHI<1.67. The percent mass of infarct indexed to area-at-risk scores (APPROACH and BARI) also tended to be higher in patients with ED (**Table 4**).

### Microvascular obstruction

ECG resolution of ST elevation was numerically inferior immediately after PCI (median 60.5% [IQR 72.0%] vs. 76.5% [IQR 41.0%]), and at 90 minutes after reperfusion (median 69.0% [IQR 53.0%] vs. 78.5 [IQR 28.0]) in patients with ED, although these differences were not statistically significant (p=0.23 and 0.07, respectively). However, residual total ST elevation was significantly higher in both ECGs performed immediately after the PCI (5.0 mV [12.0 mV, median vs. 2.5 mV [IQR 5.09 mV], p=0.048) and at 90 minutes (median 3.0 mV [IQR 7.0 mV] vs. 1.3 mV [IQR 4.0 mV]) in patients with RHI<1.67.

All angiographic measures of microvascular dysfunction after STEMI showed important trends towards worse results in patients with RHI<1.67: cTFC was higher (19.5 [12.0] vs. 16.4 [IQR7.0], p=0.07) and TMPG 2 or 3 was less frequent (56.3% vs. 86.4, p=0.09). Likewise, there was a clear trend towards higher values of IMR in patients with ED: median values 40.5 (IQR 54.4) vs. 22.0 (IQR 26.0), p=0.09. The prevalence of microvascular coronary damage, defined as an IMR >24 (median value) was almost 2 times higher in patients with ED (36.4% vs. 62.5%, p=0.11).

Contrast enhanced CMR showed a significant higher proportion of patients with MVO when ED was present (11.1% vs. 54.5%, p=0.03). The MVO mass was higher in the group with RHI<1.67 (median value 5.3 vs. 6.8), although the difference was not statistically significant.

## DISCUSSION

To our knowledge, this is the first study reporting the association of early (24 hours) measured peripheral endothelial dysfunction with microvascular coronary damage and with myocardial infarction extension in patients with STEMI treated with P-PCI.

The presence of ED in patients with ACS has been repeatedly demonstrated: Elbaz and colleagues evaluated invasively the endothelial function in non-culprit arteries in patients with NST-ACS using intracoronary infusion of acetylcholine and found that 81% of patients had a blunted response to this endothelium-dependent vasodilator.[38] Spiro and colleagues used flow-mediated dilation and low flow-mediated constriction to demonstrate that endothelial function is significantly more compromised in patients with non-ST elevation acute coronary syndromes (NST-ACS), as compared to patients with stable coronary artery disease, both before and after angioplasty.[39] More recently, Careri evaluated endothelial function in the first 12 hours both in stable and NST-ACS patients, and showed that flow-mediated dilation is significantly lower in acute patients.[18] Finally, we also demonstrated that patients with STEMI treated with P-PCI have a high prevalence of ED evaluated with PAT 3 to 5 days after the event.[19].

Importantly, peripheral endothelial function as been shown to relate closely with coronary microvascular dysfunction, with several different techniques[40–42], including PAT[43]. However, the exact role of peripheral ED (as a surrogate for coronary ED) in the early phase after P-PCI was never investigated before. Indeed, peripheral endothelial function testing is particularly challenging in acute patients, for several reasons: in addition to the unpredictable nature of the acute event onset, the unstable condition and the pain, anxiety and fear patients usually

experience, most of the times it is not possible to comply with several recommendations for endothelial function evaluation (including fasting state, morning exam, avoidance of caffeine or smoking, etc.).[44]. Additionally, patients were evaluated after an arterial puncture (for the urgent angiography) and several venous punctures (both for blood tests and for drugs administration). Finally, the time of the day was not the same for all measurements (since it depended on the hour of the primary angioplasty) and, accordingly, the patients could be or not on a fasting state.

Notwithstanding all these potential pitfalls, the RHI results we describe in this population are similar to the values reported by Schoenenberger and colleagues (1.82±0.48) in the only published study that also measured RHI 24 hours after P-PCI in STEMI patients.[21] Accordingly, the percentage of patients with an RHI<1.67 (42.1%) is very consistent with the results observed in all studies that reported this information in the acute/subacute phase of ACS: 47.9% in the study by Yamomoto *et al*[23], 43.1% in our pilot study[19] and 45.0% in the study by Schoenenberger *et al*.[21]

Patients with an RHI<1.67 had significantly higher values of both peak TnI and AUC of TnI, either total or indexed to the area-at-risk scores. Correspondingly, they had a 3 times higher risk of transmural necrosis and a clear trend to higher total and percent infarct masses in the ceCMR. In line with these results, they also had lower LVEF and higher (worse) WMSI, both in the initial echocardiogram and in the ceCMR.

Similarly, patients with an RHI<1.67 had a clear trend towards worse electrocardiographic (particularly residual ST elevation after P-PCI) and angiographic (IMR, cTFC and TMPG) indicators of microvascular reperfusion. Altogether, these data suggest a higher damage of coronary microvasculature in patients with peripheral ED, which is confirmed by the results of the ceCMR: patients with an RHI<1.67 had an almost 5 times higher probability of having MVO on the ceCMR, and tended to have higher obstruction masses.

The fact that patients with ED had a trend for more severe coronary artery disease (the prevalence of multivessel disease was almost two times higher in patients with ED and SYNTAX score also tended to be higher in these patients) is in accordance with our previous work[22] and suggests that at least the *previous* severity of coronary artery disease is related with ED. We also found a trend for lower initial TIMI flow in patients with ED, which his in agreement with a recent study by Kandhai-Ragunat and colleagues. These authors documented lower RHI values (although measured 4 to 6 weeks after the STEMI) in patients with an occluded artery before the P-PCI, as compared with patients with a patent artery (2.08±0.34 vs. 1.75±0.35; p<0.007),[24] suggesting that ED may be involved in microvascular reperfusion in STEMI patients.

However, we also found a significant negative correlation between both area-at-risk scores and RHI and there was a trend for lower RHI in patients that had the LAD as culprit artery. Both larger areas-at-risk and the LAD as culprit artery are associated with larger infarctions, implying that the documented lower RHI values may translate the extension of the infarction, as opposed to being part of the pathophysiological process that caused it. Indeed our finding that patients with ED in the first 24 hours after STEMI have larger infarcts and lower ejection fraction are in line with the evidence of neuro-humoral activation in patients with heart failure. In fact, it was already demonstrated that the reduction in stroke volume produces a lower endothelial shear stress, which causes a dysregulation in nitric oxide (NO) synthase isoforms gene expression[45,46], eventually leading to a reduced NO bioavailability.[47] Furthermore, there is an additional reduction

in NO bioavailability caused by direct NO destruction by reactive oxygen species, mainly driven by an increase in angiotensin II and aldosterone activity, and purine metabolism.[48]

While we cannot infer from our results if the ED was a *cause* or a *consequence* of the severity of the myocardial infarction, it is reasonable to conclude that peripheral ED in these patients may be a marker of the severity of the myocardial disease and, therefore, of its systemic consequences, which makes it useful to assess the effectiveness of therapy and predict events, independently of its exact pathophysiological role.

In fact, in patients with acute myocardial infarction, early treatment with statins, currently known to reduce events, was initially evaluated through its beneficial effect in the endothelial function.[49,50] Likewise, in heart failure patients, peripheral endothelial function has been described as tool to predict the response to cardiac resynchronization therapy, and ED improvement after CRT correlated with functional improvement[51], being related to an increase in cardiac output[52], likely mediated by shear-stress.[53]

In summary, albeit demonstrating the exact role of ED in STEMI patients is not possible at this point, we demonstrated that its measurement early after primary PCI is feasible and related both with the extent of infarct and MVO, which makes it a valuable tool both for identifying high risk patients and for testing new pharmacological or device-based strategies.

## STUDY LIMITATIONS

This study is limited by the small number of patients and larger studies will be necessary to confirm these results. Additionally, the EndoPAT exams were not performed according the recommendations for endothelial function evaluation: patients were not fasting, coffee or tea consumption was not excluded and the morning hour of the exam was not respected. However, measuring endothelial function at the acute phase was exactly the purpose of the study. Importantly, all the operators performing the EndoPAT evaluation were highly trained in this procedure, having participated in several previous studies.

## CONCLUSIONS

The presence of ED evaluated by PAT at 24 hours after P-PCI in patients with STEMI is related with larger infarcts, lower LVEF, higher WMSI and higher prevalence of MVO.

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# FIGURE LEGENDS

**Figure 1**. Study flow chart

**Figure 2**. Area under the curve of and peak values of troponin I (determined from 7 scheduled blood tests in the first 48 hours after P-PCI), according to the presence of endothelial dysfunction (RHI<1.67)

**Figure 3**. Left ventricular ejection fraction (mean ± standard deviation) and wall motion score index (median and interquartile range) evaluated by Echo, according to the presence of endothelial dysfunction defined as an RHI<1.67 ($^{*}$Independent T-test, $^{**}$Mann-Whitney test)

# TABLES

**Table 1**. Baseline characteristics according to the presence of endothelial dysfunction (RHI<1.67).

| Variable | Total Population (n=38) | Endothelial Dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| **Physical characteristics** | | | | |
| Age (years) [a] | 60.0±13.7 | 60.1±12.4 | 59.8±15.7 | 0.94 |
| Male gender [b] | 29 (76.3) | 18 (81.8) | 11 (68.8) | 0.58 [e] |
| BMI [a] | 27.2±4.0 | 27.4±4.5 | 27.1±3.3 | 0.82 |
| Waist circumference [a] | 100.0±11.5 | 101.1±12.6 | 98.5±9.9 | 0.55 |
| **Risk Factors and previous coronary disease** [b] | | | | |
| Hypertension | 26 (68.4) | 16 (72.7) | 10 (62.5) | 0.50 |
| Diabetes | 12 (31.6) | 8 (36.4) | 4 (25.0) | 0.46 |
| Dyslipidemia | 19 (50.0) | 11 (50.0) | 8 (50.0) | 1.00 |
| Active smoking | 13 (34.2) | 9 (40.9) | 4 (25.0) | 0.31 |
| Previous angina | 7 (18.4) | 6 (27.3) | 1 (6.3) | 0.22 [e] |
| Previous revascularization | 2 (5.3) | 1 (4.5) | 1 (6.3) | 0.61 [e] |
| **Previous medication** [b] | | | | |
| Aspirin / Clopidogrel | 3 (7.9) | 2 (9.1) | 1 (6.3) | 0.73 [e] |
| ACEi/ARBs | 13 (34.2) | 10 (45.5) | 3 (18.8) | 0.11 |
| Beta-blockers | 1 (2.6) | 1 (4.5) | 0 (0.0) | 0.85 [e] |
| Statins | 4 (10.5) | 2 (9.1) | 2 (12.5) | 0.89 [e] |
| **Admission laboratory values** | | | | |
| Creatinine (mg/dL) [c] | 0.88 (0.32) | 0.87 (0.54) | 0.90 (0.19) | 0.67 |
| NT-pro-BNP (pg/mL)[c] | 158.5 (305.0) | 154 (365) | 163 (250) | 0.95 |
| hs-CRP (mg(dL) [c] | 0.57 (0.71) | 0.29 (0.45) | 0.81 (0.98) | 0.06 |
| Glucose (mg/dL) [c] | 136.0 (63.0) | 131.0 (60.0) | 145.5 (81.8) | 0.51 |
| HbA1c (%)[c] | 5.8 (1.3) | 5.7 (1.4) | 5.9 (2.1) | 0.69 |
| **Ischemic times** | | | | |
| Pain-to-balloon time (min) [c] | 209 (173) | 209 (186) | 211 (167) | 0.94 |
| Door-to-balloon time (min) [c] | 75 (52) | 79 (46) | 57 (44) | 0.06 |
| **Angiographic characteristics** | | | | |
| Culprit artery [b] | | | | |
| Left anterior descending | 21 (53.3) | 11 (50.0) | 10 (62.5) | 0.89 [e] |
| Left circumflex | 7 (18.4) | 5 (22.7) | 2 (12.5) | |
| Right coronary artery | 10 (26.3) | 6 (27.3) | 4 (25.0) | |
| Multivessel disease [b] | 19 (50.0) | 8 (36.4) | 11 (68.8) | 0.10 [e] |
| SYNTAX score [c] | 17.8±6.2 | 16.8±6.2 | 19.0±6.0 | 0.29 |
| Initial TIMI flow 0-1 [b] | 31 (81.6) | 16 (72.7) | 15 (93.8) | 0.22 |
| **Area at risk scores** [c] | | | | |
| APPROACH score | 27.8 (3.0) | 28.1 (5.0) | 29.7 (10.0) | 0.08 |
| BARI score | 28.5 (6.0) | 26.7 (7.0) | 30.2 (10.0) | 0.07 |
| **Angioplasty variables** [b] | | | | |
| Mechanical aspiration | 16 (42.1) | 9 (40.9) | 7 (43.8) | 0.86 |
| Balloon pre-dilatation | 21 (55.3) | 13 (59.1) | 8 (50.0) | 0.58 |
| Stent implantation | 36 (94.7) | 20 (90.9) | 16 (100.0) | 0.61 |
| Balloon post-dilatation | 24 (63.2) | 14 (63.6) | 10 (62.5) | 0.94 |
| Abciximab treatment | 9 (23.7) | 4 (18.2) | 5 (31.5) | 0.58 |
| Mechanical aspiration | 16 (42.1) | 9 (40.9) | 7 (43.8) | 0.86 |

[a] Presented as mean±standard deviation; [b] Presented as number (%); [c] Presented as median (interquartile range); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yate's correction

**Table 2.** Troponin I release according to the presence of endothelial dysfunction (RHI<1.67)

| Variable [a, b] | Total Population (n=38) | Endothelial Dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No (n=22) | Yes (n=16) | |
| **Peak TnI values** | | | | |
| $TnI_{peak}$ | 95 (96) | 67 (81) | 118 (186) | 0.024 |
| $TnI_{peak}$ (APPROACH) | 25 (32) | 17 (20) | 34 (56) | 0.009 |
| $TnI_{peak}$ (BARI) | 24 (31) | 17 (22) | 33 (47) | 0.008 |
| $TnI_{peak}$ (2 scores) | 24 (31) | 17 (21) | 33 (55) | 0.009 |
| **Area under the curve of TnI** | | | | |
| $TnI_{AUC}$ | 1293 (1580) | 1076 (1042) | 2305 (2486) | 0.012 |
| $TnI_{AUC}$ (APPROACH) | 403 (522) | 315 (303) | 664 (1080) | 0.008 |
| $TnI_{AUC}$ (BARI) | 383 (448) | 314 (326) | 618 (799) | 0.007 |
| $TnI_{AUC}$ (2 scores) | 393 (482) | 314 (300) | 641 (984) | 0.007 |

[a] Presented as median (IQR); [b] Peak value and area under the curve (AUC) of 7 troponin I (TnI, in mg/dL) measurements performed in the first 48 hours after the primary angioplasty; total values and values indexed to the APPROACH, BARI or both are presented; [c] Mann-Whitney Test

**Table 3**. Echocardiographic results according to the presence of endothelial dysfunction (RHI<1.67)

| Variable | Total Population | Endothelial Dysfunction (RHI<1.67) | | *p* value [c] |
|---|---|---|---|---|
| | | No | Yes | |
| **2D measurements** | **(n=26)** | **(n=15)** | **(n=11)** | |
| LVTdV (ml) [a] | 109.8±23.4 | 106.5±30.2 | 113.8±11.4 | 0.48 |
| LVTsV (ml) [a] | 55.9±12.6 | 51.1±12.3 | 61.7±10.9 | 0.047 |
| LVEF (%)[a] | 48.6±7.1 | 51.4±4.7 | 45.3±8.3 | 0.045 |
| Wall motion score index [b] | 1.44 (0.41) | 1.35 (0.47) | 1.77 (0.47) | 0.006 |
| Left atria volume (ml/m$^2$) [a] | 36.1±11.0 | 36.7±12.1 | 35.3±10.0 | 0.78 |
| **Doppler measurements** | **(n=21)** | **(n=11)** | **(n=10)** | |
| E/A ratio [a] | 1.10±0.40 | 1.17±0.40 | 0.97±0.0.39 | 0.30 |
| E/e' ratio [a] | 8.91±3.30 | 8.83±3.91 | 9.05±2.18 | 0.89 |
| **2D speckle tracking imaging** | **(n=21)** | **(n=11)** | **(n=10)** | |
| Global longitudinal strain [a] | -13.16±2.35 | -14.32±1.72 | -11.89±2.35 | 0.014 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Independent t-test for continuous variables with a normal distribution. Mann-Whitney test for continuous variables with a non-normal distribution. LVEdV – left ventricular end diastolic volume; LVEsV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; WMSI – wall motion score index.

**Table 4**. ceCMR results according to the presence of endothelial dysfunction (RHI<1.67)

| Variable | Total Population (n=29) | Endothelial Dysfunction (RHI<1.67) | | *p* value [d] |
|---|---|---|---|---|
| | | No (n=18) | Yes (n=11) | |
| LVTdV (ml) [a] | 138.6±26.9 | 139.2±26.5 | 137.5±28.9 | 0.87 |
| LVTsV (ml) [a] | 63.5±21.0 | 59.3±19.4 | 70.4±22.6 | 0.17 |
| LVEF (%)[a] | 53.9±8.4 | 56.6±8.1 | 49.5±7.2 | 0.025 |
| Wall motion score index [a] | 1.37±0.33 | 1.28±0.31 | 1.53±0.32 | 0.05 |
| Edema mass [b] | 19.1 (19.0) | 17.2 (14.1) | 21.2 (28.6) | 0.28 |
| Transmural necrosis [c] | 12 (38.7) | 4 (22.2) | 7 (63.6) | 0.06 [e] |
| Infarct mass | | | | |
| Total [b] | 11.6 (9.3) | 10.1 (10.3) | 17.5 (15.4) | 0.08 |
| Percent [b] | 11.5 (13.7) | 10.2 (7.6) | 17.5 (21.8) | 0.10 |
| Indexed to APPROACH | 3.2 (7.0) | 2.7 (2.6) | 4.9 (11.5) | 0.10 |
| Indexed to BARI | 3.4 (5.8) | 2.3 (2.7) | 5.1 (11.5) | 0.09 |
| Salvage mass [b] | 5.0 (14.0) | 5.0 (8.8) | 4.7 (27.6) | 0.87 |
| Microvascular obstruction | | | | |
| MVO present [c] | 8 (27.6) | 2 (11.1) | 6 (54.5) | 0.03 [e] |
| MVO mass [b] | 6.1 (15.5) | 5.3 (-) | 6.8 (20.8) | 0.29 |

[a] Presented as mean±standard deviation; [b] Presented as median (interquartile range); [c] Presented as number (%); [d] Independent t-test for continuous variables with a normal distribution, Mann-Whitney test for continuous variables with a non-normal distribution, Chi-Square for categorical variables; [e] Yate's correction

## FIGURES

**Figure 1**

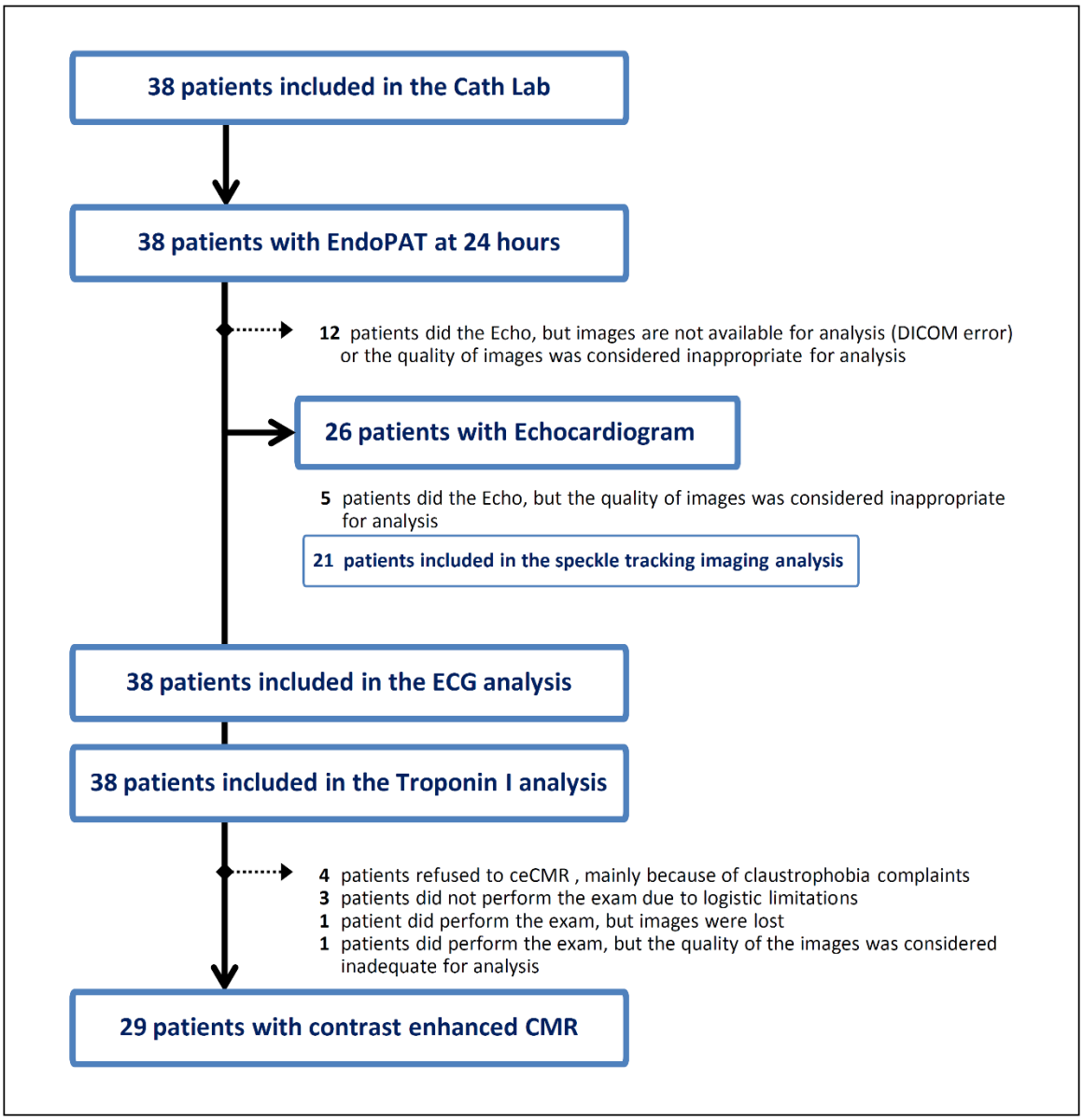


**Figure 2**

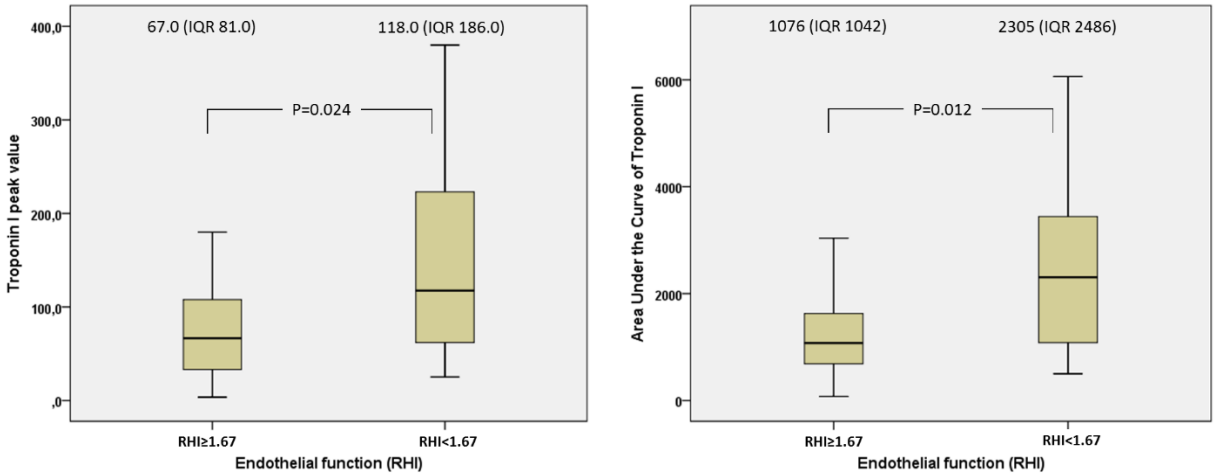
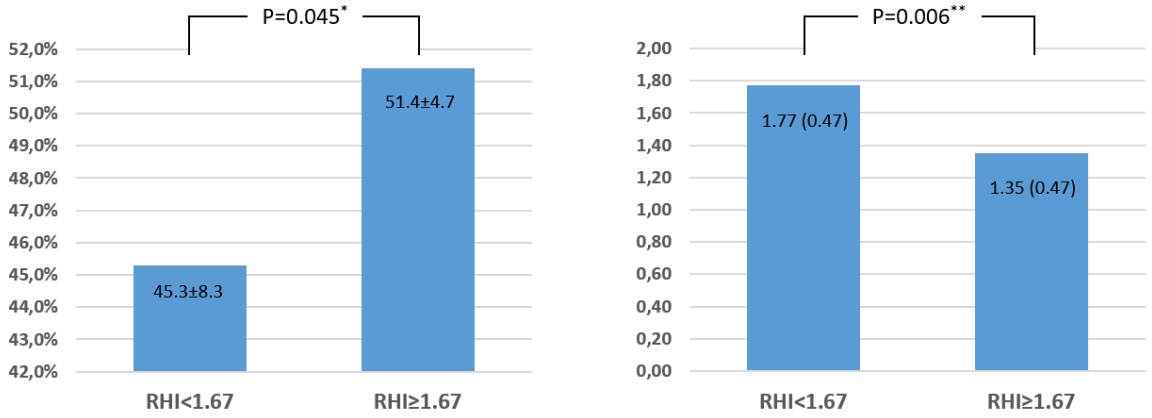



**Figure 3**




**Left ventricular ejection fraction**

**Wall motion score index**