Theragnostic Options for Microvascular Obstruction in STEMI

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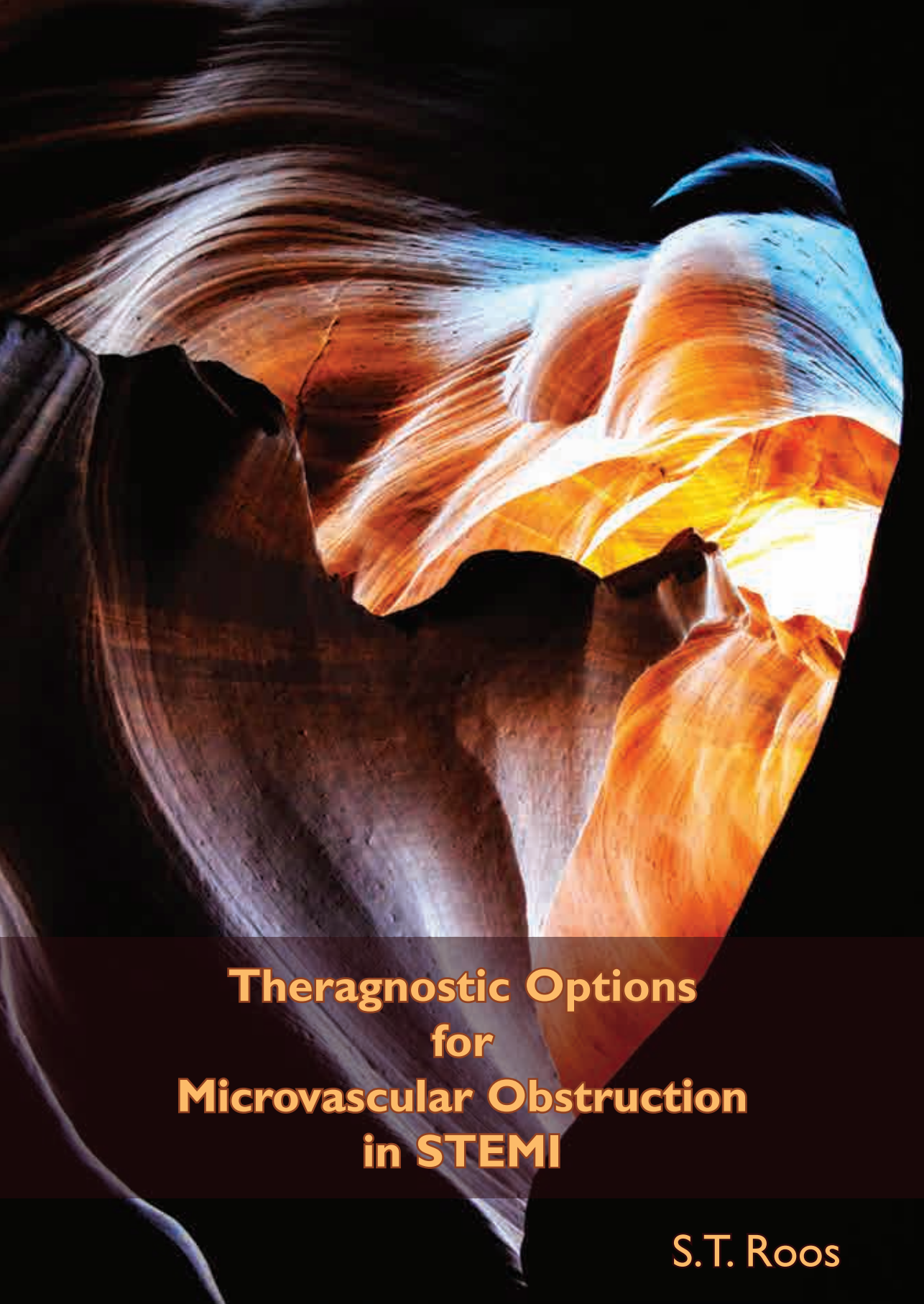

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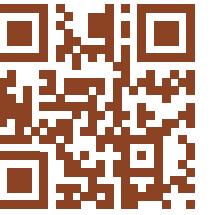

VRIJE UNIVERSITEIT

Theragnostic Options for Microvascular Obstruction in STEMI

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Sebastiaan Theo Roos

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VOOR PAPA, MAMA, STEPHANIE

VOOR ILANIT

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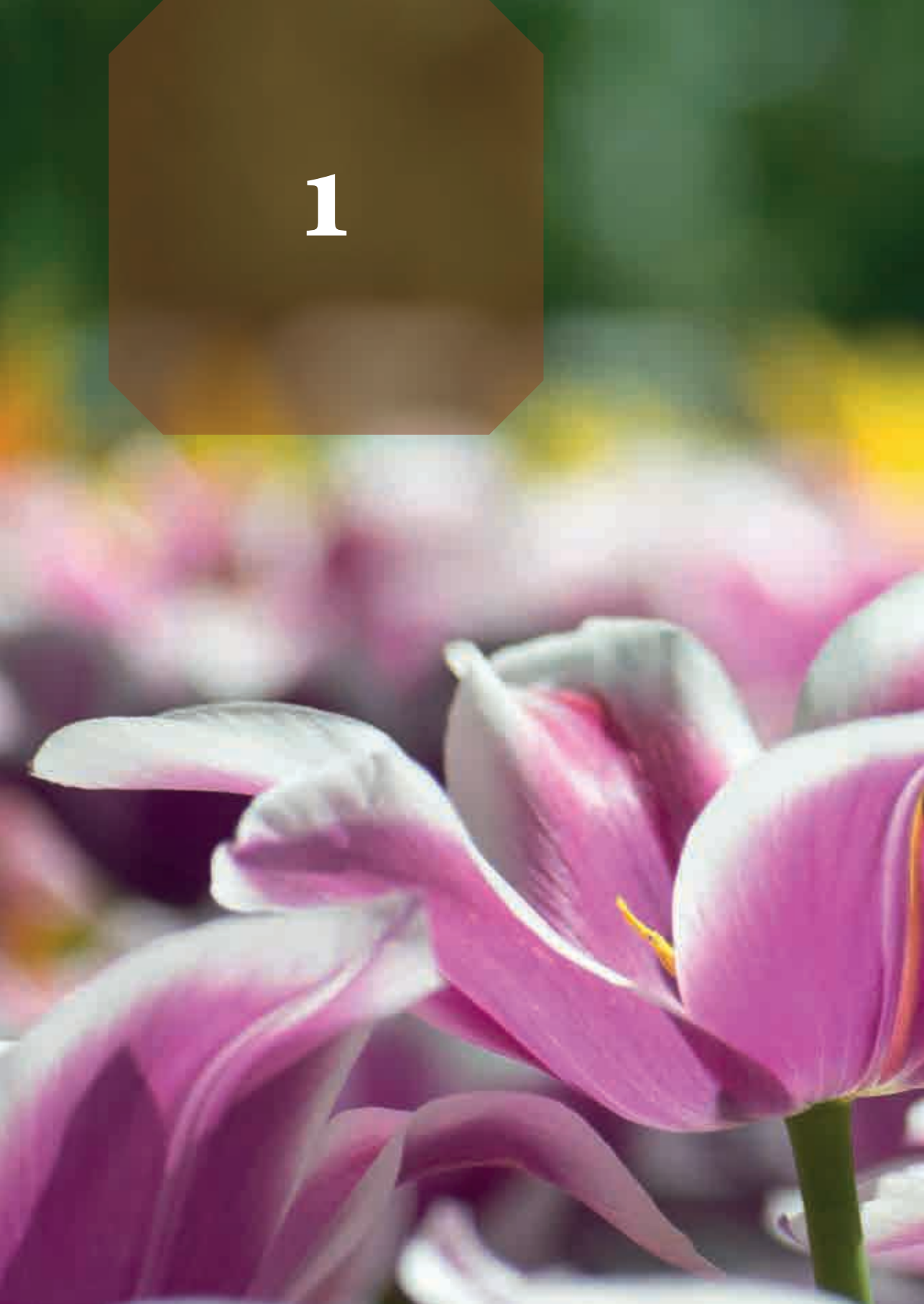

Chapter 1:
General Introduction

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1.1 Introduction

Acute ischemic arterial disease is an important cause of global mortality and morbidity. Risk factors such as obesity, diabetes mellitus, hypertension and smoking contribute to the development of arterial disease by the formation of atherosclerosis. While the pathophysiological pathways by which this occurs span a multitude of factors, the result is that over the course of decades atherosclerotic plaques are formed in the arterial vascular walls. The spontaneous rupture of such a plaque changes laminar flow to a more turbulent state. This causes the formation of a thrombus, because as we know from Virchow's triad, thrombosis occurs when there is a combination of stasis of blood flow, endothelial injury and a hypercoagulable state. The rupture of an atherosclerotic plaque therefore not only alters blood flow, it causes endothelial injury and due to local inflammation causes an increase of coagulability. Atherothrombosis, the formation of a local thrombus, now occurs, resulting in further flow restriction and possibly complete occlusion of the vessel.

This can occur anywhere in the arterial system, with varying severity of consequences. Acute occlusion of an artery can cause for example stroke or myocardial infarction, with possible life threatening consequences. Until the late seventies of the previous century, treatment with thrombus dissolving medication, called fibrinolytic agents, was the only possible treatment to decrease myocardial damage. However, efficacy was not optimal and patients experienced larger myocardial infarctions with higher complication and mortality rate.

Fortunately, advances in health care have sharply reduced the mortality and morbidity of acute cardiovascular events; especially early opening of an occluded artery through primary percutaneous coronary intervention (PCI), introduced in 1977 as a treatment option for ST segment elevation myocardial infarction (STEMI). This has greatly improved the clinical outcome of patients with STEMI. However, as the coronary artery in STEMI can be fully occluded for quite some time before primary PCI can be performed, myocardial tissue will still be damaged in a varying degree. This can, in extreme cases, cause (sub)acute complications of the myocardial infarction, but even in milder cases of less myocardial damage, over time the risk of developing heart failure is ever present. Also, while PCI is capable of re-opening the coronary artery in a large proportion of patients, achieving reperfusion, ad-

ditional tissue damage occurs because of reperfusion injury. This paradoxical phenomenon is called reperfusion injury and it is the current scientific hurdle to take to further improve cardiovascular outcomes in acute ischemic events.

1.2 Reperfusion injury

The occurrence of reperfusion injury is caused in part by the acute restoration of blood flow, delivering nutrients and oxygen to the ischemic myocardial area at risk. However, during myocardial ischemia, the local pH has steadily been decreasing due to the formation of lactic acid, as this is formed under anaerobic circumstances; intracellular hydrogen amounts are increasing at this time. The sudden restoration of pH causes a rapid influx of sodium and calcium into the cell as hydrogen is rapidly exchanged through the Na^+/H^+ exchanger. The increase of intracellular sodium causes an increase of function of the Na^+/Ca^{2+} exchanger, causing a calcium overload, leading to hypercontraction of the cell.

Furthermore, the sudden increase of reactive oxygen species due to the sudden re-oxygenation of the mitochondria, further causes cellular damage. Both calcium overload and the formation of reactive oxygen species causes opening of the mitochondrial permeability transition pore, which ultimately leads to adenosine-tri-phosphate depletion, cellular edema and rupture of cellular membranes leading to cell death or apoptosis.

Consequently, reperfusion injury will cause a strong local inflammatory response, due to release of cytokines, chemokines and reactive oxygen species. Clinically, this can cause arrhythmias, myocardial stunning and no-reflow, or also called microvascular obstruction.

1.3 Microvascular obstruction

Microvascular obstruction (no-reflow) is not only caused by edema of the capillary and small vessel wall and surrounding post-ischemic tissues, which effectively blocks the peripheral circulation, but also due to the disruption of the culprit thrombus due to PCI. As wire passage and balloon inflation occur, small portions of the fresh thrombus break off and block the distal coronary arteries. Unfortunately, while PCI is an

excellent technique for the proximal and distal large arteries, recanalization due to wire passage is currently simply not possible in the peripheral circulation, due to decreasing lumen diameters. Novel therapeutic options are therefore considered and researched, targeting this specific problem. One of these is called sonothrombolysis, a technique by which the mechanical forces created by ultrasound are strong enough to destroy small thrombi in the macro- and microvasculature, by a process called cavitation. If the mechanical index of an ultrasound beam, which is the peak negative pressure divided by the square root of the ultrasound frequency, increases, small bubbles form in the fluid, such as blood or saline, through which the ultrasound wave travels. These bubbles will start to oscillate at first, but at increasing mechanical indices, the bubble will burst violently, increasing local temperature and releasing destructive force on the surrounding tissue. This can be enhanced dramatically by the administration of ultrasound contrast agents, which are nothing more than lipid-shell gas-filled spheres, often called microbubbles. In this way, sonothrombolysis can be used to dissolve micro-thrombi forming during PCI, effectively treating microvascular obstruction.

1.4 Contents of this thesis

The main objective of this thesis is to review and investigate novel diagnostic and therapeutic (theragnostic) targets for reperfusion injury and microvascular obstruction after STEMI. For this purpose, the thesis has been divided in three parts. The first part is focusing on diagnostic features; which patients suffer from these phenomenon, do not respond to therapy and require additional treatment before heart failure occurs. The second and third part describe therapeutic targets for reperfusion injury and microvascular obstruction. The second part evaluates potential therapeutic options for reperfusion injury and the effectiveness of one of these agents, exenatide. In the third part, research and treatment of microvascular obstruction using sonothrombolysis is described.

Part 1. Diagnostic targets: angiographic flow, strain imaging and clinical outcome

In chapter 2, a novel measurement technique applied on a coronary angiographic image is investigated which seeks to determine which patient is at increased risk of post-STEMI death, achieved through a flow speeds calculation in the culprit artery on the post-PCI coronary angiogram.

Then, in chapter 3, 3-dimensional ultrasound imaging is performed in STEMI patients in order to predict the long term follow-up effects of myocardial damage. We investigated whether measurements of the myocardial strain at baseline, can predict the occurrence of adverse and reverse remodeling, on top of clinical, biochemical and volumetric data.

Part 2. Therapeutic targets: reperfusion injury

Chapter 4 will provide an update on the currently available knowledge regarding novel pharmacological agents that might provide part of the solution to reperfusion injury. A literature review is performed and a final assessment on the most promising candidates is provided.

The effectiveness of one of these agents, a glucagon-like-peptide-1 (GLP-1) receptor agonist known as exenatide, is researched in chapter 5, the EXAMI study. This study included patients with STEMI who were treated with either placebo or exenatide and PCI and assessed myocardial infarct size using magnetic resonance imaging at baseline and follow-up.

Part 3. Therapeutic targets: microvascular obstruction

Chapter 6, provides a literature review with information on the current knowledge of human trials that applied sonothrombolysis and theragnostic imaging, in both the setting of acute neuro- and cardiovascular disorders.

An in vitro experiment is then performed in chapter 7, in which the required ultrasound 'dose' is investigated in combination with currently used pharmacological agents including aspirin, heparin and tissue plasminogen activator.

Finally, these results are then applied in chapter 8, which describes a human safety and feasibility study in STEMI patients. This study was designed to use sonothrombolysis before and immediately after primary PCI in STEMI patients in order to reduce microvascular obstruction.

Part 1:
Diagnostic targets: angiographic flow, strain imaging and clinical outcome


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Chapter 2:
Fluoroscopy Assisted Scoring of Myocardial Hypoperfusion (FLASH) ratio as a novel predictor of mortality after primary PCI in STEMI patients

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Abstract

Introduction

The aim of this study was to investigate whether FLuoroscopy Assisted Scoring of myocardial Hypoperfusion (FLASH) enabled a more accurate assessment of coronary blood flow and prediction of cardiac mortality after primary PCI (pPCI), than the presently used angiographic scores of reperfusion.

Methods

We included 453 STEMI patients who received pPCI at our hospital. Using the novel FLASH algorithm, based on contrast passage time and Quantitative Coronary Analysis, FLASH flow was measured after pPCI and was used to calculate FLASH ratio of culprit and reference artery. In 28 of the 453 patients, FLASH flow was compared to Doppler-derived-flow.

Results

FLASH flow had a good correlation with Doppler derived flow (Pearson's R=0.65, $p<0.001$) and had a high inter-observer agreement (ICC = 0.83). FLASH flow was significantly lower in patients that died of cardiac death within six months (25.9±17.7 ml/min vs. 38.2±18.8 ml/min, p=0.004). FLASH ratio had a high accuracy of predicting cardiac mortality with a significant higher area under the curve as compared with CTFC and QuBe (p=0.041 and p=0.008) FLASH ratio was an independent predictor of mortality at 6 months (HR=0.98 per 1% increase, p=0.014).

Conclusion

FLASH is a simple non-invasive method to estimate coronary blood flow and predict mortality directly following pPCI in STEMI patients, with a higher accuracy compared to presently used angiographic scores.

2.1 Introduction

Impaired epicardial coronary flow is an important complication after primary percutaneous coronary intervention (pPCI). [1,2] Angiographically impaired coronary flow, traditionally called "no-reflow", is linked to increased mortality [3–7] and is seen in approximately 5-15% of cases. [8–11] Epicardial coronary flow can be assessed by TIMI flow grade (TFG) [12] and corrected TIMI frame count (CTFC). [13] Although several studies [3–7] demonstrated the value of TFG for predicting mortality, this method has several important limitations. TFG provides a categorical instead of a continuous value therefore having less discriminating value when used as measurement of reperfusion. [14] In addition, TFG has high inter-observer variability and poor inter-observer agreement in grading TIMI 2. [13]

In contrast, CTFC provides a quantitative index to assess coronary flow by counting the number of frames required for contrast to reach a standardized distal landmark. Advantages of CTFC are its high reproducibility, low intra- and inter-observer errors and it furthermore enables a quantitative estimation of flow. [14–16] Although Gibson et al. [17,18] showed a relatively high predictive value of CTFC for mortality, this could not be demonstrated in other studies. [19,20] CTFC requires more dedicated cineangiographic filming because the standardized distal landmarks need to be visualized before contrast arrival and filming must be performed in specific projection angles. Moreover, although correction was made based on differences in coronary vessel length in the total study population, CTFC does not take the individual variances of coronary length into account. The same is true for the diameter of the coronary artery, even though luminal diameter is a critical determinant of flow resistance. [21] Besides epicardial flow, Vogelzang et al. [22] described a novel assessment of myocardial perfusion through computer-assisted myocardial blush quantification by quantitative blush evaluator (QuBE). Although QuBE was an independent predictor of mortality in the work by Vogelzang et al., these results have yet to be investigated by other groups. Furthermore, approximately 20% of angiograms could not be assessed using QuBE due to overlapping vessels or panning movements. [22,23]

The present study describes a new non-invasive method to assess coronary blood flow using a combination of contrast passage time and quantitative coronary analysis (QCA) and investigates its predictive value for mortality.

2.2 Methods

Patient population

Five hundred and eighty-three consecutive patients presenting to the VU University Medical Center in Amsterdam with STEMI treated with primary PCI within 12 hours were retrospectively assessed for eligibility between January 2011 and December 2012. Patients with triple vessel disease (3VD) or previous coronary artery bypass graft (CABG) were excluded. Other exclusion criteria were significant stenosis in the reference artery, TIMI 0 after procedure or insufficient image quality. In addition, as a control group, we included 38 patients who underwent elective coronary angiography because of stable coronary artery disease. The local medical ethics committee (VU University Medical Center, Amsterdam) approved data collection and management.

FLuoroscopy Assisted Scoring of myocardial Hypoperfusion (FLASH)

Angiograms were stored and analyzed using Xcelera (Philips Medical Systems, The Netherlands) with QCA software (CAAS II, PIE Medical, Maastricht, the Netherlands). Assessment of mean surface area and length of the coronary artery was performed offline using geometric analysis. Methodological principles of QCA have been previously described. [24,25] The measurements were calibrated based on the known width of the catheter in a frame where the tip of the catheter was filled with contrast. The coronary arteries were measured in a single frame that included the coronary ostium and the, by the observer selected, distal point. FLASH allows the distal point to be at any point along the coronary artery distally to the stent, but preferably had to be as distal as possible with the ostium still visible in the same frame. Measurements started at the ostium and continued up to the distal point in the same frame. Similar to CTFC, the frame in which the dye had fully entered the artery was selected as starting frame. [13] The number of frames required to reach the most distally visible point was counted.

Next, a central line was drawn along the coronary artery of interest after which the software applied automatic contour detection of the artery. Poor edge detection of certain areas was manually corrected by comparing the recording with other CAG angles. Both the

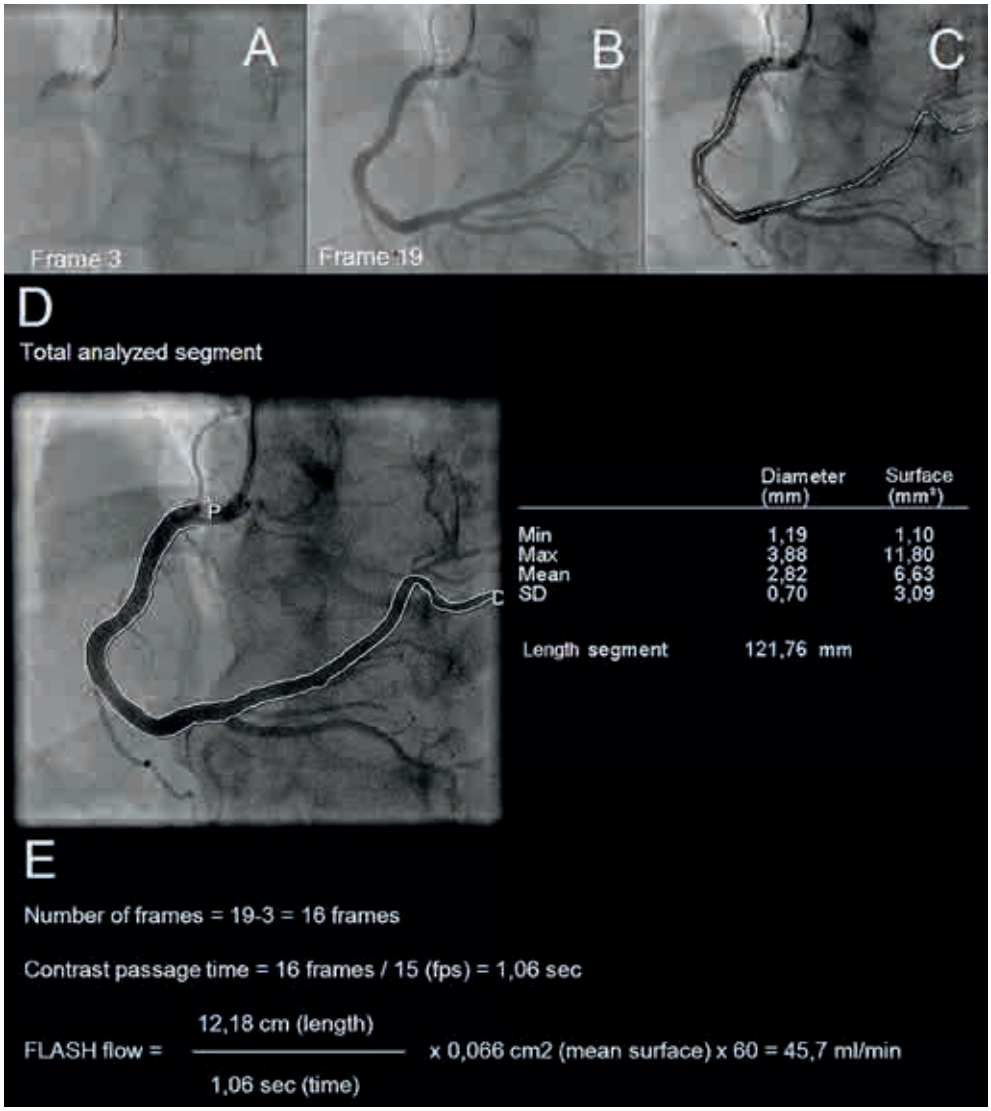

Figure 1: Fluoroscopy Assisted Scoring of Myocardial Hypoperfusion (FLASH) algorithm. A) Selection of the first frame where the contrast has filled the ostium of the vessel. B) Selection of the second frame where the dye has reached the self-appointed distal end point. C) A central line was then drawn along the coronary artery. D) QCA software determines the vessel length and vessel cross sectional area after automatic edge detection. E) Values were then entered into the formula in order to calculate FLASH flow in milliliters per minute.

infarct related artery and a reference artery were measured using this method (Figure 1). Moreover, FLASH flow was measured in 85 unobstructed coronary arteries of the 38 control patients.

FLASH flow, an estimate of coronary blood volume flow in milliliter per minute, was calculated using the passage time, vessel length and mean cross sectional area. FLASH ratio was expressed as the relative difference of FLASH flow in the infarct related artery (IRA) compared to that in the reference artery. FLASH ratio will have a negative value if the FLASH flow in the IRA after pPCI is decreased compared to the reference artery. FLASH ratio was corrected for the difference in average flow in the reference artery of STEMI patients found in our cohort. (Figure 2)

The reference FLASH flow was therefore multiplied by 1.15 when LAD was used and by 1.34 when LCx was used as a reference. Parameters can be calculated using the following formulas:

- Contrast passage time (sec) = Counted frames / Frame rate (fps)
- FLASH flow (ml/min) = [(Distance (cm) / Time (sec)) * mean cross sectional area (cm^2)] * 60
- FLASH ratio (%) = [(FLASH flow IRA - FLASH flow reference)/ FLASH flow reference] * 100%

Angiographic parameters of reperfusion

TIMI flow grade (TFG) was scored by the operator directly following pPCI and entered into our database. TFG scores were retrospectively extracted for use in the present study. [12] CTFC was assessed offline in all 583 patients by a single blinded observer as previously described (SB). [13] Computer-assisted myocardial blush quantification was also assessed offline in all patients and in a blinded fashion using the 'Quantitative Blush Evaluator' (QuBE) computer program. [22] In short, on each angiogram, an independent observer (MH) indicated a polygonal shape that contained the distal infarct-related area, after which the computer program determined the quantitative blush evaluator values.

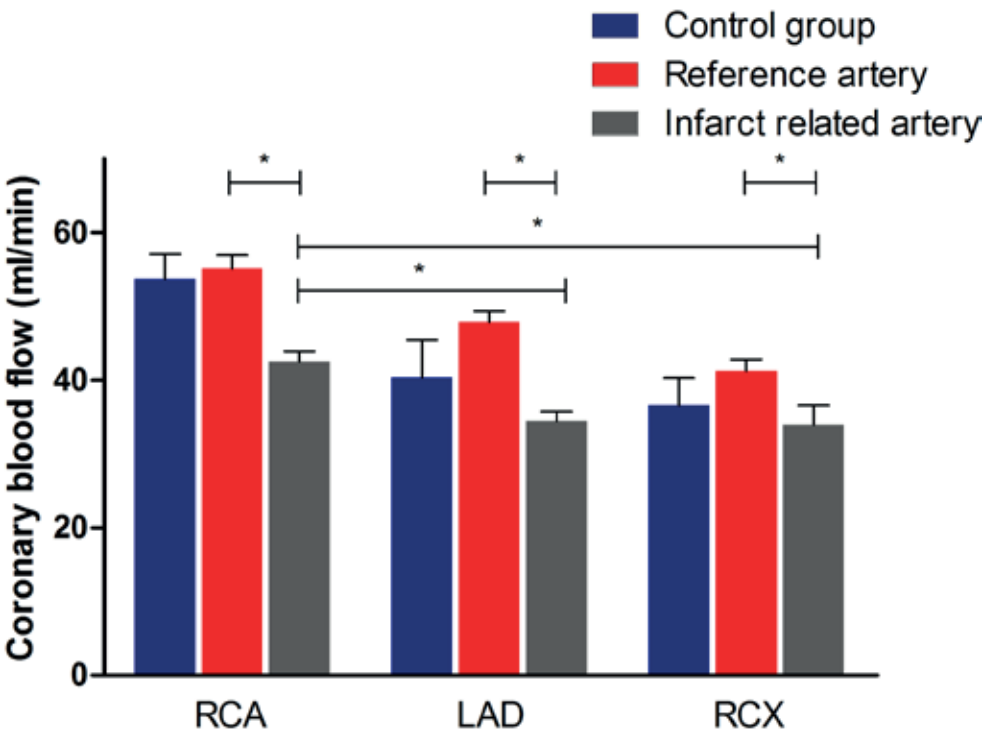

Figure 2: FLASH flow in coronary arteries of STEMI patients. FLASH flow in the infarct related artery was significantly lower than in the reference artery. The FLASH flow in the reference arteries was significantly higher in the RCA than in the LAD and RCX.

Doppler flow velocity

Doppler flow velocity data were available in 49 patients, which had been included in the PREDICT-MVO study [26], out of the 453 patients in our cohort. In brief, the PREDICT-MVO study aimed to investigate hyperemic microvascular resistance as predictor for the occurrence of cardiac magnetic resonance imaging defined microvascular obstruction. 28 of the 49 were included for the final analysis after stringent quality selection of the baseline Doppler flow velocity tracings to ensure an adequate representation of true baseline coronary blood flow. The PREDICT-MVO study conformed to the Declaration of Helsinki by the World Medical Association and each patient gave informed consent.

In the PREDICT-MVO study, immediately following standard pharmacological treatment according to ESC clinical guidelines and angiographically successful primary PCI, a 0.014-inch wire equipped with both a pressure and Doppler flow velocity sensor (Combo-

wire XT, Volcano Corp, San Diego, CA, USA) was placed in the culprit artery just distally to the stent. [27] Subsequently, instantaneous pressure and Doppler flow velocity measurements were obtained under resting conditions. In the present study, we used the baseline Doppler flow velocity, which was averaged over at least 5 consecutive heartbeats for comparison to FLASH flow and CTFC. True resting conditions were ensured by avoiding preceding intracoronary saline or contrast injections. Analysis and quality selection of the Doppler flow velocity tracings were performed off-line by a single observer (MH) blinded to the FLASH and CTFC results using custom software (written in Delphi v. 2010; Embarcadero, San Francisco, CA, USA).

Doppler derived blood flow was calculated by multiplying the intracoronary Doppler velocity (cm/min), measured just distally to the stent, by the cross-sectional area of the inflated stent (cm^2). The cross-sectional area of the stent was derived from the pressure compliance table supplied by the manufacturer.

Follow-up

Survival status at 6 months was determined using the Municipal Personal Records Database. Cause of death was determined using medical records or by contacting other hospitals if patient was transferred. The general practitioner was contacted when patient died after discharge from the hospital to determine the cause of death.

Statistical analysis

Continuous variables are presented as mean (range), mean ± S.D. or percentages and FLASH ratio is presented in tertiles. Variables were compared using Student's t test or ANOVA. Peak levels of CK, CK-MB and troponin were log transformed to obtain a normal distribution. Mann-Whitney U test was used with nonparametric data. Dichotomous variables were compared using chi-square statistics. Absolute inter-observer agreement was analyzed using Intraclass Correlation Coefficient (ICC). Outliers were identified using the ROUT method in GraphPad Prism (GraphPad Software 5, La Jolla, CA, USA) and were excluded from statistical analysis. Receiver operating characteristics were calculated for angiographic parameters. Comparison of ROC curves for each angiographic parameter was performed using Hanley & McNeil methodology in MedCalc (MedCalc Software 12.7.1, Ostend, Belgium). Univariate predictors of mortality were determined using Cox proportional hazard regression. Univariate predictors of mortality with $p<0.01$ were included in a multivariable

forward regression model. Kaplan Meier curves were plotted for survival between groups with different FLASH. Furthermore, FLASH groups were plotted for survival after stratification by IRA. Subsequently, Log-rank test was used to test the difference between these groups. Values of p less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS statistics (IBM SPSS Statistics 20, Chicago, IL, USA) and GraphPad Prism (GraphPad Software 5, La Jolla, CA, USA).

2.3 Results

Four hundred and fifty-three STEMI patients were included in the present study. Clinical demographics and procedural characteristics are shown in Table 1. Figure 3 shows the flow diagram of all STEMI patients. Of all angiograms 99% (453/459) was analyzable for FLASH, 69% (311/453) for CTFC and 79% (356/453) for QuBE. Survival status could not be obtained in 18 (4%) patients who did not reside in the Netherlands. At 6 months all-cause mortality was 6% (25/435) and cardiac mortality was 5% (20/435). FLASH ratio, enzymatic myocardial infarct size and cardiac mortality were similar in patients with either analyzable or non-analyzable angiograms for CTFC or QuBE. In patients with non-analyzable angiograms, however, the infarct related artery was significantly more frequent the LAD. (Tables 2 & 3)

Validation of FLASH as a method to assess coronary blood flow

Doppler derived blood flow measured in the culprit vessel was significantly correlated with FLASH-flow (Pearson's $R=0.65$, $p<0.001$). A weak relationship, though not significant, existed between CTFC and Doppler derived blood flow (Pearson's $R=-0.47$, $p<0.055$) as shown in figure 4. Angiograms were evaluated by two independent observers (PB and SR) in a randomly selected sample of patients. The inter-observer variability of FLASH flow was 15.1±13.9 ml/min with an intraclass correlation coefficient of 0.83 (figure 4C). The inter-observer variability of CTFC was 3.6±4.4 frames with an intraclass correlation coefficient of 0.86.

FLASH flow in coronary arteries of STEMI patients

The FLASH flow was significantly lower in the IRA (37.8 ± 18.7 ml/sec) n comparison to the reference artery (48.9 ± 21.9 ml/sec, $p<0.001$). (Figure 2)

The FLASH flow in the reference artery was significantly higher in the RCA (55.1±24.7 ml/min) compared to the LAD (47.8±19.6 ml/min, $p=0.003$) and LCx (41.2±1.6 ml/min, $p<0.001$).

Table 1: Clinical, procedural and angiographic characteristics. BMI = Body Mass Index; CK = creatine kinase; TnT = Troponin; CK-MB = creatine kinase-MB fraction

	1st FLASH ratio tertile	2nd FLASH ratio tertile	3rd FLASH ratio tertile	P-value
n	151	151	151	
FLASH value (mean, range), %	-65 (-92 to -48)	-33 (-48 to -15)	39 (-14 to 370)	
Age, y	67 (31-94)	62 (36-97)	63 (31-95)	0.002
BMI, kg/m2	26 (19-40)	26 (19-43)	27(18-45)	0.750
Male sex, %	67 (101/151)	64 (97/151)	66 (99/151)	0.889
Heart rate, bpm	72 (32-124)	75 (27-202)	75 (33-139)	0.580
Systolic blood pressure, mm Hg	121 (56-205)	121 (62-193)	120 (57-186)	0.939
Diastolic blood pressure, mm Hg	71 (17-116)	71 (15-103)	71 (28-110)	0.982
Ischaemic time, min	210 (51-1416)	225 (58-1620)	177 (57-1405)	0.281
Risk factors, %				
Current smoker	32 (43/134)	50 (67/134)	45 (58/128)	0.001
Hypertension	31 (41/133)	36 (49/135)	31 (38/124)	0.537
Hypercholesterolemia	15 (17/112)	30 (34/114)	19 (21/109)	0.022
Diabetes Mellitus	14 (20/145)	9 (13/141)	11 (15/139)	0.462
Positive family history	34 (43/127)	45 (58/130)	36 (43/118)	0.181
Cardiogenic shock on arrival, %	11 (16/151)	7 (10/151)	5 (8/151)	0.191
Resuscitation on arrival, %	10 (15/151)	6 (9/151)	5 (7/151)	0.165
Multivessel disease, %	41 (62/151)	42 (63/151)	47 (71/151)	0.519
Culprit vessel, %				0.125
RCA	34 (51/151)	40 (60/151)	50 (75/151)	

	1st FLASH ratio tertile	2nd FLASH ratio tertile	3rd FLASH ratio tertile	P-value
LAD	46 (69/151)	42 (64/151)	33 (49/151)	
RCx	9 (14/151)	10 (15/151)	11 (16/151)	
Other	11 (17/151)	8 (12/151)	7 (11/151)	
Preprocedural TIMI flow grade, %				0.036
0/1	63 (91/145)	64 (91/150)	58 (87/150)	
2	16 (23/145)	7 (11/150)	11 (17/150)	
3	21 (31/145)	31 (47/150)	31 (46/150)	
Postprocedural				
TIMI ≤ 2, %	20 (30/151)	5 (8/151)	2 (3/151)	<0.001
QuBE value	15.5 (5.0-47.9)	17.7 (6.5-38.9)	20.8 (6.2-42.0)	<0.001
CTFC	34 (4-124)	23 (6-82)	18 (6-42)	<0.001
FLASH flow, ml/min	23 (4-59)	39 (15-104)	52 (20-106)	<0.001
Laboratorium				
Peak CK	1593 (68-17378)	1206 (66-8913)	1447 (182-89125)	0.467
Peak TnT	3.49 (0.02-141.00)	1.47 (0.06-19.50)	2.04 (0.08-21.89)	0.010
Peak CK-MB	139 (2-1000)	95 (8-575)	122 (6-977)	0.263
Cardiac death*, %	11 (17/149)	1 (1/145)	1 (2/141)	<0.001

Table 2: Difference in clinical demographics and procedural characteristics between patients with analyzable CTFC and unanalyzable CTFC. BMI = Body Mass Index; CK = creatine kinase; TnT = Troponin; CK-MB = creatine kinase-MB fraction

	CTFC analyzable		P- value
	Yes	No	
Age, y	64 (31-95)	62 (36-97)	0.039
BMI, kg/m2	27 (18-43)	26 (19-45)	0.470
Male sex, %	64 (200/311)	68 (97/142)	0.406
Heart rate, bpm	73 (32-202)	75 (27-139)	0.617
Systolic blood pressure, mm Hg	121 (56-205)	121 (62-182)	0.939
Diastolic blood pressure, mm Hg	70 (17-110)	73 (15-116)	0.026
Ischaemic time, min	201 (51-1620)	213 (53-1416)	0.647
Cardiogenic shock on arrival, %	8 (25/311)	6 (9/142)	0.524
Resuscitation on arrival, %	8 (24/311)	5 (7/142)	0.276
Multivessel disease, %	42 (130/311)	47 (66/142)	0.351
Culprit vessel, %			<0.001
RCA	57 (177/311)	6 (9/142)	
LAD	27 (83/311)	70 (99/142)	
LCx	10 (32/311)	9 (13/142)	
Other	6 (19/311)	15 (21/142)	
Preprocedural TIMI flow grade, %			0.022
0/1	56 (172/305)	70 (98/140)	
2	14 (42/305)	6 (9/140)	
3	30 (91/305)	24 (33/140)	
Postprocedural			
TIMI ≤ 2, %	11 (34/311)	5 (7/142)	0.039
QuBE value	18.4 (5.2-47.9)	17.0 (5.0-41.8)	0.125
CTFC, n	NA	NA	NA
FLASH flow, ml/min	37 (4-106)	39 (6-92)	0.285
FLASH ratio, %	-21 (-92 to 370)	-18 (-84 to 201)	0.320
Laboratorium			
Peak CK	110	145	0.215
Peak TnT	2.03	3.19	0.095
Peak CK-MB	1328	1712	0.203
Cardiac death*, %	4 (13/301)	5 (7/134)	0.677

Table 3: Difference in clinical demographics and procedural characteristics between patients with analyzable QuBE and unanalyzable QuBE. BMI = Body Mass Index; CK = creatine kinase; TnT = Troponin; CK-MB = creatine kinase-MB fraction

	QuBE analyzable		P-value
	Yes	No	
Age, y	64 (36-97)	63 (31-91)	0.498
BMI, kg/m2	26 (18-43)	28 (21-45)	0.003
Male sex, %	65 (230/356)	69 (67/97)	0.412
Heart rate, bpm	73 (27-202)	77 (33-157)	0.214
Systolic blood pressure, mm Hg	121 (56-203)	118 (62-205)	0.401
Diastolic blood pressure, mm Hg	71 (17-116)	70 (15-103)	0.706
Ischaemic time, min	218 (51-1620)	141 (56-381)	0.019
Cardiogenic shock on arrival, %	9 (31/356)	3 (3/97)	0.063
Resuscitation on arrival, %	8 (28/356)	3 (3/97)	0.099
Multivessel disease, %	46 (163/356)	34 (33/97)	0.038
Culprit vessel, %			0.041
RCA	42 (150/356)	37 (36/97)	
LAD	37 (132/356)	52 (50/97)	
LCx	11 (40/356)	5 (5/97)	
Other	10 (34/356)	6 (6/97)	
Preprocedural TIMI flow grade, %			0.310
0/1	59 (206/352)	69 (64/93)	
2	12 (43/352)	9 (8/93)	
3	29 (103/352)	23 (21/93)	
Postprocedural			
TIMI ≤ 2, %	9 (33/356)	8 (8/97)	0.756
QuBE value	NA	NA	NA
CTFC, n	24 (4-82)	30 (6-124)	0.011
FLASH flow, ml/min	37 (4-104)	41 (7-106)	0.040
FLASH ratio, %	-22 (-92 to 370)	-11 (-86 to 267)	0.568
Laboratorium			
Peak CK	1417 (66-89410)	1441 (142-8275)	0.933
Peak TnT	2.20 (0.02-85.90)	2.47 (0.08-140.0)	0.656
Peak CK-MB	124 (2-1000)	110 (6-743)	0.563
Cardiac death*, %	5 (17/340)	3 (3/95)	0.449

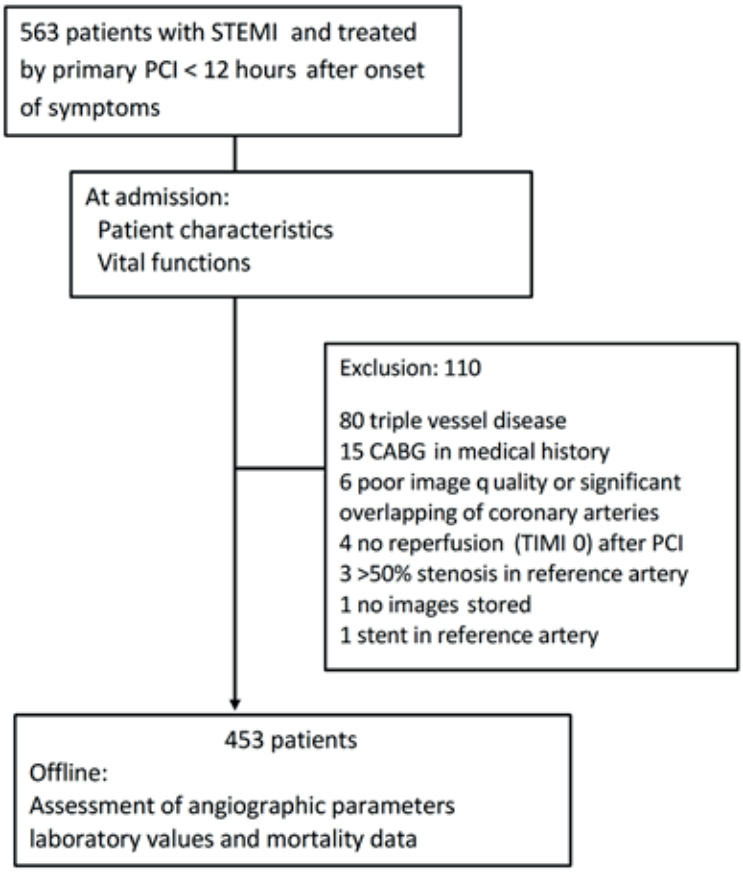

Figure 3: Study flow diagram

FLASH flow was significantly higher in unobstructed coronary arteries of STEMI patients than control patients (49.11±1.03 ml/min vs. 43.74±2.50 ml/min, p=0.04). Heart rate, systolic blood pressure and rate-pressure-product during coronary angiography had no significant association with FLASH flow (r=-0.003, p=0.958; r=0.083, p=0.129 and r=0.065, p=0.241 respectively) or FLASH ratio (r=-0.021, p=0.409; r=-0.044, p=0.697 and r=-0.05 p=0.360 respectively).

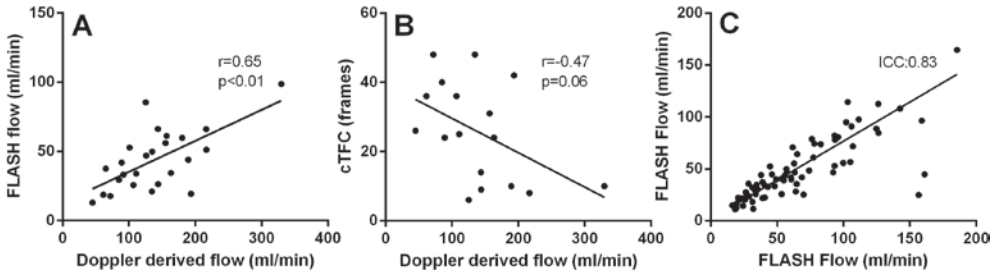

Figure 4: Validation of FLASH algorithm. A) Correlation between FLASH flow and Doppler flow velocity B) correlation between CTFC and Doppler flow velocity C) FLASH flow by two independent observers.

Relationship between angiographic parameters and outcome

FLASH ratio was significantly more disturbed in patients that died within six months after pPCI (-57%±26 vs. -20%±55, p<0.001). FLASH flow of the IRA was also significantly lower in this cohort of patients (25.9±17.7 ml/sec vs. 38.2±18.8 ml/sec, p=0.004). There was a trend to a higher CTFC (33.3±20.8 vs. 24.8±16.2, p=0.07) in patients who died within six months but QuBE values did not differ significantly between groups (15.8±5.5 vs. 18.0±7.9, p=0.25). Furthermore, patients with FLASH ratio values in the first tertile had significant larger enzymatic myocardial infarct size based on plasma levels of MB creatine kinase (MB-CK) and Troponin T as shown in Table 1.

FLASH as a predictor of cardiac mortality at 6 months

The predictive accuracies of FLASH ratio, TFG, CTFC and QuBE for 6 month cardiac mortality are shown in Figure 5. FLASH (AUC: 0.75) had a significantly higher accuracy for the prediction of 6 month cardiac mortality than either CTFC (AUC: 0.57) and QuBE (AUC: 0.51) (p=0.041, p=0.008 respectively) but was not significantly higher than TFG (AUC: 0.64, p=0.314).

Moreover, the predictive accuracy of FLASH ratio was higher than that of FLASH flow in the infarcted related artery (AUC: 0.69). (Figure 6)

The optimal cut-off value for FLASH was determined at -49%, which yields a sensitivity and specificity of 85% and 69% respectively. 32% (146/453) of the patients had a FLASH ratio below this cut-off value. Kaplan Meier curves were plotted for survival and showed a significant difference of survival between these two groups (log-rank p<0.001). (Figure 7).

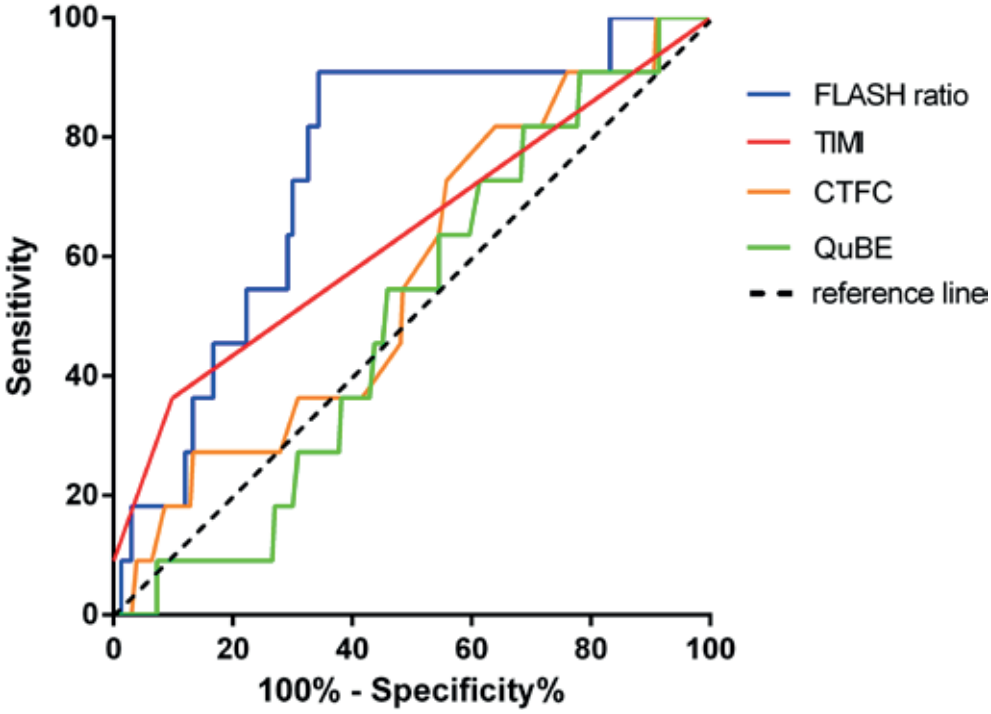

Figure 5: Receiver Operating Characteristic Curves of angiographic parameters. Blue line indicates FLASH (AUC: 0.75), red line indicates TFG (AUC: 0.64), orange line indicates CTFC (AUC: 0.57) and green line indicates QuBE (AUC: 0.51). FLASH had a significant higher AUC than CTFC and QuBE (p=0.041 and p=0.008 respectively).

This difference in survival between FLASH groups remained true after stratification by IRA, but only reached statistical significance in the LAD group (Logrank: p<0.001). (Figure 7)

FLASH, age, heart rate, IRA and the blood values; glucose, creatinin, leukocytes and cholesterol were univariate predictors of mortality. (Table 4)

FLASH ratio remained an independent predictor of cardiac mortality after correction using multivariate analysis, as well as heart rate, age and creatinine plasma levels. (Table 4)

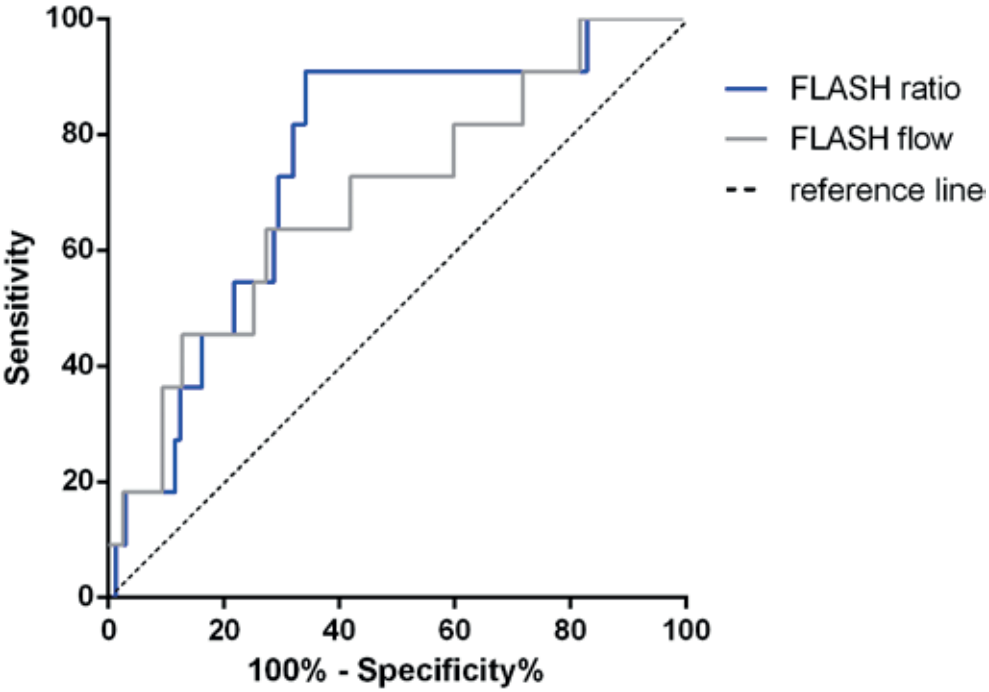

Figure 6: Receiver Operating Characteristic Curves of FLASH ratio and FLASH flow. Blue line indicates FLASH ratio (AUC: 0.75), while grey line indicates Flash flow (AUC: 0.69).

2.4 Discussion

The main findings of our study are that FLASH flow is better correlated with coronary blood flow than currently used angiographic scores, and that FLASH ratio is an independent predictor of 6 month cardiac mortality in STEMI patients without 3VD who received pPCI. FLASH ratio had a significantly higher accuracy for the prediction of cardiac mortality compared to CTFC and QuBE.

Technical validation of FLASH

Novels methods must pass through two important stages, i.e. technical validation and clinical validation. The FLASH algorithm uses vessel parameters provided by QCA with geometric coronary analysis, which already have been validated in several studies and

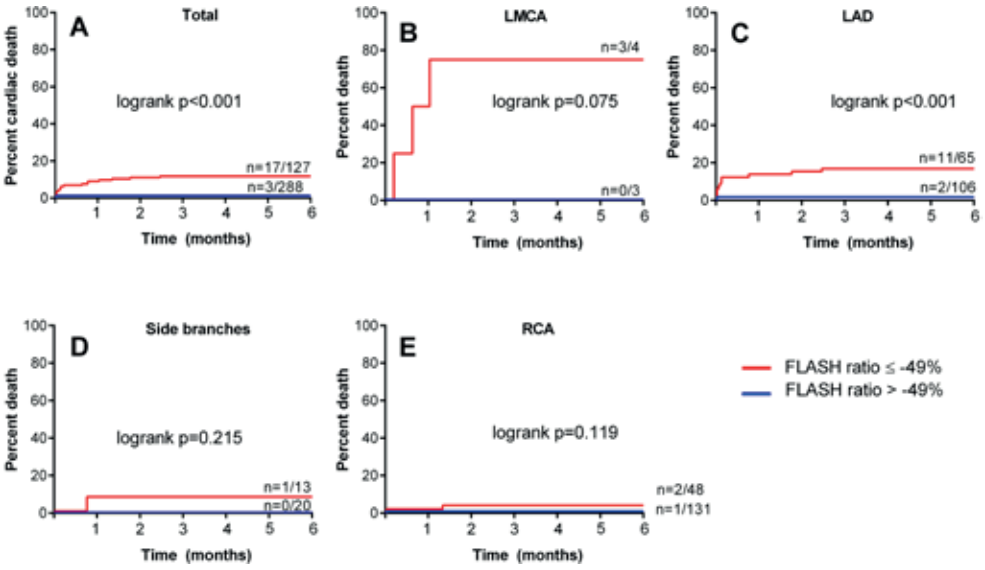

Figure 7: Kaplan Meier survival curves. FLASH cut-off value demonstrated a significant difference in cardiac death at 6 months (A). This difference in survival between FLASH groups remained true after stratification by IRA: B = left main coronary artery, C = LAD, D = side branches and E = RCA. None of the patients with LCx related infarcts died within six months.

provides high reproducibility and reliability. [28,29] In the present study, we found a high inter-observer agreement in both FLASH measures and CTFC between two independent observers. The absolute differences in CTFC between two independent observers closely resembles the inter-observer variability found in previous studies. [13,30,31] An important limitation of coronary angiography is the fact that it provides a two-dimensional view of a three-dimensional vessel. To counteract this limitation, we preferably chose an image without overlap of vessels and with the coronary artery in a lateral view. [32] Doppler flow velocities and QCA measurements in our group of patients are comparable to what is known from other studies. The culprit artery in our study had a mean vessel diameter of 2.4±0.4 mm which is similar to findings of Sahin et al. [33] who reported a mean vessel diameter of 2.2±0.5mm measured by QCA. In addition, the mean Doppler flow velocity in the culprit artery of 20.9±8.7 cm/s in our study closely resembles the mean Doppler velocity of 20.0±11.1 cm/s as reported in a study by Kern et al. [34]

Table 4: Univariate and multivariable analysis for predicting cardiac mortality. HR = Hazard Ratio; BMI = Body Mass Index

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, y	1.07 (1.03-1.10)	<0.001	1.06 (1.02-1.11)	0.002
IRA				
RCA		0.001		
LAD	4.62 (1.32-16.20)	0.017		
LCx	NA	NA		
Side branches	1.80 (0.19-17.3)	0.609		
LMCA	28.9 (5.8-143.3)	<0.001		
Ischemic time, min	1.00 (1.00-1.00)	0.789		
Heart rate pre PCI, bpm	1.02 (1.01-1.03)	0.002	1.03 (1.01-1.05)	0.002
CTFC (per 10-frame increase)	1.24 (0.97-1.60)	0.086		
FLASH ratio, per percent increase	0.97 (0.95-0.99)	<0.001	0.98 (0.96-0.99)	0.014
QuBE	0.96 (0.89-1.03)	0.225		
Glucose, mmol/L	1.20 (1.12-1.28)	<0.001	1.31 (1.18-1.46)	<0.001
Creatinine, umol/L	1.02 (1.00-1.03)	0.001		
Leukocytes, 10e9/L	1.05 (1.00-1.10)	0.008		
Cholesterol, mmol/L	0.65 (0.42-0.99)	0.044		

Clinical validation of FLASH

We validated FLASH flow by using a combined measure from Doppler wire data and the known cross-sectional area of the inflated stent. FLASH flow appeared to be well correlated with intracoronary flow measurements and therefore seems to provide an accurate estimation of coronary blood flow. Interestingly, only a weak and not significant correlation between intracoronary flow measurements and CTFC existed. This finding is in concordance with a study conducted by Barcin et al. [35], in which coronary blood flow was assessed in similar fashion, by combining Doppler wire measurements and QCA. Possibly, the poor correlation between intracoronary measurements and CTFC may partly be explained by the lack of measurement of luminal diameter. Luminal diameter has a substantial effect on vessel resistance (4^{th} power) which in turn has an important influence on blood flow. [21]

FLASH flow was better correlated with intracoronary measurements than CTFC, but

values were substantially lower than the intracoronary flow measurements. There may be several explanations for this finding.

First, due to branching of vessels, blood flow in distal segments of a vessel are lower than those measured in a proximal segment. [36] Since FLASH flow averages the blood flow over the traced length of the coronary artery and not at a single point as in Doppler flow velocity, the FLASH flow may therefore have been substantially lower.

Second, for intracoronary flow measurements we used the cross sectional area of the inflated stent determined by the compliance tables of the stent manufacturers. In a study by de Ribamar et al [37], the actual stent diameter determined by intravascular ultrasound appeared to be lower than the stent diameter as predicted by the manufacturer. This may potentially have contributed to an overestimation of the coronary blood flow as determined by intracoronary flow measurements.

A third factor that might explain the difference between Doppler and FLASH flow is the higher viscosity of contrast agents. The higher viscosity causes flow to be more sluggish compared to blood, causing flow speed to be underestimated in the coronary angiographic analysis of the images. Moreover, dye was injected via hand-held injections, which may have led to a variation in dye injection rates. Nevertheless, since dye injection rates do not affect CTFC, it is unlikely that hand-held injection will have had a significant effect on FLASH measures. [15,38]

With the differences in absolute flow between FLASH and the intracoronary flow measurements, it is important to point out that FLASH was not developed with the purpose to calculate exact coronary blood flow, but to enable an accurate estimation of coronary blood flow in a noninvasive and simple matter, that can be used in the catheterization laboratory to guide subsequent treatment.

Value of FLASH flow in predicting outcomes

In order to translate the FLASH flow into clinical practice, we conducted a patient study and evaluated its prognostic value. In the present study, FLASH was able to predict cardiac mortality after 6 months in STEMI patients, with a higher accuracy compared to presently used angiographic scores. We speculate that the superior predictive accuracy of FLASH ratio may partially be related to the inclusion of a reference artery, as its predictive accuracy appeared to be higher than that of FLASH flow in the IRA. Possibly, adjustment

for reference coronary blood flow allows for a better discrimination between pathophysiological reduction in blood flow caused by the myocardial infarction, and (global) variations in coronary blood flow caused by factors such as myocardial oxygen demand, sympathetic stimulation, circulating hormones, and drugs. [21]

In concordance with findings by Bhatt et al. [19], we did not find a significant association between mortality and CTFC. This is in contrast to several other studies like the TIMI 4 trial. [17,20] However, the TIMI 4 trial had a larger patient population and patients were treated by thrombolysis instead of PCI.

Furthermore, the predictive power of CTFC and its relationship with coronary blood flow has been questioned in several studies. [19,20] Vogelzang et al. [22] showed a correlation between mortality and QuBE value, however we could not reproduce these findings in our cohort. Patients who died within 6 months did not have a significantly lower QuBE value directly following pPCI. This lack of correlation is possibly attributable to the relatively small population used in the present study.

Clinical implications

The FLASH algorithm enables the ability to accurately estimate coronary blood flow in a non-invasive manner and to predict mortality in STEMI patients. QCA software is currently available in many interventional clinics and FLASH can be calculated within minutes. Therefore FLASH may be implemented as a surrogate endpoint in reperfusion trials instead of the commonly used CTFC and TFG. Furthermore, automatic frame counting and coronary length measuring has already been shown in a study by ten Brinke et al. [39] and potentially allows for an automated FLASH flow measurement.

Study limitations

In this study, the correction factor used in the calculation, derived from the difference in average flow in the reference arteries, was calculated from the same study cohort in which the FLASH ratio was calculated. Although a methodological limitation, it allowed for a larger cohort of patients to be included. However, an independent and prospective study is required to validate our results.

Only 69% and 79% of the cines were analyzable for respectively CTFC and QuBE, mostly caused by absence of distal landmark visualization in CTFC and too short cine filming or no specific blush sequence in QuBE, especially true for when the LAD was the

infarct related artery. It can be argued that this has negatively influenced the predictive accuracy of CTFC and QuBe. This percentage is however similar to previous studies and thus probably reflects the actual feasibility to perform the methods in a non-selected pPCI population. [19,22] Secondly, because FLASH ratio requires a non-obstructed reference artery to serve as a control, we excluded patients with 3VD. Therefore, results from our study cannot be extrapolated to this group of patients.

2.5 Conclusion

In the present study we describe the novel FLASH algorithm and show that FLASH flow correlates better with coronary blood flow, as determined by Doppler flow velocity and stent diameter, than current angiographic parameters. Furthermore, FLASH ratio proves to be a powerful predictor of cardiac mortality in STEMI-patients without 3VD CAD. FLASH ratio had a higher accuracy of predicting cardiac mortality within 6 months than CTFC and QuBE. Investigating the relationship between FLASH ratio and clinical follow up could further enhance the clinical relevance of FLASH.

3

Chapter 3:
Added value of 3D ultrasound deformation imaging in STEMI patients for early detection of left ventricular remodeling

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Submitted

Abstract

Introduction

Patients with ST-elevation myocardial infarction [STEMI] are at risk for left ventricular [LV] adverse remodeling [AR]; an inadequate myocardial response in order to optimize cardiac output. In contrast, reverse remodeling [RR] is defined as an improvement of cardiac function over time. 3D ultrasound [3D-US] can be used to detect left ventricular remodeling and subsequent patient prognosis. Our aim was to examine prognostic parameters in the development of AR and RR using 3D-US in STEMI patients.

Methods

Clinical, biochemical and LV volumetric parameters were collected at baseline. 3D-US was performed at baseline and at 4 months follow-up in patients with STEMI (<6h) treated with primary coronary intervention. Basic US parameters, as well as global longitudinal strain [GLS], global circumferential strain [GCS] and other 3D-US parameters were measured.

Results

Patients (n=91, 76% male, on average 57 years) suffered from anterior infarction in 30% of cases. In total, 26.4% (n=24) of patients developed AR, 28.6% (n=26) developed RR. Baseline GLS was significantly worse in patients that ultimately developed AR (-14.1±3.6), compared to those who did not (-16.7±3.7, p=0.01). Baseline GCS was better in patients who developed RR (-26.3±5.9) compared to patients without RR (-22.5±4.8 p=0.01). Multivariate analysis showed GLS was a statistically significant independent predictor of the occurrence of AR (OR 0.83, p=0.035) and GCS was a significant predictor of RR (OR 0.84 p=0.036).

Conclusion

A reduced GLS at baseline was found to be predictive of the development of AR after 4 months in STEMI patients. Furthermore, a GCS on the upper limit of normal at baseline was predictive for RR at 4 months. Both parameters measured with 3D-US were stronger predictors than clinical, biochemical and LV volumetric parameters.

3.1 Introduction

The treatment of patients with a ST elevation myocardial infarction (STEMI) has improved sharply in the past decades due to primary percutaneous coronary intervention (PCI) and additional medical treatment focused on inhibition of thrombus formation. However, even after a successful PCI long-term morbidity and mortality are still relatively high.

About 6% of patients develop heart failure within 2 years after their first STEMI. [40,41] Heart failure is related to left ventricular (LV) remodeling after an initial successful PCI. Still, the related mechanisms are complex and both adverse (AR) and reverse remodeling (RR) of the left ventricle may occur. [4,42]

Ventricular AR occurs due to necrosis and disproportionate thinning of the infarcted myocardium. This is related to microvascular obstruction or no-reflow after a successful PCI. Patients with microvascular obstruction after STEMI are more likely to suffer from AR as a larger area is left irreparable. The infarcted area is weakened and cannot withstand both pressure and volume load as adequately as healthy tissue.

Dilation of the left ventricular (LV) chamber occurs, changing the left ventricle from an elliptical to a more spherical shape. Consequently, this results in an increase of ventricular mass and volume, with a reduction in ejection fraction, cardiac output and may even cause functional mitral regurgitation in dilated (ischemic) cardiomyopathy. [43–46] Furthermore, myocardial stunning occurs during ischemia and also as part of the reperfusion injury pathway. [47] This can cause otherwise viable tissue to appear damaged. This may lead to an overestimation of initial infarct size.

Ventricular RR on the other hand, is a complex biochemical process in which the LV decreases in volume and increases in ejection fraction. It can be induced through pharmacological intervention, as well as with mild to moderate intensity exercise training. Currently, drugs that are capable of preventing cardiac dilatation, such as beta-blockers and renin-angiotensin system inhibitors are already part of the guidelines for treatment of STEMI.

Determining which patients benefit most from additional (medical) therapy that focusses on preventing AR and increasing RR is crucial early in the treatment process after primary PCI. Therefore, optimal imaging techniques and the use of parameters that are able to predict AR and RR in STEMI patients are urgently needed.

Two-dimensional (2D-US) is widely available and mostly used after STEMI, to assess remaining LV function and remodeling at follow-up. A frequently used parameter is the global longitudinal strain (GLS), which is the representation in time of movement of the myocardial tissue, relative to the myocardial wall thickness. It is thus a vector of myocardial deformation through the cardiac cycle and indicative of local wall strength.

A decrease in GLS points to an impairment of longitudinal left ventricular function and is a useful parameter to identify subclinical dysfunction. Recently, a strong association was found between peak GLS and AR. [48] GLS is derived from 2D or 3D speckle tracking echocardiography and is a semiautomatic tool to assess myocardial mechanics in a reproducible manner.

Three-dimensional echocardiography (3D-US) overcomes geometric 2D assumptions and is proven to be more accurate in determining quantitative left ventricular (LV) volumetric and deformation parameters compared to 2D-US. [49–52] As STEMI patients might benefit from an early start of medical treatment following PCI to prevent further deterioration of ejection fraction, we hypothesize that early deformation (strain) imaging using 3D-US is more accurate in predicting both AR and RR compared with quantitative volumetric LV variables in STEMI patients.

3.2 Methods

Patient population

Data from STEMI patients included in another clinical trial [53], who were successfully treated according to the ESC STEMI guidelines [54] with primary PCI and dual antiplatelet therapy, was used in this study. The addition of glycoprotein IIb/IIIa inhibitors was left to the discretion of the interventional cardiologist.

Inclusion criteria were: acute STEMI (<6 hours old) defined as detection of alteration in cardiac necrosis biomarker values associated with elevation of the ST segment on the initial electrocardiography of 2 mm or more in 2 consecutive leads successfully treated with PCI within 6 hours after onset of complaints.

Exclusion criteria were primarily: prior myocardial infarction or coronary artery bypass grafting, a clinically unstable patient (i.e. cardiac shock, ventricular rhythm disorders

and Killip class > 1 excluded), diabetes mellitus, multi-vessel disease requiring bypass grafting, atrial fibrillation, frequent extrasystoles or other significant arrhythmias and inadequate echocardiographic image quality.

All patients underwent 2D and 3D serial echocardiographic studies at baseline and at 4 months follow-up.

Echocardiographic imaging and analysis

3D-US imaging was performed in the apical position using a commercially available scanner (IE33 xMATRIX, Philips Healthcare, the Netherlands) with a fully sampled matrix array transducer (X5-1 xMATRIX array, Philips Healthcare, the Netherlands). Wide angle acquisitions were recorded consisting of wedge shaped volumes acquired during single breath hold. Depth and sector width were decreased as much as possible to improve spatial and temporal resolution of the images. An average of 25-30Hz was used for the 3D-US image acquisition. 3D-US images were analysed offline using TomTec 4D LV analysis (Image-Arena Version 4.6.3.9, TomTec Imaging Systems, Germany). Both systolic and diastolic endocardial and epicardial borders were automatically detected by the 3D wall motion tracking software. These borders were manually adjusted if necessary. The system automatically calculated LV end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), GLS and global circumferential strain (GCS). Normal values for GLS (-15.9 to -22.1%) and GCS (-20.9 to -27.8%) were obtained from the literature. [55] Adverse remodeling was defined as either a >5% decrease in LVEF at follow-up, or an increase of LV EDV more than 15%. Reverse remodeling was defined as an improvement of LVEF of 5%, or decrease of LV EDV by more than 15%. [49]

Statistical analysis

Independent sample t-test was used for continuous variables. Chi square test and Fisher Exact were used for categorical data. 1-way ANOVA with Bonferroni post-hoc testing was be used to compare subgroups of the study population. Logistic regression analysis was used to test significant predictors of remodeling. Continuous data are presented as mean ± standard deviation (SD). Categorical data are presented as count (n) and percentage (%). Statistical significance was defined as a probability value of less than 0.05. Data were analysed using SPSS version 23.

Ethics

All patients gave written informed consent. The local ethics committee approved of the protocol. This study was performed in accordance to the declaration of Helsinki.

3.3 Results

Study population

A total of 114 STEMI patients were enrolled in this study. Due to poor echocardiographic window and image quality, 12 baseline echocardiographic datasets had to be excluded from analysis. At 4 month follow-up, an additional 11 patients were excluded due to insufficient imaging quality.

Important baseline characteristics are comparable to other STEMI trials and can be found in Table 5. The mean age of the remaining 91 patients was 57.4 years and 76% of patients were male. About 59% of patients were smokers and 48% had a family history positive for cardiovascular disease. The left anterior descending artery was culprit artery in 30% of patients. Glycoprotein IIb/IIIa inhibitors were administered in 30% of patients. Most primary PCI procedures (96%) resulted in final TIMI 3 flow, which was 7% prior to PCI. Average CKMB max was 256±170μg/L. At discharge, 90% of patients received beta-blockers and 83% of patients received ACE inhibitors, without differences between patients with AR, RR and unchanged LVEF and EDV. At 4 month follow-up, no major adverse cardiac events had occurred. One patient had received a pacemaker due to high grade AV-nodal block.

All baseline pooled echocardiographic data is shown in Table 6. LVEF at baseline using 3D-US was 52.8±7.8% for all patients. Out of all patients, 26.4% developed adverse remodeling (n=24), reverse remodeling was present in 28.6% of patients (n=26). The remainder of patients (n=41) did not show a marked change in EDV or LVEF. Overall, patients had a significantly higher LVEF on follow-up, albeit the absolute difference was small (52.78±7.78 vs 55.68±7.67 %, p=0.027). Also, on average for all patients, GLS improved significantly at follow-up (-15.6±3.6 baseline vs -16.9±3.7 follow-up, p=0.024), which was also true for GCS (-358±5.7 baseline vs -26.2±5.2 follow-up, p=0.01).

Figure 8 shows an example of the difference in longitudinal wall movement over time in 2 patients, 1 with AR and 1 with RR.

Table 5: Baseline clinical characteristics and effect on adverse remodelling. Numbers as 'mean (± SD)' or 'n (%)' where applicable. N = number, SD = Standard Deviation, BMI = Body Mass Index, Hb = Hemoglobin, CRP = C-Reactive Protein, eGFR MDRD = Estimated Glomerular Filtration Rate Modification of Diet in Renal Disease, CKMB = Creatine Kinase Muscle Brain, CK = Creatine Kinase, FMC=First Medical Contact, TIMI = Thrombosis In Myocardial Infarction, PCI = Percutaneous Coronary Intervention

	All patients (n=91)	Adverse Remodelling (n=24)	Unchanged (n=41)	Reverse Remodeling (n=26)	P-value
Age (years)	57.4 (±10.1)	58.7 (±9.9)	57.3 (±10.55)	56.2 (±9.4)	Ns
Male (%)	69 (76%)	18 (75%)	30 (73%)	21 (81%)	Ns
BMI (kg/m^2)	26.9 (±3.6)	26.5 (±3.8)	27.1 (±3.1)	27.2 (±3.2)	Ns
Risk factors					
Past/Current Smoker	54 (59%)	9 (38%)	33 (80%)	12 (46%)	Ns
Hypertension	15 (16%)	5 (21%)	5 (12%)	5 (19%)	Ns
Hypercholes-terolemia	21 (23%)	7 (29%)	10 (24%)	4 (15%)	Ns
Positive family history	44 (48%)	10 (42%)	25 (61%)	9 (35%)	Ns
Lab results					
Hemoglobin (mmol/L)	8.9 (±1.3)	9.1 (±0.8)	8.8 (±1.6)	9.0 (±1.3)	Ns
Creatinin (µmol/L)	78.8 (±20.2)	81.3 (±20.6)	75.4 (±18.7)	83.1 (±22.8)	Ns
NTproBNP (ng/L)	112.2 (±310.3)	107.9(±169.2)	103.8 (±314.5)	133.3 (± 443.1)	Ns
CKMB Max (µg/L)	244.7 (±165.1)	275.3 (±173.3)	227 (±173.3)	247.6 (±140.6)	Ns
FMC-to-balloon time (min)	77 (±24)	78 (±17)	77 (±26)	77 (±25)	Ns
TIMI 0-1 pre PCI	81 (89%)	21 (88%)	37 (83%)	23 (88%)	Ns
TIMI 3 post PCI	87 (96%)	23 (96%)	41 (100%)	23 (88%)	Ns
Anterior in-farction n (%)	29 (31.8)	9 (37.5)	15 (36.6)	5 (19.2)	Ns

Table 6: 3D Ultrasound characteristics at baseline and 4 month follow-up for all patients. LV = Left Ventricle

Characteristics	Baseline (N=91)	Follow-up (n=91)	P-value
LV End Diastolic Volume (ml)	117.25 (±31.07)	121.76 (± 31.27)	0.39
LV End Systolic Volume (ml)	55.72 (±13.74)	54.61 (±19.21)	0.724
LV Ejection Fraction (%)	52.78 (±7.78)	55.68 (±7.67)	0.027
Stroke Volume (ml)	61.54 (±17.46)	66.82 (±18.83)	0.058
LV Mass (gr)	181.75 (±46.43)	184.59 (±56.84)	0.743
LV Mass indexed (gr/m^2)	84.60 (±29.13)	88.99 (±28.99)	0.819
Systolic Dyssynchrony Index	5.18 (±1.59)	5.24 (±1.53)	0.826
Global Longitudinal Strain	-15.6 (±3.6)	-16.9 (±3.7)	0.024
Global Circumferential Strain	-24.57 (±5.33)	-26.2 (±5.12)	0.064
Torsion	1.71 (±0.95)	2.21 (±2.77)	0.141
Twist	14.18 (±7.78)	14.64 (±7.85)	0.731

Adverse remodeling

When compared to patients who developed no AR, the clinical and angiographic parameters did not seem to be prognostic for AR, such as complaint-to-balloon time, or culprit coronary artery. Patients ultimately developing AR, all had TIMI 3 flow grade after primary PCI. Clinically, patients with lower CK max after the myocardial infarction were less likely to develop AR (2149±1984 vs. 3136±2045 U/L, p=0.071), while this was not reflected in the peak myoglobin fraction of CK (CKMB max) (p=0.188). (Table 5). No difference existed in use of medication after STEMI or in the occurrence of TIMI 0 or 1 prior to procedure between groups. The initial 16-segment systolic dyssynchrony index (16-SDI) was slightly higher for patients that ultimately developed AR. At follow-up LV mass increased significantly in patients with AR (36.9±31.2 gr), versus a decrease of 17.2±26.4gr in patients without AR (p<0.001), which is reflected by the change in end diastolic volume. At 4 month follow-up, GLS in patients with AR was persistently worse compared to patients without AR

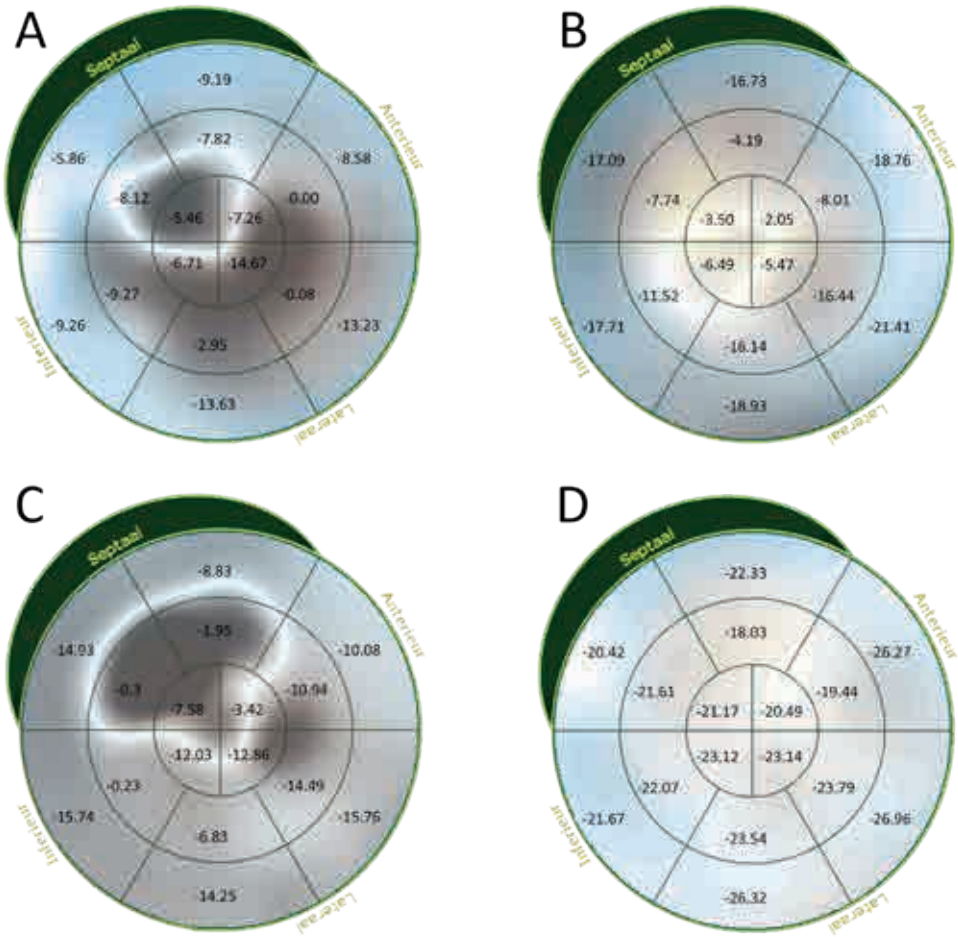

Figure 8: Bullseye diagram with end diastolic longitudinal myocardial wall movement at baseline (A & B) and follow-up (C & D). Patient 1 (panels A & C) with an anterolateral infarction due to occlusion of the mid left anterior descending (LAD), with a CKMB max of 100.5ug/L, but ultimately developed adverse remodeling. GLS was -7.80 and -8.80 at baseline and follow-up respectively. Patient 2 (panels B & D) with an anterolateral infarction with a CKMB max of 150.9ug/L due to an occlusion of the mid LAD. This patient showed reverse remodeling, initial global longitudinal strain (GLS) was -11.70 and at follow-up improved to -22.40.

(-15.8±4.1 vs -17.7±3.4 respectively, p=0.048). All echocardiographic characteristics of AR patients are compared to patients with unchanged EF and LVEDV and RR patients, depicted in Table 7.

Table 7: 3D Ultrasound characteristics at baseline and 4-months follow-up for patients with and without adverse remodeling (AR). LV= Left Ventricle, EF = Ejection Fraction, SDI = Systolic Dyssynchrony Index, GLS = Global Longitudinal Strain, GCS = Global Circumferential Strain

	With AR (n=24)	Without AR (n=67)	P-value
LV EDV baseline (ml/m²)	52.45 (±10.93)	59.45 (±12.04)	0.205
LV EF baseline (%)	53.80 (±7.63)	54.53 (±8.21)	0.146
LV mass baseline (gr)	167.53 (±43.92)	187.15 (±47.81)	0.112
16-SDI baseline	5.76 (±1.73)	5.01 (±1.50)	0.071
GLS baseline	-14.09 (±3.59)	-16.67 (±3.74)	0.010
GCS baseline	-24.41 (±6.65)	-24.86 (±5.10)	0.765
Torsion baseline	1.79 (±1.12)	1.65 (±0.93)	0.690
Twist baseline	13.88 (±8.67)	14.23 (±7.69)	0.858

Univariate logistic regression analysis showed that only 3D calculated baseline GLS was an independent predictor of AR, with an odds ratio of 0.851 (p=0.035). This analysis can be found in Table 8. Using the receiver operator characteristic curve, an optimal cut-off point of GLS was found at -15.7 producing a sensitivity of 75% and specificity of 62% for predicting the development of AR. GCS showed a trend towards being a predictor of AR, with an odds ratio of 0.882 (p=0.089).

Reverse remodeling

In total, 28.6% of patients experienced RR. Baseline LVEF was significantly lower for patients ultimately developing RR, with baseline GLS and GCS being higher in those patients. (Table 9) At follow-up, ESV decreased significantly in patients with RR compared to patients without RR (Δ -7.7±9.5ml vs. 6.0±11.6ml respectively, p<0.001). Furthermore, both GLS (Δ-3.3±4.2 vs. 0.2±2.7, p<0.001) and GCS (Δ -4.4±3.9 vs 0.6±3.4, p<0.001) improved significantly in patients with RR compared to patients without RR. GCS values were within the normal range, but binary logistic regression showed that a higher GCS significantly increased the odds of developing RR (β; 0.136 Odds; 1.15 p=0.016). In contrast to patients with AR, no significant change in LV mass was found in patients with RR (169.4±60.1 gr. vs. 193.3±54.2 gr respectively, p=0.11). Univariate analysis provided 3 individual predictors (GCS, GLS and 16-SDI) of the occurrence of RR, however, combining these predictors in a multivariate analysis showed that in patients with RR, only GCS was a statistically significant predictor with an odds ratio of 0.81 (p=0.036). (Table 10)

Table 8: Univariate regression analysis for patients with adverse remodeling (AR). CKMB = Creatinin Kinase Myoglobin, BNP = Brain Natriuretic Peptide, SDI = Systolic Dyssynchrony Index, GLS = Global Longitudinal Strain, GCS = Global Circumferential Strain

	Univariate analysis		
	Odds	Beta	P-value
CKMB max	0.998	-0.002	0.481
NTProBNP	1	0	0.563
16-SDI	0.804	-0.218	0.457
GLS	0.851	-0.161	0.035
GCS	0.882	-0.125	0.089
Torsion	0.957	-0.044	0.918
Twist	0.985	-0.015	0.776

Table 9: Echocardiographic patient characteristics at baseline in patients with and without reverse remodeling (RR). LV= Left Ventricle, EF = Ejection Fraction

	With RR (n=26)	Without RR (n=65)	P-value
LV EDV (ml)	112.7 (±29.5)	116.8 (±32.6)	0.613
LV EF (%)	48.1 (±5.9)	55.4 (±8.4)	0.016
LV mass (gr)	175.6 (±46.7)	182.3 (±47.5)	0.590
16-SDI	5.9 (±1.8)	5.0 (±1.4)	0.185
GLS	-16.3 (±4.3)	-14.7 (±3.0)	0.089
GCS	-26.3 (±5.9)	-22.5 (±4.8)	0.010
Torsion	1.8 (± 1.1)	1.7 (± 0.99)	0.839
Twist	14.6 (± 9.0)	14.0 (±7.9)	0.853

Table 10: Regression analysis for patients with reverse remodeling.

	Univariate analysis			Multivariate analysis		
	Odds	Beta	P-value	Odds	Beta	P-value
CKMB max	0.999	-0.001	0.591			
NTProBNP	1.001	0.001	0.604			
16-SDI	0.694	-0.365	0.046	0.870	-0.139	0.493
GLS	0.810	-0.211	0.003	0.927	-0.076	0.505
GCS	0.810	-0.210	0.019	0.844	-0.170	0.036
Torsion	0.946	-0.055	0.839			
Twist	0.990	-0.010	0.755			

3.4 Discussion

In our study, we demonstrated that baseline 3D-US can be used clinically to predict AR and RR in STEMI patients. We found that baseline GLS is an independent predictor of AR and baseline GCS a predictor for RR. This means that the use of 3D-US can provide clinicians important information in order to differentiate which STEMI patients might need to receive more aggressive medical therapy in order to prevent heart failure and improve their prognosis.

Adverse remodeling

The only baseline clinical and biochemical parameter that correlated in some degree with worse outcome after myocardial infarction and development of AR was high CK. This is in accordance to prior studies and CK is already used in the clinical daily practice in order to determine which patients are most eligible for early advanced heart failure therapy.

We found no correlation between other clinical and angiographical parameters, such as symptom-to-balloon time or medication used, and development of AR and RR. Treatment of myocardial infarction using primary PCI in the Amsterdam region in the Netherlands occurs very rapidly due to the use of an ambulance-interventional protocol called LIFENET [56], with average symptom-to-balloon times well under 2 hours. Also, as we studied patients included in a previous clinical trial that excluded patients with diabetes, infarct size may also be smaller than usual, as glucose control is an important factor in patient recovery after STEMI. [57] These two factors limit the total infarct size, therefore most patients have a relatively small myocardial scar size compared to other international regions and therefore adverse remodeling occurs less often. The current results are therefore most likely an underestimation compared with regions where symptom-to-balloon time is higher. This is reflected by our finding that patients with adverse remodeling had a GLS that fell just outside of the previously established normal range. [55] This gives an indication that the infarcted area in our patient population was relatively small, as even in patients with adverse remodeling, GLS still was borderline normal. In regions without the LIFENET protocol, or without primary access to PCI, infarct sizes are expected to be bigger, increasing the importance of GLS measurements for predicting AR. Compared with the commonly used LVEF, GLS achieved with 3D-US is a more accurate prognostic marker. [58] 3D speckle tracking has also been proven superior in assessing wall motion abnormalities compared to 2D speckle

tracking. [59] The predictive value of GLS has been described earlier in the setting of LVEF improvement. [49,60] A strength of our current study is that we have measured 3D strain also at follow-up, which has not been performed before, in order to compare changes over time in global strain in patients with acute myocardial infarction.

Reverse remodeling

With regard to development of RR, we found that a higher baseline GCS was a predictor of the occurrence of RR. This might be attributed to the fact that patients with RR have a lower baseline EDV, with a more elliptically shaped LV compared to the spherical shape of heart failure patients, something already suggested by Hung et al in their 20-month follow-up study using 2D ultrasound in patients with myocardial infarction. [61] Baseline GCS at the upper limit of normal is therefore more likely to be predictive of patients that will show improved LV function and is indicative of a more localized sub-endocardial infarction. Also, patients with lower LVEF at baseline were more likely to develop RR at follow-up. This is interesting, because it may indicate that patients that showed a large increase in LVEF at follow-up, had relatively larger areas of myocardial stunning post STEMI and were thus capable of showing improvement. [62]

Limitations

Although this study used data from a randomized clinical trial, several limitations should be mentioned. Our study was a post-hoc analysis and therefore results should be interpreted with caution. Similarly to other trials [50], only 80% of patients had an analysable 3D echocardiographic exam that was of sufficient quality. Consequently, as not all ultrasound exams were useful for the analysis of data, this diminishes the strength of our study. Also, as we used data from patients included in a prior randomized trial, our study population is relatively small, especially in comparison with other 2D ultrasound studies. However, our findings and baseline characteristics are in line with previous studies so these data seem to reflect a normal STEMI population.

Furthermore, while we distinguished AR and RR as separate entities in this trial, statistical analysis was performed comparing AR with all other patients, including both those with RR and unchanged LVEF and EDV. Similarly, patients with RR were compared with unchanged and AR patients. A direct comparison between AR and RR however would be of less clinical relevance, as this would exclude almost 50% of patients from our analysis. This

was purposefully done, however it is possible that some parameters that would otherwise have been significantly different between AR and RR groups are now missed.

While the relationship between AR, low ejection fraction and heart failure is clear, follow-up in our study was only 4 months. There were no major adverse cardiac events in this study period, longer follow-up is therefore needed to determine the true risk a patient with AR has in developing heart failure.

Our echocardiographic analysis has been performed using TomTec software. These values might not be directly comparable to the analysis using software from other manufacturers. Additional studies are also warranted in a patient population with mediocre image quality.

3.5 Conclusions

Baseline GLS using 3D-US was found to be predictive of the occurrence of AR after 4 months in STEMI patients treated with primary PCI. Furthermore, 3D derived GCS was shown to be predictive of RR in our patient population. Both strains were stronger predictors than clinical, biochemical and LV volumetric parameters. Future studies are warranted focussing on the clinical long term implications of these data.

Part 2:
Therapeutic targets: reperfusion injury

4

Chapter 4:
Progression in attenuating myocardial reperfusion injury: an overview

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Abstract

Reperfusion by means of percutaneous coronary intervention or thrombolytic therapy is the most effective treatment for acute myocardial infarction, markedly reducing mortality and morbidity. Reperfusion however induces necrotic and apoptotic damages to cardiomyocytes that were viable prior to reperfusion, a process called lethal reperfusion injury. This process, consisting of many single processes, may be responsible of up to half of the final infarct size. A myriad of therapies as an adjunct to reperfusion have been studied with the purpose to attenuate reperfusion injury. The majority of these studies have been disappointing or contradicting, but recent proof-of-concept trials show that reperfusion injury still is a legitimate target. This overview will discuss these trials, the progression in attenuating myocardial reperfusion injury, promising therapies, and future perspectives.

4.1 Introduction

The mortality rate as a consequence of acute myocardial infarction (MI) has dropped dramatically due to the introduction of reperfusion therapy by means of thrombolysis or primary percutaneous coronary intervention (pPCI). Reperfusion in an early stage improves salvage of the myocardium and limits the final infarct size. [63] And by limiting the infarct size, the prognosis for development of heart failure, the quality of life of patients and deaths is improved. [64,65] As with many successful therapies however, there is a drawback. The restoration of blood flow, which provides oxygen and nutrients necessary for survival of the ischemic area, paradoxically causes additional damage to the myocardium as well. This process is called reperfusion injury. It can manifest itself as arrhythmias, myocardial stunning, no-reflow, and cardiomyocyte death. [66] This overview will focus on the latter, also termed lethal reperfusion injury. Lethal reperfusion injury is an interaction of multiple processes ultimately leading to necrosis and apoptosis of cardiomyocytes, which were viable prior to reperfusion. [66] In that way reperfusion injury reduces the successful effect of reperfusion therapies on the final infarct size. The improved outcome that can be achieved by reducing infarct size is motivation for many to search for therapies that are able to inhibit the process of lethal reperfusion injury. Over the past years, the processes involved in reperfusion injury have become more and more elucidated. Thus far, multiple therapies appeared to be effective in attenuating reperfusion injury in the experimental setting. Translation into clinical practice, however, has been demonstrated to be complicated. This overview outlines the current progression in targeting lethal myocardial reperfusion injury in the clinical setting.

Processes in ischemia–reperfusion injury

To understand the rationale behind these therapies, it is important to appreciate the many processes that are set in motion at the onset of ischemia and subsequent reperfusion. Comprehensive reviews already illustrate these mechanisms in detail. [66–68] Here, we provide a mere short description of the concept, based on these reviews. The main mechanisms are also summarized in Figure 9.

Ischemia develops upon occlusion of one or more coronary vessels. The perfusion territory of the occluded vessel is deprived of a steady supply of oxygen, necessary for survival. The normal myocardium depends highly on aerobic metabolism to generate energy. Under

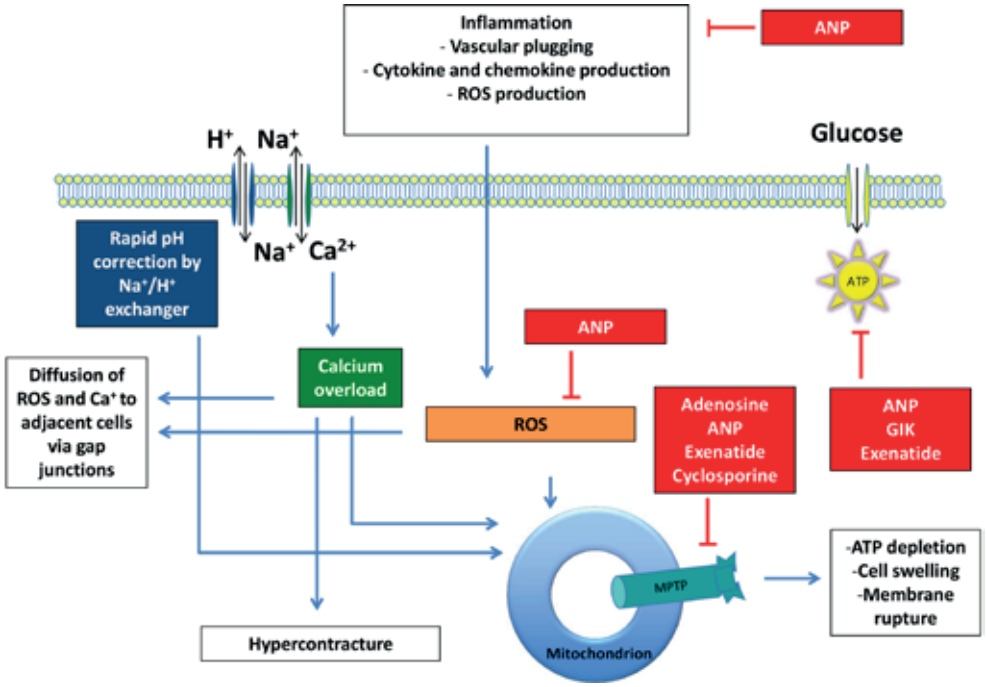


Figure 9: Mechanisms involved in myocardial ischemia reperfusion injury. This figure constitutes a simplistic display of the main mechanisms involved in myocardial ischemia reperfusion injury as described in Processes in ischemia–reperfusion injury. The red boxes indicate the most promising adjunct pharmacological therapies and point to their specific targets by which they reduce lethal myocardial reperfusion injury. Abbreviations: ANP = atrial natriuretic peptide; ATP = adenosine triphosphate; GIK = Glucose–Insulin–Potassium; GLP-1 = glucagon-like peptide-1; MPTP = mitochondrial permeability transition pore; ROS = reactive oxygen species.

physiological conditions, the heart utilizes not only non-esterified fatty acids (NEFA), but also glucose, and to a lesser extent, lactate, ketones, amino-acids and pyruvate in order to produce sufficient adenosine triphosphate (ATP) to sustain contractile function. NEFA consumption requires more oxygen for the generation of ATP in comparison with glucose. [68, 69] Consequently, under ischemic circumstances, the reliance on NEFA will render the myocardium vulnerable, and intracellular ATP is depleted in this process. Thus, the ischemic myocardium is forced to shift predominantly to anaerobic metabolism, leading to formation of lactate, which results in a fall of intracellular pH. [68] The cardiomyocyte reacts by removing H^+ via the Na^+/H^+ exchanger, and Na^+ accumulates within the cell. Because of ATP-depletion, the cardiomyocyte has difficulty removing calcium, leading to intracellular calcium

overload. [68] Calcium overload induces hypercontracture (with rupture of muscle fibers) and activation of apoptotic processes. [68] Upon reperfusion, the acutely restored blood flow immediately delivers nutrients and oxygen to the ischemic myocardium, thus improving the conditions required for survival. At the same time however, the rapid normalization of intracellular pH causes an H^+-gradient, leading to Na^+/H^+ exchange, and accumulation of intracellular Na^+. The subsequent action of an Na^+/Ca^{2+} exchanger not only removes the excess Na^+, but also leads to an influx of Ca^{2+}, leading to an additional Ca^{2+} overload (Figure 9). [67,68] Moreover, the low pH under ischemic conditions provided a protective effect, which is now abolished. [67] Re-oxygenation of the mitochondria leads to a burst of reactive oxygen species (ROS), which may cause cardiomyocyte injury. [66] The negative consequences of Ca^{2+} overload and ROS actions also might extend to adjacent tissue through gap junctions. [68] In addition, ischemia and reperfusion both activate inflammatory responses, such as inflammatory cell activation, vascular plugging, production and release of cytokines, chemokines and ROS. [66,70] The rapid pH correction, Ca^{2+} overload, and the formation of ROS cause opening of the mitochondrial permeability transition pore (MPTP). [66,71] During ischemic circumstances, this channel of which the molecular basis is still unknown remains closed. [72] Opening of the MPTP leads to ATP-depletion, cell swelling, and eventually rupture of membranes, causing necrosis. [66,71] All of these processes interact and result in ischemia–reperfusion injury. [66] By targeting a single process, the complete mechanism might be influenced. Therefore, different adjuvant therapies targeting isolated processes are investigated, with the goal to enhance myocardial salvage after reperfusion. These adjuvant therapies to reperfusion will be described in the following sections. Therapies primarily aimed at improving myocardial perfusion, such as anti-thrombotics, are beyond the scope of this overview.

4.2 Targeting the processes in ischemia/reperfusion injury

The multiple adjuvant therapies studied can roughly be divided into two major groups, mechanical ischemic conditioning and pharmacological interventions. Also, the use of supersaturated oxygen delivery yielded promising results and is discussed further in this review.

Ischemic conditioning

Ischemic preconditioning

The observation that patients who experienced symptoms of angina pectoris prior to the actual (index) ischemic event seemed to have a better outcome than patients without prior symptoms was reason for Murry and co-workers to apply brief cycles of ischemia in dogs. This intervention, in which repetitive cycles of 5 min of ischemia before the index ischemic event are supplied by a balloon occlusion in the culprit vessel, was called ischemic pre-conditioning and reduced infarct size by 75%. [73]

Until this day, pre-conditioning is thought of as the most powerful cardioprotective mechanism. The factors responsible for this protective effect include a cascade of actions mediated by stimulation of receptors of adenosine, bradykinin, and opioids. [68] Via complex mechanisms these substances finally converge on a common target, i.e. the activation of protein kinase C (PKC). [68] PKC in its turn belongs to the so-called reperfusion injury salvage kinases (RISK), which inhibit opening of the MPTP. In patients undergoing CABG, the application of ischemic preconditioning by repetitive aortic cross clamping results in lower troponin release, as well as fewer ventricular arrhythmias, less inotropic requirements and a shorter intensive care unit stay. [74] The invasive nature of the procedure and the increased thrombo-embolic risk, however, have hampered the performance of large prospective clinical studies. Ischemic preconditioning is not suitable for STEMI, as the preconditioning stimulus requires to be applied prior to the index myocardial ischemic event. These obstacles have been overcome by the introduction of ischemic postconditioning, an endogenous cardioprotective strategy which can be applied at the time of myocardial reperfusion, and remote ischemic perconditioning, which can be applied after the onset of the myocardial ischemia to another organ or tissue remote from the heart.

Ischemic postconditioning

In 2003, Zhao and co-workers showed for the first time that the cardioprotective effect of ischemic conditioning in humans is not restricted to the pre-ischemic period. They demonstrated that repetitive 30-second cycles of LAD re-occlusion and reflow after 60 min of LAD occlusion reduced infarct size by 43% in canine hearts. [75] Since a balloon is already in place during pPCI, this protocol is easily applicable in the clinical situation for STEMI patients. Indeed, shortly after, the first clinical results were introduced by Staat et al. in 2005 in a small proof-of-concept trial of 30 patients in which ischemic postconditioning was compared to a sham protocol. The result was reduced by a creatine kinase area under the curve (AUC). [76]

This landmark study provided evidence that infarct size reduction was possible in humans by an intervention initiated during reperfusion, and therefore for the existence of lethal reperfusion injury as a distinct mediator of cardiomyocyte death in patients with STEMI undergoing pPCI. Results of subsequent studies using echocardiography [77], SPECT [78,79] and cardiac magnetic resonance (CMR) endpoints [80] were overall positive. Lønborg et al. were the first to assess the effect of ischemic postconditioning using CMR. In 118 patients they demonstrated a 19% decrease in infarct size and a 31 increase in the myocardial salvage index. [80] Sorensson et al., however, showed a positive effect of ischemic postconditioning in patients with a large AAR (>30%) or LAD infarct, but did not reach a significant difference in infarct size (creatine kinase and troponin area under the curve and CMR) in their total study population (n = 76). [81] Also Tarantini recently reported negative results and even stated that ischemic postconditioning might be harmful, since a trend was observed towards larger infarct size and more adverse events. [82]

The reason for these discordant findings is not clear yet. It might be attributed to confounding factors that have been demonstrated to be of influence on the benefit of myocardial conditioning in preclinical and clinical studies. These include comorbidities, ischemic time, thrombolysis in myocardial infarction (TIMI) flow in the infarct related coronary artery, the size of the AAR and coronary collateralization. The present studies are too small to draw definite conclusions at the moment, and larger studies are needed to identify those patients who will benefit from ischemic postconditioning and who will not. Overall, however, ischemic postconditioning seems cardioprotective in STEMI patients treated with pPCI. An important Danish large multicenter study powered for clinical endpoints, the DANAMI-3

study (NCT01435408), is currently on-going. Although promising, ischemic postconditioning also has negative aspects. It is an invasive procedure that prolongs the pPCI procedure. Also, in many of the STEMI patients there is a delay between reperfusion and the postconditioning protocol, because optimal or suboptimal TIMI flow is already achieved before the PCI due to the antithrombotic therapy delivered in the ambulance. These drawbacks could be overcome by another endogenous cardioprotective strategy: remote ischemic preconditioning.

Remote ischemic preconditioning

The first evidence that repetitive induction of ischemia and reperfusion in a remote region can provide cardioprotection was delivered in 1993 by Przyklenk et al. [83] A conditioning protocol in the circumflex coronary artery reduced infarct size in a myocardial infarction induced by LAD occlusion. This concept of remote ischemic preconditioning was quickly extended to conditioning of other organs than the heart such as the kidney and intestine. [84] Despite intensive investigation, the exact mechanistic pathways linking the preconditioning organ or tissue to the heart are unclear. A murine study provided evidence for both neural and humoral pathways. [85] Remote ischemic conditioning of the upper and lower limbs can be readily applied in all settings of MI without invasive manoeuvres, and could therefore be of great value in attenuating myocardial reperfusion injury. In patients undergoing CABG, remote ischemic preconditioning of the upper arm reduced enzymatic infarct size and all-cause mortality over 1.5 years. [86] The remote conditioning protocol also appeared to be effective then applied after the onset of myocardial ischemia. Indeed, remote ischemic preconditioning by four 5 minute inflations of a cuff placed on the upper arm in the ambulance increased myocardial salvage in STEMI patients as determined with SPECT (0.75 vs 0.55, $p = 0.033$). [87] Larger randomized studies are required to confirm the effect of remote ischemic preconditioning in STEMI patients and to assess the effect on clinical outcome.

Supersaturated oxygen delivery

In experimental studies, the delivery of supersaturated oxygen in the infarct related artery immediately after successful reperfusion reduced infarct size, by decreasing capillary endothelial cell swelling, reducing ROS formation and inhibiting leukocyte activation and adherence. [88] The first results in humans were obtained by Dixon and colleagues in 2002,

who observed local wall motion improvements using echocardiography in a study composed of 22 patients. [89] In the AMIHOT I trial, 269 patients with acute anterior or large inferior AMI undergoing primary/rescue PCI (<24 h from symptom onset) were randomly assigned to receive hyperoxemic reperfusion (during 90 min intracoronary infusion of aqueous oxygen) or normoxemic blood autoreperfusion. The study was overall negative, but a possible treatment effect was observed in anterior AMI patients reperfused <6 h of symptom onset. [90] The subsequent AMIHOT II trial investigated this subgroups of patients (n = 301) and demonstrated a reduction in infarct size (20% vs 27%) measured with SPECT. [91]

Pharmacological interventions

In reaction to the identification of the many different pathways responsible for myocardial reperfusion injury, a myriad of pharmacological compounds have been subject to experimental investigation with the goal to attenuate reperfusion injury. As in ischemic conditioning, the most effective target of a pharmacological intervention would be aimed at inhibiting the MPTP. Accordingly, pharmacological compounds aimed at inhibition of rapid restoration of the low intracellular pH, generation of ROS, Ca^{2+} overload, and interfering with the RISK pathway have been investigated. Several agents have already been studied in the clinical setting, often yielding disappointing or conflicting results. These include nicorandil, Ca^{2+} modulators, H^+/Na^+ exchanger inhibitors, anti-inflammatory compounds, iron chelation, free-radical scavengers or inhibitors, ATP sensitive K^+ channel openers, and statins. [66,92–104] Also for erythropoietin the results are disappointing thus far. [105,106] The EPO-AMI II study is currently recruiting 600 STEMI patients with reduced left ventricular (LV) ejection fraction to investigate whether erythropoietin improves systolic LV function. [107] Other agents seem to be more promising. These are reviewed in the following section. For a detailed design of the reported trials, including methods of reperfusion therapy applied, we refer to Table 11. The most promising compounds and their specific targets are also illustrated in Figure 9.

Promising pharmacological agents

Atrial natriuretic peptide (ANP)

Although not directly aimed at the targets described above, atrial natriuretic peptide (ANP) is a hormone with a wide range of effects, which might attenuate myocardial reperfu-

sion injury (see also Figure 9). As the name suggests, ANP is produced in the cardiac atria. It has diuretic and vasodilatory effects, and plays an important role in improving the hemodynamic status in patients with heart failure. In addition, ANP has an inhibitory effect on the renin–angiotensin–aldosterone-system (RAAS), sympathetic nerve activity, inflammation, ROS generation, and apoptosis. [125] ANP was also demonstrated to increase glucose uptake under hypoxic circumstances in rat cardiomyocytes, thereby improving the metabolic efficiency of the myocardium. [126] The largest clinical trial as of yet is the J-WIND trial. [100,125] This trial was divided into two separate clinical trials investigating nicorandil and ANP in patients with an acute MI undergoing pPCI. A reduction in infarct size of 14.7% was observed (as measured with creatine kinase AUC) in 277 patients receiving ANP intravenously for 72 h, initiated after reperfusion, vs. 292 patients receiving placebo. At 6–12 months the LV ejection fraction was higher in the ANP treatment-group (44.7% vs. 42.5%, $p = 0.024$). The rate of the composite of cardiac death and readmission for heart failure was also lower in the ANP treatment-group. Twenty-nine patients receiving ANP experienced severe hypotension, probably as a result of the vasodilatory effect. Although promising, these findings need to be confirmed in additional large-scale trials.

Adenosine

When it became clear that activation of adenosine receptors causes a cascade of actions that eventually inhibit the opening of the MPTP, investigators began to study the effect on reperfusion injury of the compound adenosine itself. The underlying idea being that activation of adenosine receptors can mimic ischemic conditioning and subsequently result in smaller infarct size. Adenosine is distributed throughout the human body, and plays an important role in biochemical processes, including energy metabolism as it is a major component of ATP. Upon ischemia, adenosine levels rise markedly, and affect many processes, including apoptosis. [127] Experimental evidence suggests that adenosine is able to attenuate reperfusion injury, although the exact cardio protective mechanism remains to be resolved. [127] Clinical trials have focused on two routes of adenosine administration, intracoronary and intravenous. Marzilli and co-workers demonstrated a beneficial effect of intracoronary administration of adenosine on coronary flow, clinical course and LV function in a proof-of-concept study in 54 STEMI patients undergoing pPCI. [108] In a similar randomized controlled trial with 110 patients (56 to receive adenosine versus 54 placebo), no

effect on myocardial salvage was detected with CMR. [109] The AMISTAD II trial recruited 2118 patients with anterior MI, who were randomized to high dose adenosine, low dose adenosine or placebo, administered 15 min before reperfusion by either pPCI or thrombolytic therapy, followed by 3 h infusion. [111] The design of this trial was based on the results of the first AMISTAD trial, in which a reduction in infarct size was found in patients with an anterior MI. [110] Because an increase in adverse events was observed in the population with non-anterior infarction, these patients were excluded in the second AMISTAD trial, which indeed confirmed the infarct size reducing effect of high dose adenosine. [111] Moreover, a post-hoc analysis of AMISTAD II demonstrated a potential reduction of mortality in patients, who received reperfusion within 3.17 h. [128] A recent meta-analysis showed adenosine to reduce the incidence of post-procedural no-reflow, but no improvement in clinical outcome. [129] In summary, adenosine remains a promising compound to attenuate lethal reperfusion injury at least in a subset of patients with acute MI. Additional studies should confirm this effect and demonstrate the impact on clinical outcome.

Cyclosporine

Another pharmacological agent aimed at the inhibition of opening the MPTP is the immunosuppressant cyclosporine. [130] Although experimental research demonstrated variable and inconsistent results [131], a first proof-of-concept trial (cyclosporine vs placebo) in 58 patients with an acute MI undergoing pPCI showed a 40% reduction in infarct size in the cyclosporine treatment-group, as measured with creatine kinase AUC. [112] Cyclosporine has pleiotropic effects, and concerns were raised that long-term treatment could lead to adverse LV-remodeling and heart failure. [132] However, in a subset of 28 patients who participated in the above-mentioned proof-of-concept trial, CMR analysis at 5 days and 6 months showed that a single bolus of cyclosporine had no adverse effect on LV remodeling. [132] Currently, a large double-blinded randomized trial (CIRCUS, NCT01502774) with a combined endpoint of mortality, hospitalization for heart failure and left-ventricle remodeling is recruiting patients to investigate the effects of cyclosporine on clinical outcome, and is expected to complete by the end of 2013. The positive clinical results achieved with cyclosporine also formed the rationale to investigate another MPTP inhibitor, TRO40303, in the clinical setting. This MITOCARE study is a European multicenter trial and is currently recruiting. [133]

Glucose–Insulin–Potassium (GIK)

The most extensive research in this field has been done with glucose, insulin and potassium (GIK). Already in 1962 GIK infusion was introduced in the setting of ischemia and reperfusion. [134] Ischemic and reperfused myocardium is thought to benefit from GIK in two ways: (i) insulin suppresses lipolysis and consequently lowers circulating NEFA-levels, thereby removing toxic elements to the myocardium, and (ii) by offering glucose as a substrate for ATP-production as an energy source. [69,135,136] Potassium is added in order to keep the potassium levels in balance, which can be disturbed because of glucose- and insulin-infusion. A dosing scheme of GIK was introduced in 1981 that maximizes myocardial glucose uptake and decreases NEFA levels. [137] Many clinical trials have been performed since, and although a vast majority of these, mostly small proof-of-concept trials were positive, the overall results of the GIK trials are contradicting. [138,139] Especially the larger trials show disappointing results. The POL-GIK trial, published in 1998 including 954 non-diabetic patients with an acute MI, either receiving thrombolytic therapy or conservative treatment, showed no reduction in cardiac death, occurrence of cardiac events or creatine skinase elevations. In fact, total mortality at 35 days was higher in the GIK-group (8.9% versus 4.8%, $p = 0.01$). [113] In the same year however, the ECLA trial with 407 patients with suspected acute MI, who received thrombolytic therapy, PCI or conservative treatment, revealed a reduction of mortality in patients receiving GIK, who underwent reperfusion therapy. [114] This study was followed by the Create-ECLA trial, a mega trial including 20,201 patients with an acute MI, receiving comparable reperfusion therapy as in the ECLA trial. [115] The Create-ECLA however did not show any benefit of GIK on mortality or other clinical outcomes. Also the GIPS II study including 889 patients with an acute MI could not replicate the positive effects of GIK on reperfusion injury in patients without heart failure [117,140], that were demonstrated earlier in the GIPS I trial. [141] Furthermore, although strictly not a GIK-trial, the DIGAMI 1 trial including 620 patients showed that strict glucose control with glucose and insulin decreased mortality of patients with MI. [118] DIGAMI 2 however, including 1253 patients, did not confirm this. [57] The most recent study investigating the effect of GIK on ischemia and reperfusion injury is the IMMEDIATE trial. [119] Their hypothesis was that the timing of GIK-infusion was responsible for the inconsistent results of prior GIK-trials. Therefore, instead of including patients in a hospital setting and commencing the treatment as late as 24 h after onset of symptoms, 871 patients with a sus-

Table 11: Summary of the most promising pharmacological agents as adjunct to reperfusion therapy.

[a] Due to excess hypoglycemia in the GIK group after enrolment of 369 patients, the insulin dose was decreased to 20 U.

[b] Study investigating tight glycemic control (no potassium involved).

[c] Patients allocated to 4 pre-defined strata: 1. low-risk, no insulin; 2. high-risk, no insulin; 3. low-risk, insulin; and 4. high-risk, insulin.

Abbreviations and acronyms: ACS = acute coronary syndrome; (A)MI = (acute)myocardial infarction; AMISTAD = Acute Myocardial Infarction Study of Adenosine; AUC = area under the curve; CABG = coronary artery bypass graft; CAG = coronary angiogram; (C)HF = (congestive) heart failure; CK(MB) = creatine kinase (muscle and brain fraction); CMR = cardiovascular magnetic resonance imaging; CREATE-ECLA = Clinical Trial of Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation Estudios Cardiológicos Latinoamérica; CV = cardiovascular; DIGAMI = diabetes mellitus insulin–glucose infusion in acute myocardial infarction; DM = diabetes mellitus; ECG = electrocardiogram; ECLA = Estudios Cardiológicos Latinoamérica; EMPIRE = exenatide myocardial protection in revascularization; EXAMI = effect of additional treatment with exenatide in patients with an acute myocardial infarction; g = gram; GIPS = Glucose–Insulin–Potassium Study; h = hour; IC = intra-coronary; IMMEDIATE = immediate myocardial metabolic enhancement during initial assessment and treatment in emergency care; IV = intravenous; J-WIND-ANP = the Japan working group studies on acute myocardial infarction for the reduction of necrotic damage by human atrial natriuretic peptide; KCl = potassium chloride; kg = kilogram; LVEF = left ventricle ejection fraction; mEq = milli Equivalent; µg = microgram; min = minute; mL = milliliter; mmol = millimole; NaCl = sodium chloride; PCI = percutaneous coronary intervention; POL-GIK = Polish Glucose–Insulin– Potassium trial; Ref ID = reference identification; GLP-1 = recombinant glucagon-like peptide-1; SC = subcutaneous; SPECT = single photon emission computed tomography; STEMI = ST-elevation myocardial infarction; TC = time curve; U = unit

Study	Trial design	Patients	Treatment protocol	Primary endpoints	Outcome	Comments
Atrial natriure peptide (ANP)						
Kitakaze et al. 2007 / J-WIND-ANP [100]	Single-blind randomized (n = 569)	STEMI undergoing PCI <12 h symptoms	Initiation: after reperfusion IV 0–025 µg/kg/min ANP during 3 days IV 5% glucose solution during 3 days	Infarct size (AUC&TC of CK) LVEF (LV angiography at 6–12 months)	14.7% decrease infarct size. Higher LVEF	Less cardiac death and admission for HF (combined)
Adenosine						
Marzilli et al. 2000 [108]	Randomized (n = 54)	STEMI undergoing PCI <3h symptoms	Initiation: during reperfusion IC 4 mg adenosine in 2 mL saline during 1 min IC 2 mL saline during 1 min	Feasibility & safety. TIMI flow & no-reflow	Safe, feasible. Higher TIMI flow, less no-reflow	Beneficial effect on ventricular function and clinical course

Study	Trial design	Patients	Treatment protocol	Primary endpoints	Outcome	Comments
Desmet et al. 2011 [109]	Double blind randomized (n = 110)	STEMI undergoing PCI <12 h symptoms	Initiation: during reperfusion IC 4 mg adenosine in 5 mL 0.9% NaCl IC 5 mL 0.9% NaCl	Myocardial salvage (CMR at 2-3 days)	No difference	
Mahaffey et al. 1999 / AMISTAD I [110]	Open-label placebo controlled randomized (n = 236)	STEMI undergoing thrombolysis <6h symptoms	Initiation: before reperfusion IV 70 µg/kg/min adenosine during 3 h IV 70 µg/kg/min saline during 3 h	Infarct size (SPECT at 5-7 days)	67% less infarct size in anterior infarction	Increase adverse in-hospital CV events
Ross et al. 2005 / AMISTAD II [111]	Double-blind randomized (n = 2118)	Anterior STEMI undergoing thrombolysis and PCI <6h symptoms	Initiation: within 15 min at start of fibrinolysis or before PCI IV 70 µg/kg/min adenosine during 3 h IV 50 µg/kg/min adenosine during 3 h Placebo	Occurrence of in hospital CHF, re hospitalization for CHF or all cause mortality in 6 months	No difference	57% decrease infarct size in high-dose regimen
Cyclosporine						
Piot et al. 2008 [112]	Single-blind randomized (n = 58)	STEMI undergoing PCI <12 h symptoms	Initiation: before reperfusion IV bolus 2.5 mg cyclosporine (25 mg/mL) IV bolus 2.5 mg saline	Infarct size (AUC of CK & troponin I)	CK 40% less	Lower infarct size measured with CMR in cyclosporine group
Glucose-Insulin-Potassium (GIK)						
Mehta et al. 2005 / CREATE-ECLA [115]	Randomized controlled (n = 20,201)	STEMI treated conservatively, with thrombolysis or PCI DM& non-DM <12 h	Initiation: immediately after randomization IV 1000 mL 25% glucose, 50 U insulin, 80 mmol KCl (1.5 mL/kg/h during 24 h) Standard care	All-cause mortality at 30 days	No difference	

Study	Trial design	Patients	Treatment protocol	Primary endpoints	Outcome	Comments
[a] Ceremuzynski et al. 1999 / POL-GIK [113]	Randomized controlled (n = 954)	STEMI undergoing thrombolysis or conservative treatment Non-DM <24 h symptoms	Initiation: during or immediately after reperfusion IV 1000 mL 10% glucose, 32 U insulin, 6.0 g KCl (42 mL/h, during 24 h) IV 1000 mL sodium(42 mL/h during 24 h)	Cardiac mortality at 35 days, cardiac arrest, CHF, reinfarction, arrhythmia, conductance disturbances, CAG, PCI, CABG	Increased mortality in GIK group	
Diaz et al. 1998 / ECLA [114]	Randomized controlled (n = 407)	Suspected AMI treated conservatively, with thrombolysis or PCI DM& non-DM <24 h symptoms	Initiation: immediately after randomization IV 1000 mL 25% glucose, 50 U insulin, 80 mmol KCl (1.5 mL/kg/h during 24 h) IV 1000 mL 10% glucose, 20 U insulin, 40 mmol KCl (1.0 mL/kg/h during 24 h) Standard care	Feasibility Effect on clinical endpoints to develop rationale for performance of large scale trial	Feasible, clinical benefit in patients with reperfusion strategy	
Van der horst et al. 2003 / GIPS I [116]	Open-label randomized (n = 940)	STEMI treated conservatively, with PCI of CABG DM& non-DM <24 h symptoms	Initiation: before reperfusion IV 500 mL 20% glucose, 80 mmol KCl (3 mL/kg/h during 8–12 h) + on-pump 50 U insulin in 50 mL sodium adjusted to keep blood-glucose levels be- tween 7.0 and 11.0 mmol/L Standard care	Mortality at 30 days	No difference	Lower mortality in patients without HF
Rasoul et al. (2007) GIPS II [117]	Open-label randomized (n = 889)	Suspected AMI treated conservatively, with thrombolysis or PCI	Initiation: before reperfusion IV 20% glucose, 80 mmol KCl (2 mL/kg/h during 12 h) + short acting insulin based on admission glucose and hourlymeasured glucose Standard care	Mortality at 30 days and 1 year	No difference	

Study	Trial design	Patients	Treatment protocol	Primary endpoints	Outcome	Comments
b Malmberg et al. 1995 / DIGAMI I [b] [118]	Randomized controlled (n = 620)	Suspected AMI treated conservatively or with thrombolysis DM <24 h suspected AMI	Initiation: immediately after randomization IV 500 mL 5% glucose, 80 U insulin started at 30 mL/h, adjusted according to glucose levels. Duration ≥24 h, followed by subcutaneous insulin 4 times daily ≥3 months Standard care	Mortality at 3 months (patients allocated to strata)[c]	Lower mortality in stratum 1	Increase in hypoglycemic episodes
[b] Malmberg et al. 2005 / DIGAMI II [57]	Randomized open-label (n = 1253)	Suspected AMI treated conservatively, PCI or CABG DM <24 h suspected AMI	Initiation: immediately after randomization IV 500 mL 5% glucose, 80 U insulin started at 30 mL/h, adjusted according to glucose levels. Duration ≥24 h, followed by tight glucose-control during follow-up IV 500 mL 5% glucose, 80 U insulin started at 30 mL/h, adjusted according to glucose levels. Duration≥24 h, followed by standard diabetes care Standard care	Mortality comparison during follow-up between groups 1 and 2	No difference	Hyperglycemia strong independent mortality predictor
Selker et al. 2012 / IMMEDIATE [119]	Double-blind randomized (n = 871)	Suspected ACS treated conservatively, with thrombolysis, PCI or CABG DM& non-DM	Initiation: in ambulance IV 30% glucose, 50 U/L insulin, 80 mEq of KCl/L (1.5 mL/kg/h during 12 h) IV 5% glucose	Progression of suspected ACS toMI within 24 h (biomarkers & ECG evidence)	No difference	Less cardiac arrest & in hospital mortality Lower infarct size at 30 days (SPECT)
Glucagon-like peptide-1 (GLP-1)						
Nikolaidis et al. 2004 [120]	Nonrandomized (n = 21)	STEMI with killip class of HF II–IV and LVEF <40%, treated with PCI DM& non-DMb6h symptoms	Initiation: after reperfusion and LVEF assessment IV rGLP-1 (1.5 pmol/kg/min during 72 h) No adjunct therapy	Improvement of global and regional LV-function 6–12 h after infusion (echo)	Higher LVEF, higher regional recovery peri-infarct zone	No differences between diabetic and non-diabetic patients

Study	Trial design	Patients	Treatment protocol	Primary endpoints	Outcome	Comments
Read et al. 2011 [121]	Double-blind randomized (n = 20)	Elective PCI No MI <3 months Non-DM	Initiation: after 1st balloon occlusion IV GLP-1 (1.2 pmol/kg/min during complete PCI-procedure) IV saline (during complete PCI-procedure)	LV function (biochemistry & hemodynamic measurements)	Less LV dysfunction, less myocardial stunning	
Lonborg et al. 2012 [122]	Double-blind randomized (n = 172)	STEMI undergoing PCI DM& non-DM <12 h symptoms	Initiation: 15 min before reperfusion IV bolus exenatide (0.12 μg/min during 15 min), followed by IV (0.043 μg/min during 6 h) IV saline + albumin	Myocardial salvage index after 3 months (CMR)	15% increase myocardial salvage index	19% increase myocardial salvage index in anterior infarction
Bernink et al. 2013 / EXAMI [123]	Double-blind randomized (n = 39)	STEMI undergoing PCI Non-DM <6h symptoms	Initiation:≈30 min before reperfusion IV bolus exenatide (5 μg in 30 min, followed by IV 0.014 μg/min during 72 h) IV saline + albumin	Safety & feasibility	Safe & feasible	Trend towards lower infarct size / AAR ratio in exenatide group
Woo et al. 2013 / EMPIRE [124]	Single-blind randomized (n = 58)	STEMI undergoing PCI DM & non-DM	Initiation 5 min prior to reperfusion: 10 μg exenatide SC and 10 μg IV, followed by 10 μg SC twice daily during 72 h Placebo	Infarct size (CK-MB and troponin I) release during 72 h & CMR at 1month Conventional & speckle tracking echo	Lower infarct size, higher sub clinical LV function	

pected acute coronary syndrome (ACS) were randomized to GIK-infusion or placebo in the ambulance, thereby significantly shortening system delay. Although the primary endpoint, progression of ACS to MI within 24 h was not reached, a statistical significant reduction in infarct size measured with SPECT (2% GIK, 10% placebo) was seen in the GIK group and the composite of cardiac arrest and in-hospital mortality was lower (6.1% GIK, 14.4% placebo). The final infarct size was measured in a relatively small sub-population (49 receiving GIK, 61 receiving placebo), and the infarct-limiting effect of GIK might have been even larger, if final infarct size was chosen as primary endpoint. Long-term follow-up is under way.

Although GIK-infusion during the ischemia–reperfusion period has a mechanistic rationale and although several studies are encouraging, this intervention entails drawbacks that should be mentioned. For delivery of sufficient quantities of glucose, insulin and potassium to the ischemic and reperfused myocardium, a relative large volume (ranging from 30 mL per hour up to 210 mL per hour a person of an average weight of 70 kg) has to be administered. In patients with pre-existing heart failure the burden of receiving such a relative high volume load might be detrimental and abolish the potential positive effect of GIK. This was reason for the GIPS II and IMMEDIATE investigators to exclude this patient population. [119,142] In addition, the IMMEDIATE investigators limited infusion time to a maximum of 12h. [119] Furthermore, GIK infusion is associated with difficulties in maintaining a balance in potassium and glucose values. [117] These drawbacks do not count for the glucagon-like peptide (GLP-1) and the GLP-1 receptor agonists.

Glucagon-like peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) is an incretin hormone, which in response to food intake, lowers blood glucose by stimulating insulin secretion and production and suppressing glucagon in a glucosedependent manner, reducing the risk of hypoglycemic overshoot. [143] Because of these features, GLP-1 receptor agonists are currently used as a glucose-lowering agent in patients with type 2 diabetes mellitus. [144] Besides its action on the pancreatic islets, receptors for GLP-1 are present in many organs, including the heart. [145] In experimental studies, activation of cardiac GLP-receptors promoted myocardial glucose uptake, similarly to GIK, resulting in improvements in metabolic efficiency in ischemic and reperfused myocardium. [146] Moreover, induced activation of pro-survival, anti-apoptotic pathways was shown among the mechanisms. [147] Two clinical studies have been perfor-

med with GLP-1 in the setting of myocardial ischemia and reperfusion injury. Nikolaidis and co-workers were the first to demonstrate that a 72-hour infusion with GLP-1 improved LV function measured by echocardiography in 21 patients with acute MI and severe systolic dysfunction. [120] Read and co-workers then showed that GLP-1 infusion reduces myocardial stunning during elective PCI of the LAD in 20 non-diabetic patients. [121] The clinical application of native GLP-1 is limited however, due to its rapid degradation in vivo by the ubiquitous enzyme dipeptidyl-peptidase-4 (DPP-4), resulting in a circulating half-life of 1–2 min. [148]

Glucagon-like peptide-1 receptor agonists (GLP-1RA)

The problem of rapid degradation of GLP-1 by DPP-4 could be overcome by inhibiting the action of DPP-4. Adding DPP-4 inhibitors as sitagliptin or vildagliptin proved successful in enhancing the GLP-1 potential. [148] To our knowledge however, no clinical studies have been performed with regard to lethal reperfusion injury with DPP-4 inhibitors. The GLP-1 receptor agonists exenatide, approved in 2005 in the United Stated of America (USA), and liraglutide, approved in 2010 in the USA, are currently used as blood-glucose lowering agents in the treatment of type 2 diabetes mellitus. [144] Exenatide, synthetic exendin-4, derived from the saliva of the Gila monster, is resistant to DPP-4 degradation. [148] Exenatide shares a 53% amino-acid homology to native GLP-1, but is a potent agonist of the GLP-1 receptor. [144,148] Liraglutide, a synthetic analog of the human GLP-1, shares 97% homology of native GLP-1. Due to acylated modifications, liraglutide is resistant to DPP-4 degradation and has a long half-life. [144]

In a large animal study, intravenous exenatide just prior to reperfusion activated anti-apoptotic pathways and reduced oxidative stress, resulting in 40% infarct size reduction. [149] In a similar model, liraglutide pretreatment before ischemia reperfusion injury without an additional bolus did not reduce infarct size. [150] Thus far, exenatide is the only GLP-1 receptor agonist that has been studied in a clinical setting, with regard to reperfusion injury in patients with an acute myocardial infarction.

Lønborg and co-workers performed the first clinical study with exenatide. [122] In this double-blinded randomized clinical trial 172 predominantly non-diabetic patients with an acute MI and thrombolysis in myocardial infarction (TIMI) 0 or 1 flow were allocated to either a intravenous loading dose exenatide or placebo 15 min prior to PCI for the duration

of 15 min, after which infusion rate was reduced and continued for 6 h. The primary endpoint, myocardial salvage index measured by CMR, was evaluated in 105 patients, which demonstrated a 15% higher MSI in the exenatide group. The infarct size corrected for the area at risk (AAR) was 23% smaller in the exenatide group. Moreover, a post-hoc analysis of 74 patients (38 patients receiving exenatide) with a short system delay (<132 min) showed a reduction in infarct size of 30% in the exenatide group. [151]

Woo et al. recently published their clinical trial in which they demonstrate a reduction of final infarct size (not corrected for the AAR) by treatment with an intravenous exenatide bolus, followed by 3 days of subcutaneous treatment. [124]

Parallel to these studies, we investigated the effect of exenatide versus placebo in 40 non-diabetic patients in a pilot study with STEMI undergoing primary PCI. [123] The primary goal of this randomized double blinded clinical pilot trial was to demonstrate safety and feasibility of a high intravenous exenatide bolus and subsequent intravenous therapy for the duration of 72 h. Nausea a common side effect of exenatide was more often observed in the exenatide group, but did not lead to termination of the study protocol in any patient. Although not powered for differences in efficacy endpoints, a trend towards a lower infarct size/AAR ratio was seen in a subpopulation of 23 patients with TIMI 0 and 1 flow receiving exenatide. The larger follow-up of the EXAMI study, with infarct size/AAR ratio measured with CMR as primary endpoint, is ongoing.

Cardiac magnetic resonance as a surrogate endpoint

Most trials that have been conducted on this topic are small and are powered for surrogate endpoints. The mostly used surrogate endpoint is final infarct size (creatine kinase and troponin area under the curve, SPECT, CMR), as this is a strong predictor for morbidity and mortality. [64] Specifically CMR has emerged as a very important imaging modality for infarct size measurement. [152] It is safe in the first week after primary PCI, microvascular obstruction, intramyocardial hemorrhage, left ventricular dimensions and function, and left ventricular thrombus. Most importantly, however, it allows for the assessment of the AAR (i.e. the area of edema in the perfusion area of the infarct related artery).

The determination of the AAR is essential for the calculation of myocardial salvage and corrects for differences in perfusion territory of the infarct related artery among the treatment arms, which is important in relatively small proof-of-concept trials. It has to be

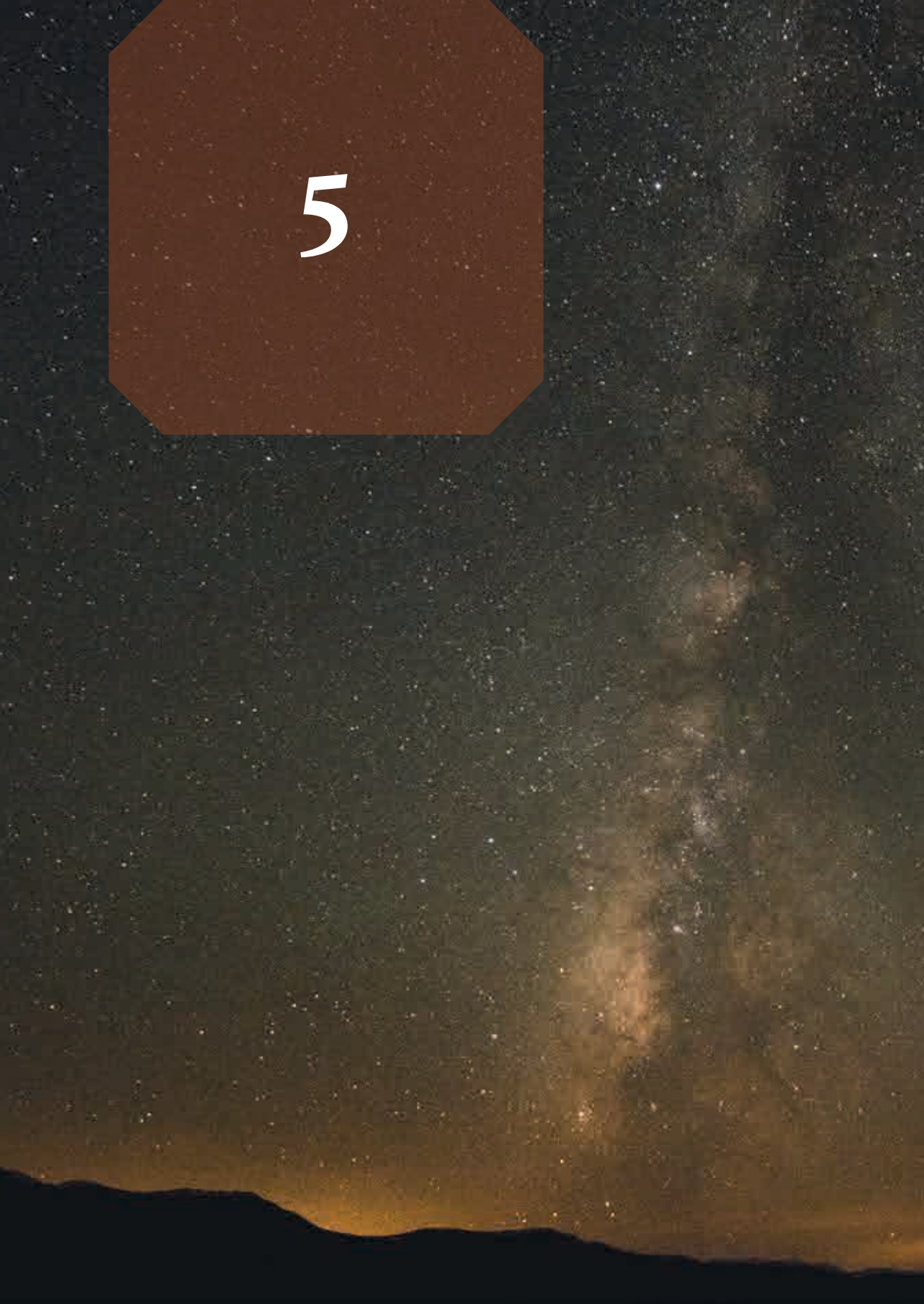
mentioned, however, that there is some controversy about the robustness of cardiac MRI to assess the AAR. [152,153]. Improvements in T2 mapping sequences are warranted to enhance the accuracy of AAR determination using cardiac MRI.

4.3 Future perspectives

Despite multiple promising results with strategies to reduce lethal reperfusion injury in the experimental setting, results in clinical studies have been mostly disappointing. Both clinical as preclinical barriers are responsible for this, as reviewed previously. [154] Pre-clinical studies should be designed to serve the clinical purpose and consequent results should be obtained with a new cardioprotective strategy before preceding onto clinical studies. Promising novel therapies should first be tested in small proof-ofconcept trials, preferably powered for myocardial salvage index or infarct size corrected for the AAR measured by means of CMR. Such studies afford and provide a first indication of treatment effect. Only the most promising trials should be followed by larger randomized multicenter trials to demonstrate the efficacy on clinical endpoints. These studies should also help in identifying the patients that would benefit most from additional cardioprotective therapies. Current evidence points to patients with a large AAR, LAD infarctions, a short system delay and TIMI 0–1 flow on coronary angiogram. [81,87,89,110,151] Novel advances in antithrombotic therapy in the pre-hospital setting will probably result in improved myocardial reperfusion before arrival at the hospital. In addition, efforts are made to enhance fibrinolytic potency of early pre-hospital thrombolytic therapy by using microbubbles and ultrasound. [155] Such developments will shift the initiation of reperfusion from the hospital setting to the pre-hospital setting. It will therefore become more important to test cardioprotective strategies in the pre-hospital setting, e.g. by remote ischemic preconditioning or treatment with a pharmaceutical compound in the ambulance.

4.4 Conclusions

Despite early reperfusion by primary PCI and advances in antithrombotic therapy, the morbidity and mortality of patients with acute MI remain significant. It is likely that lethal reperfusion injury, induced by the biological and chemical changes induced by restoration of the blood flow to the ischemic area, contributes to this. Although initially a wide range of therapies aimed at reducing lethal reperfusion injury have been tested negatively in the clinical setting, more recent proof-of-concept clinical trials are promising. The most promising results are obtained with ischemic postconditioning, remote ischemic perconditioning, ANP, adenosine, cyclosporine and exenatide. These developments justify the conduction of large multicenter studies to investigate whether prevention of lethal reperfusion injury improves clinical outcome in patients with STEMI undergoing primary PCI.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Chapter 5:
No benefit of additional treatment with exenatide in patients with an acute myocardial infarctiont

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Abstract

Introduction

This double blinded, placebo controlled randomized clinical trial studies the effect of exenatide on myocardial infarct size. The Glucagon-like peptide-1 receptor agonist exenatide has possible cardioprotective properties during reperfusion after primary percutaneous coronary intervention for ST-segment elevation myocardial infarction.

Methods

One-hundred and ninty one (191) patients were randomly assigned to intravenous exenatide or placebo initiated prior to percutaneous coronary intervention using 10 μg/h for 30 minutes followed by 0.84 μg/h for 72h. Patients with a previous myocardial infarction, Trombolysis In Myocardial Infarction flow 2 or 3, multi-vessel disease, or diabetes were excluded. Magnetic resonance imaging (MRI) was performed to determine infarct size, area at risk (AAR) (using T2-weighted hyperintensity (T2W) and late enhancement endocardial surface area (ESA)). The primary endpoint was 4-month final infarct size, corrected for the AAR measured in the acute phase using MRI.

Results

After exclusion, 91 patients (age 57.4±10.1 years, 76% male) completed the protocol. There were no baseline differences between groups. No difference was found in infarct size corrected for the AAR in the exenatide group compared to the placebo group (37.1 ± 18.8 vs. 39.3 ± 20.1%, p=0.662). There was also no difference in infarct size (18.8 ± 13.2 vs. 18.8 ± 11.3% of left ventricular mass, p=0.965).

Conclusion

Exenatide did not reduce myocardial infarct size expressed as a percentage of AAR in ST elevated myocardial infarction patients successfully treated with percutaneous coronary intervention.

5.1 Introduction

ST elevated myocardial infarction is a leading cause of mortality and morbidity, caused by acute occlusion of one or more of the epicardial coronary arteries. Therapy is focussed on fast restoration of antegrade flow preferably by means of a primary percutaneous coronary intervention. [156,157] Successful reperfusion however, paradoxically also induces death of cardiomyocytes. This is mediated by a multitude of factors that eventually culminate in loss of mitochondrial integrity and hypercontracture, leading to cardiomyocyte death. [66] This phenomenon is called reperfusion injury and contributes for up to 40% to the final myocardial infarct size [158], which is an important determinant of clinical outcome in patients with ST elevated myocardial infarction. [159] Therapies to prevent reperfusion injury are therefore of utmost importance.

Glucagon-like-peptide-1 (GLP-1) is an incretin hormone with insulinotropic and insulinomimetic properties. The GLP-1 receptor is also present on cardiomyocytes and infusion of GLP-1 has been shown to activate anti-apoptotic pathways and increase myocardial metabolic efficiency in preclinical and clinical studies. [120,147,160]

Exenatide is a long acting GLP-1 receptor agonist and is used widely for improving glycemic control in patients with type 2 diabetes mellitus. [74] In preclinical models of myocardial ischemia and reperfusion injury exenatide reduces myocardial apoptosis and oxidative stress, resulting in reduced infarct size and preserved cardiac function. [149,161]

Recently, exenatide therapy was shown to increase myocardial salvage [122] and decrease final infarct size [124] in ST elevated myocardial infarction patients successfully treated with percutaneous coronary intervention. Exenatide is therefore considered one of the most promising compounds to reduce infarct size. [47] The current study was designed to investigate the effect of exenatide on myocardial infarct size as a percentage of the area at risk (AAR) in patients with ST elevated myocardial infarction who underwent successful percutaneous coronary intervention.

5.2 Methods

Overview

The study protocol has been published previously. [162] This multi-centre, prospective, randomized, placebo controlled clinical trial was executed at the VU University Medical Centre, Amsterdam, and the University Medical Centre Utrecht, Utrecht, the Netherlands. All patients gave oral informed consent prior to percutaneous coronary intervention and written informed consent after percutaneous coronary intervention. The local ethics committees approved of the protocol. This study was performed in accordance to the declaration of Helsinki. No financial support was provided from the manufacturer. The study was registered at https://clinicaltrials.gov identifier: NCT01254123

Patient population

Consecutive adult patients with ST elevated myocardial infarction with a symptom duration of less than 6 hours were enrolled in the study. Exclusion criteria were primarily: a known history of diabetes mellitus, prior myocardial infarction or coronary artery bypass grafting, a clinically unstable patient (ie. Cardiac shock, ventricular rhythm disorders and Killip class >1 excluded) and any known contra-indications to magnetic resonance imaging. Randomization took place using envelops, created by the primary investigator, in block sizes of 6. A research nurse was unblinded upon enrolment of a patient to prepare the study medication. The study medication was then transferred to a blinded nurse, who administered the study medication to the patient. Investigators, patients and other care providers remained blinded. After randomization to placebo or exenatide, patients were treated with percutaneous coronary intervention and standard drug therapy according to local and hospital guidelines valid at the time of admission. Prior to or during percutaneous coronary intervention, additional exclusion criteria could arise; patients were excluded if they had multi-vessel disease in need of acute coronary artery bypass grafting or additional percutaneous coronary intervention, because significant multi-vessel disease could potentially have impact on the AAR assessment. Patients were also excluded if no culprit lesion was found or if the culprit vessel had Thrombosis in Myocardial infarction 2 / 3 flow. In these patients, treatment using exenatide or placebo was discontinued immediately upon reaching one of the

angiographic exclusion criteria. The study protocol was continued in patients that remained eligible for the study after primary percutaneous coronary intervention. The current study also includes patients enrolled in our pilot safety study. [123] These patients met the same in- and exclusion criteria.

Treatment protocol

On admission, patients were immediately randomized to double-blind treatment with exenatide or placebo. The study medication was prepared as follows: a 50 ml syringe was filled with 49 ml of NaCl 0.9% and 1 ml human serum albumin with or without 15 μg of exenatide, leading to a concentration of 0.3 μg/ml. All patients received a loading dose (5 μg) in 30 minutes using a 33.3 ml/h intravenous infusion, followed by a 2.8 ml/h (20 μg/day) infusion for the remainder of the 3 days. The syringe was replaced every 8 hours.

Study endpoints

The primary endpoint of this study was final infarct size measured by magnetic resonance imaging at 4 months after myocardial infarction, expressed as a percentage of the area at risk (AAR) measured with T2W magnetic resonance imaging in the first week after ST elevated myocardial infarction. (Figure 10) Secondary endpoints included final infarct size, myocardial salvage index (MSI), ejection fraction at baseline and 4 months assessed by magnetic resonance imaging and major adverse cardiac events (major adverse cardiac events, defined as cardiac death, myocardial infarction, coronary artery bypass grafting or repeat percutaneous coronary intervention) in 4 months.

Creatine kinase muscle brain was measured on admission and every 6 hours following percutaneous coronary intervention. In the first 20 patients treated with exenatide, plasma levels of exenatide were measured 4 hours and 24 hours after lowering the initial study medication infusion rate.

Magnetic resonance imaging

Magnetic resonance imaging was performed at 3-7 days after ST elevated myocardial infarction and at 4 months follow-up. The protocol included Cine, T2 weighted (T2W) and late gadolinium enhancement (LGE) imaging. [162] Parameters acquired consisted of left ventricular function (ejection fraction, volumes and left ventricular mass), area at risk using T2W and the endocardial surface area (ESA), microvascular obstruction (MVO) and

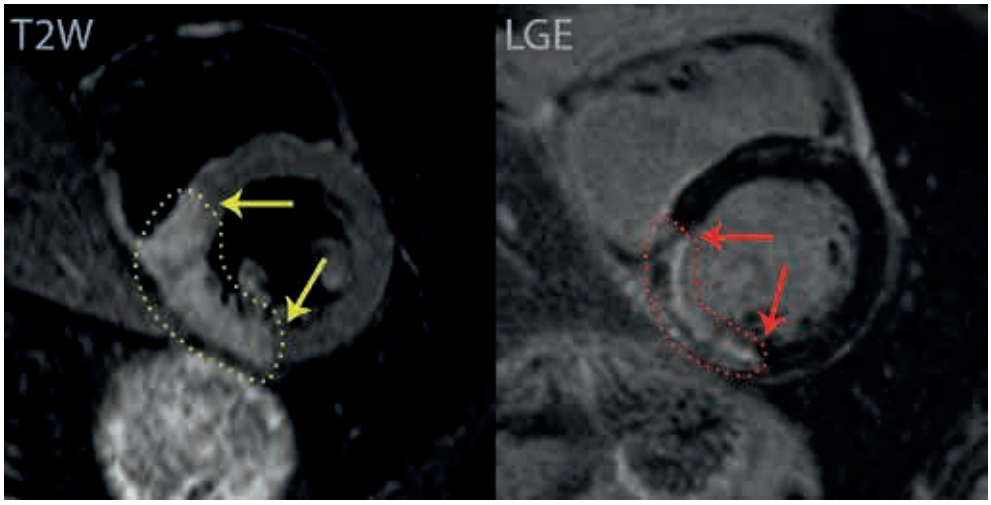


Figure 10: Measurement of the primary endpoint. The primary endpoint of final infarct size as percentage of the AAR was calculated using T2W images at baseline and LGE images at follow-up. Contours delineate myocardial oedema (Yellow) and infarct size (Red). Arrows point to the region of interest. The computer calculated oedema and infarct size within the region of interest.

infarct size at baseline. MVO was defined as the low signal intensity region within the high intensity infarct zone on LGE images. At follow-up, left ventricular function and infarct size were measured. Parameters were indexed for body surface area and calculated as percentage of left ventricular mass where applicable. The MSI was calculated in 2 ways: using the AAR measured with T2W imaging (MSI_{T2W}) [163] at baseline and ESA measured on LGE baseline images (MSI_{ESA}). [164] The formula to determine MSI was (AAR-infarct size) / AAR. Infarct size was measured in the acute phase (baseline) and at 4 months follow up magnetic resonance imaging using LGE images.

Sample size

With a 5% type 1 error risk, a power of 90% and an anticipated dropout of 10%, 108 patients (54 per group) were needed to detect a 15% improvement of the primary endpoint.

Statistical analysis

All patients were analysed using intention to treat protocol. Data was tested for normal distribution using kurtosis and skewness, values between -2 and 2 were considered to be normally distributed data. Independent sample t-test was used for continuous variables. Chi

square test and Fisher Exact were used for categorical data. 1-way ANOVA with Bonferroni post-hoc testing was be used to compare subgroups of the study population. Kolmogorov-Smirnov testing was performed when applicable for nonparametric data.

5.3 Results

Study population

Between November 2009 and September 2014, a total of 191 out of 412 screened patients with ST elevated myocardial infarction undergoing primary percutaneous coronary intervention fitted the pre-angiographic inclusion criteria and were randomly assigned to treatment with exenatide or placebo. After percutaneous coronary intervention, 108 patients (51 exenatide, 57 placebo) remained in the study due to exclusion criteria met during angiography. At follow-up, a 19% dropout led to 91 patients (42 exenatide, 49 placebo) that completed the study protocol. Most dropouts were due to insufficient imaging quality available for analysis of the primary endpoint. (Figure 11) The trial was ended as sufficient patients were included before MRI analysis took place. There were no baseline differences between both groups. (Table 12) The mean age was 57.4 years and 76% of patients were male. Symptom to balloon time was 170±83 vs. 188±91 min (p=0.35) for exenatide and placebo respectively. The left anterior descending artery was culprit artery in 30% of patients. Unfractionated heparin together with a loading dose of a P2Y12 inhibitor and intravenous aspirin were administered prior to percutaneous coronary intervention in all patients according to current European Society for Cardiology guidelines.(18) Glycoprotein IIb/IIIa inhibitors were administered in 30% of patients. Most percutaneous coronary intervention procedures (90%) resulted in final Thrombolysis In Myocardial Infarction 3 flow. A complete overview of procedural data is provided in Table 13.

Myocardial infarct size and myocardial salvage

The primary endpoint of infarct size as a percentage of the AAR did not differ between patients treated with exenatide and placebo (37.1 ± 18.8 vs. 39.3 ± 20.1%, p=0.662). There was also no difference in final infarct size and myocardial salvage. (Table 14) Infarct location, body mass index, gender and initial therapeutic treatment differences showed no confounding effects.

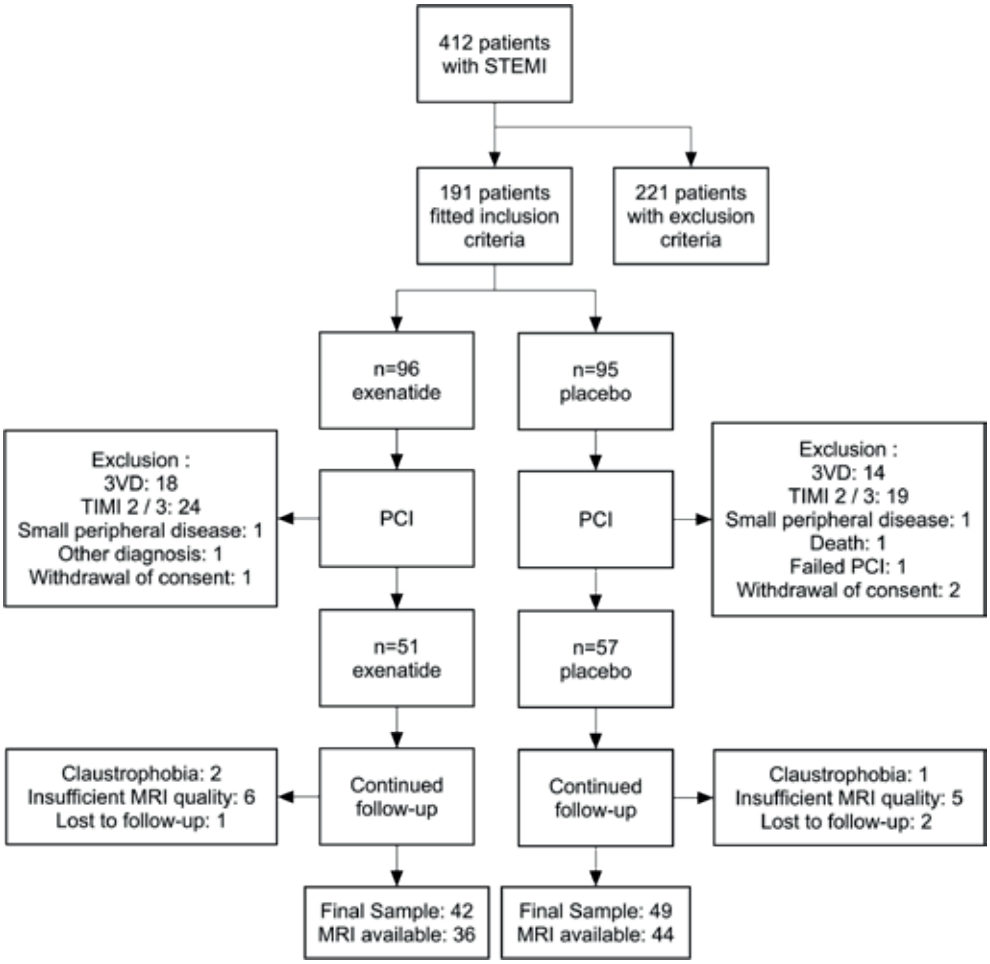


Figure 11: Study flowchart

Other endpoints

There were no differences in baseline and follow-up left ventricular volumes, functional parameters and occurrence of MVO. A significant difference in left ventricular mass was found, patients that received exenatide had higher left ventricular masses, but this difference disappeared after adjusting for body surface area. (Table 14) Creatine kinase muscle brain-max was 239 ± 146 and 249 ± 191µg/L (p=0.39) for exenatide and placebo respectively. Median plasma levels of exenatide 4 hours after percutaneous coronary intervention were 0.14 [0.01 – 1.73] nmol/L.

Table 12: Baseline characteristics. Numbers as 'mean (± SD)' or 'n (%)' where applicable. N = number, SD = Standard Deviation, BMI = Body Mass Index, Hb = Hemoglobin, CRP = C-Reactive Protein, eGFR MDRD = Estimated Glomerular Filtration Rate Modification of Diet in Renal Disease, CK-MB = Creatine Kinase Muscle Brain, CK = Creatine Kinase, HbA1c = Hemoglobin A1c [a], nonparametric Kolmogorov Smirnov test was used.

	Exenatide (n=42)	Placebo (n=49)	P-value
Age (years)	57.23 (±10.2)	57.48 (±10.1)	0.906
Male (%)	33 (79%)	36 (73%)	0.576
BMI (kg/m^2)	27.47 (±4.1)	26.39 (±3.2)	0.167
Body surface area	2.57 (±0.22)	1.99 (± 0.20)	0.311
Risk factors			
Past/current smoker	18 (45%)	26 (55.3%)	0.688
Hypertension	9 (22.5%)	6 (13.3%)	0.274
Hypercholesterolemia	9 (23.1%)	12 (27.9%)	0.622
Positive family history	18 (43.9%)	26 (56.5%)	0.245
Laboratory results			
Hemoglobin (mmol/L)	9.01 (±0.8)	8.87 (±1.6)	0.593
CRP (mg/L)[a]	3.98 (±3.8)	7.67 (±22.6)	0.357
Cholesterol (mmol/L)	5.56 (±1.1)	5.96 (±1.0)	0.084
Creatinin (µmol/L)	78.9 (± 19.0)	77.8 (±23.4)	0.822
eGFR MDRD (ml/min/1.73 m^2)	93.8 (± 22.4)	94.4 (±33.9)	0.919
NTproBNP (ng/L)[a]	75.2 (±138.2)	145.9 (±407.8)	0.294
CKMB max (µg/L)[a]	326 (±593)	249 (±191)	0.389
CK Max (U/L)[a]	2510 (±1888)	2650 (±2076)	0.738
Troponin T max (VUmc) (ng/L)	4690 (±4000)	4310 (±5600)	0.76
Troponin I max (UMCU) (ng/L)	36.6 (±38.2)	43.6 (±40.0)	0.694
Blood glucose (mmol/L)[a]	8.20 (±1.8)	8.01 (±2.1)	0.655
HbA1c (mmol/mol)[a]	38.59 (±4.4)	39.56 (±5.4)	0.363

Side effects and safety

Nausea, a notorious side effect of exenatide, occurred significantly more often in patients receiving exenatide (38 vs 8%, p=0.001) No changes had to be made to the infusion rate of study medication in these patients. Hypoglycemic episodes occurred equally between groups (24 vs 18%, p=0.53), but hyperglycemia occurred more often in the placebo treatment arm (7 vs 20%, p=0,064). (Table 15) Two patients receiving exenatide developed an exanthema after > 36 hours of infusion, resulting in the preventive cessation of study therapy

Table 13: Procedural data from PCI. Numbers as 'mean (± SD)' or 'n (%)' or 'mode [±range]' where applicable. N = number, SD = Standard Deviation, PCI = Percutaneous Coronary Intervention, FMT = First Medical Contact, LAD = Left Anterior Descending, RCX = Right Circumflex, RCA = Right Coronary Artery, TIMI = Thrombolysis In Myocardial Infarction, BMS = Bare Metal Stent, DES = Drug Eluting Stent, GP IIb/IIIa = GlycoProtein IIb/IIIa

	Exenatide (n=42)	Placebo (n=49)	P-value
Treatment pre-PCI			0.451
Heparin	42	49	
Aspegic	42	49	
Clopidogrel	14 (33%)	11 (22%)	
Prasugrel	15 (36%)	25 (51%)	
Ticagrelor	13 (31%)	13 (27%)	
GP IIb/IIIa	12 (32%)	13 (28%)	0.635
Procedural data			
Symptom-to-balloon time (min)	170 (±83)	188 (±91)	0.345
FMC-to-balloon time (min)	73 (±15)	84 (±18)	0.588
Door-to-balloon time (min)	41 (±12)	49 (±19)	0.058
Thrombosuction	36 (86%)	36 (71%)	0.101
Culprit artery			0.585
LAD	13 (31%)	14 (29%)	
RCX	6 (14%)	10 (20%)	
RCA	23 (55%)	25 (51%)	
TIMI grade before procedure			0.738
0	37 (88%)	42 (86%)	
1	5 (12%)	7 (14%)	
TIMI grade after procedure			1.0
2	4 (9%)	5 (10%)	
3	38 (91%)	44 (90%)	
Stent type			0.524
BMS	10 (%)	9 (18%)	
DES	32 (%)	40 (82%)	

and successful administration of an antihistaminic agent. At 4 months follow-up, no major adverse cardiac events had occurred. One patient had received a pacemaker due to AV block.

Table 14: Imaging results MRI and primary endpoint. Numbers as 'mean (± SD)' or 'n (%)' or 'mode [±range]' where applicable. N = number, SD = Standard Deviation, BSA = Body Surface Area, LV = Left Ventricle, EDV = End Diastolic Volume, ESV = End Systolic Volume, EF = Ejection Fraction, MVO = Microvascular Obstruction, AAR = Area At Risk, ESA = Endocardial Surface Area. EDV, ESV and mass are corrected for BSA

	Exenatide	Placebo	P-value
LV EDV (ml, n=87)	184.15 (± 38.44)	174.89 (± 40.07)	0.277
LV ESV (ml, n=91)	84.54 (± 30.71)	81.23 (± 34.17)	0.630
LV mass (gr)	115.65 (± 29.55)	107.46 (± 25.27)	0.172
LV EDV indexed	88.13 (± 13.58)	87.61 (± 16.64)	0.875
LV ESV indexed	40.51 (± 13.82)	40.65 (± 16.30)	0.964
LV mass indexed	55.33 (± 11.55)	53.78 (± 11.11)	0.529
LV SV (ml)	91.53 (± 19.97)	85.54 (± 19.16)	0.240
LV EF (%, n=86)	52.18 (± 7.25)	51.17 (± 7.35)	0.525
MVO present (n=79)	19 (50%)	22 (54%)	0.745
AAR T2W (gr, n=66)	36.79 (± 17.54)	31.72 (± 18.95)	0.267
MSI ESA (n=73)	0.59 (± 0.21)	0.55 (± 0.22)	0.491
MSI T2W (n=66)	0.63 (± 0.19)	0.61 (± 0.20)	0.662
Follow-up LV EDV (ml, n=87)	196.34 (± 36.95)	180.72 (± 39.33)	0.075
Follow-up LV ESV (ml, n=91)	94.78 (± 27.98)	87.47 (± 29.92)	0.272
Follow-up LV mass (gr)	104.46 (± 26.08)	89.77 (± 22.67)	0.010
Follow-up LV SV (ml)	99.10 (± 17.31)	92.65 (± 19.62)	0.218
Follow-up LV EF (%, n=86)	52.42 (± 8.34)	52.66 (± 8.35)	0.897
Follow-up LV EDV indexed	93.58 (± 14.66)	91.39 (± 16.09)	0.532
Follow-up LV ESV indexed	45.20 (± 12.95)	44.01 (± 13.59)	0.696
Follow-up LV mass indexed	49.56 (± 10.29)	45.31 (± 10.05)	0.073
Final infarct size (gr, n=77)	13.12 (± 9.21)	12.75 (± 9.41)	0.868
Final infarct size as % of LV mass	13.30 (± 8.97)	15.06 (± 10.53)	0.460
Final infarct size as % of AAR T2W	37.08 (± 18.78)	39.27 (± 20.12)	0.662

Table 15: Adverse events. Numbers as 'mean (± SD)' or 'n (%)' or 'mode [±range]' where applicable. MACE = Major Adverse Cardiac Events

	Exenatide (n=42)	Placebo (n=49	P-value
Nausea	16 (38%)	4 (8%)	0.001
Need for anti-emetics	14 (33%)	3 (6%)	0.001
Hypoglaecemic episode	10 (24%)	9 (18%)	0.530
Hyperglaecymic episode	3 (7%)	10 (20%)	0.064
MACE	2 (5%)	2 (4%)	0.876

5.4 Discussion

In contrast to previous clinical trials using exenatide in ST elevated myocardial infarction patients, our trial shows no benefit of using exenatide on top of primary percutaneous coronary intervention in ST elevated myocardial infarction patients. This may indicate that the cardioprotective effect of exenatide is less than previously thought, or that it depends on several specific conditions. Therefore, it is of utmost importance to understand the differences between this trial and the previous studies.

Trial differences

Two previous trials investigated the cardioprotective effect of exenatide in patients with ST elevated myocardial infarction undergoing primary percutaneous coronary intervention and reported a beneficial effect on myocardial salvage in a Danish study [122] and final infarct size in a Korean study. [124] Most baseline clinical characteristics, procedural characteristics and average AAR are comparable with the Danish and Korean studies. Another report by Lønborg et al. in 2012 showed that exenatide reduced infarct size in patients with a short system delay, i.e. < 132 minutes, and not in patients with a system delay > 132 minutes. [151] Most likely the ischemic area is beyond repair if the ischemic duration is too long. In our trial the system delay was shorter (76 minutes vs 132 minutes) and the symptom to balloon time was comparable with the Danish trial. Despite the short system delay, we were not able to confirm a cardioprotective effect of exenatide. Other factors must have played a role.

For example, in our study less patients with anterior infarctions were included. While the average infarct size as a percentage of AAR (38%) and final infarct size (13gr) were similar to the previously published trials, patients included in our trial suffered from anterior infarctions in only 30% of cases, which was 40% in the Danish trial. [122] In the Danish trial, myocardial salvage was more pronounced in anterior MI than in non-anterior infarct location. This might be of interest in determining the exact subgroup of patients with myocardial infarction that benefits from exenatide treatment.

Our study also included more smokers (Table 12) and fewer patients were treated with glycoprotein (GP) IIb/IIIa inhibitors in our study compared to the Danish study (30% vs 90%; no data provided in the Korean study). We did not observe an interaction between

smoking and GPIIb/IIIa inhibitors and infarct size, but a relationship cannot be ruled out because our study was not powered for subgroup analysis. GP IIb/IIIa inhibitors can be considered in patients if no-reflow occurs after percutaneous coronary intervention. [157] There is evidence for reduced infarct size in ST elevated myocardial infarction patients receiving abciximab. [165] A potential synergistic effect between abciximab (or other GP IIb/IIIa inhibitors) and exenatide could explain the difference in outcome between our study and the Danish study.

Diabetes

Patients with known diabetes mellitus were excluded from our study, for the arbitrary reason to exclude a potential effect from glucose control instead of a direct effect on apoptosis. Exenatide might be more effective in patients with diabetes mellitus, as glucose control might contribute to an improved clinical outcome. [166] Because of the preclinical evidence, and the relatively low number of patients with diabetes mellitus in the previous trials (4-9% in the Danish and 25-28% in the Korean) it is unlikely that exenatide mediated cardioprotection is exclusively present in patients with diabetes mellitus. Consequently, this doesn't explain the different outcomes between the clinical trials.

Underestimation of effect

Furthermore, potential favourable effects of exenatide in this study might have been underestimated, because of the assessment of the AAR using T2W magnetic resonance imaging. This modality of AAR assessment is based on myocardial oedema. Since exenatide might also reduce myocardial oedema, the AAR could have been underestimated in the exenatide treatment arm, and therefore the infarct size in relation to the AAR overestimated. This effect might be enhanced by the time-dependence of oedema in the first week after ST elevated myocardial infarction that adds to the large variability of AAR assessment using T2W imaging. [167] Lønborg et al. however used the same modality and observed a favourable effect of exenatide. Also, the ESA does not have these limitations and also did not show a difference between our patient groups.

Treatment protocol

The most obvious difference between the trials is the exenatide treatment protocol. Our initial bolus dose was chosen based on our unpublished previous experience with he-

althy subjects and was demonstrated to be the highest well tolerated dose, not inducing severe nausea. The maintenance dose and duration were based on results of our previous preclinical study [149] and a clinical study with GLP-1 [120], in order to achieve a potential beneficial effect on metabolic efficiency and cardiac function. We previously demonstrated this protocol to be safe and feasible for application in patients with ST elevated myocardial infarction. [123]

We administered an exenatide bolus of 5 μg in 30 minutes IV followed by an infusion of 20 μg per day for 3 days, whereas by Lønborg et al. an initial bolus of 1.8 μg IV (in 15 minutes) was given and another infusion of 15.5 μg over the next 6 hours. These protocols resulted in exenatide plasma levels of 0.01-1.73 nmol/L (mean 0.14 nmol/L, measured 4½ hours after initiation of treatment) in our study and of 0.1-0.39 nmol/L (mean 0.177 nmol/L; measured 15 minutes after initiation of the treatment) in the Danish study. Unfortunately, plasma levels cannot be easily compared due to the different time points. Woo et al treated patients with a 10 μg exenatide bolus intravenously and a 10 μg subcutaneous dose 5 minutes before reperfusion, followed by a 10 μg twice daily subcutaneous injection for three days, in accordance with our preclinical study [149], but plasma levels were not measured.

The cascade of events resulting in reperfusion injury is initiated in the first minutes after reperfusion. [66] Therefore it is important to obtain a therapeutic plasma level before the onset of reperfusion. In all 3 studies, the treatment was initiated before reperfusion. Woo et al administered the highest intravenous dose before reperfusion (10 μg). In the Danish study, all participants received at least 1.8 μg before reperfusion. On average, our 42 patients in the treatment arm received 4.82 (± 1.09) μg of exenatide prior to balloon inflation. Thus, a higher dose of exenatide was administered before reperfusion in our study than in the Danish study. Nonetheless, the Danish investigators observed a reduction in myocardial infarct size, whereas we did not. A biphasic dose-effect relationship of exenatide has been suggested for exenatide in an isolated rat heart model, with a loss of a cardioprotective effect with plasma levels exceeding 3.0 nmol/L. [161] Unfortunately, we don't have exenatide plasma levels available around the time of reperfusion. It cannot be ruled out that plasma levels were too high, possibly resulting in a loss of cardioprotection. However, in the Korean study by Woo et al even a higher intravenous dose of 10 μg was administered before reperfusion.

Although no correction was made for the AAR in this study, the reduction in final infarct size suggests a cardioprotective effect of exenatide using a very high bolus dose. Ano-

ther possibility is that exenatide exerts its most important cardioprotective actions not so much in the first minutes, but in the first hours after reperfusion. Both Lønborg and Woo administered a higher total dose in the first 6 hours after reperfusion than we did in the present study.

Tolerability

An important concern regarding a high treatment dose is the tolerability. Nausea is a well-known side effect of exenatide described to occur in up to 40-50% of the patients. [168] Severe nausea requiring the need for anti-emetics in most cases occurred in 38% of the patients receiving exenatide in this study, following the initial 30 minute bolus dose, versus 8% in patients receiving placebo. The previous studies do not report data on the occurrence of nausea.

Limitations

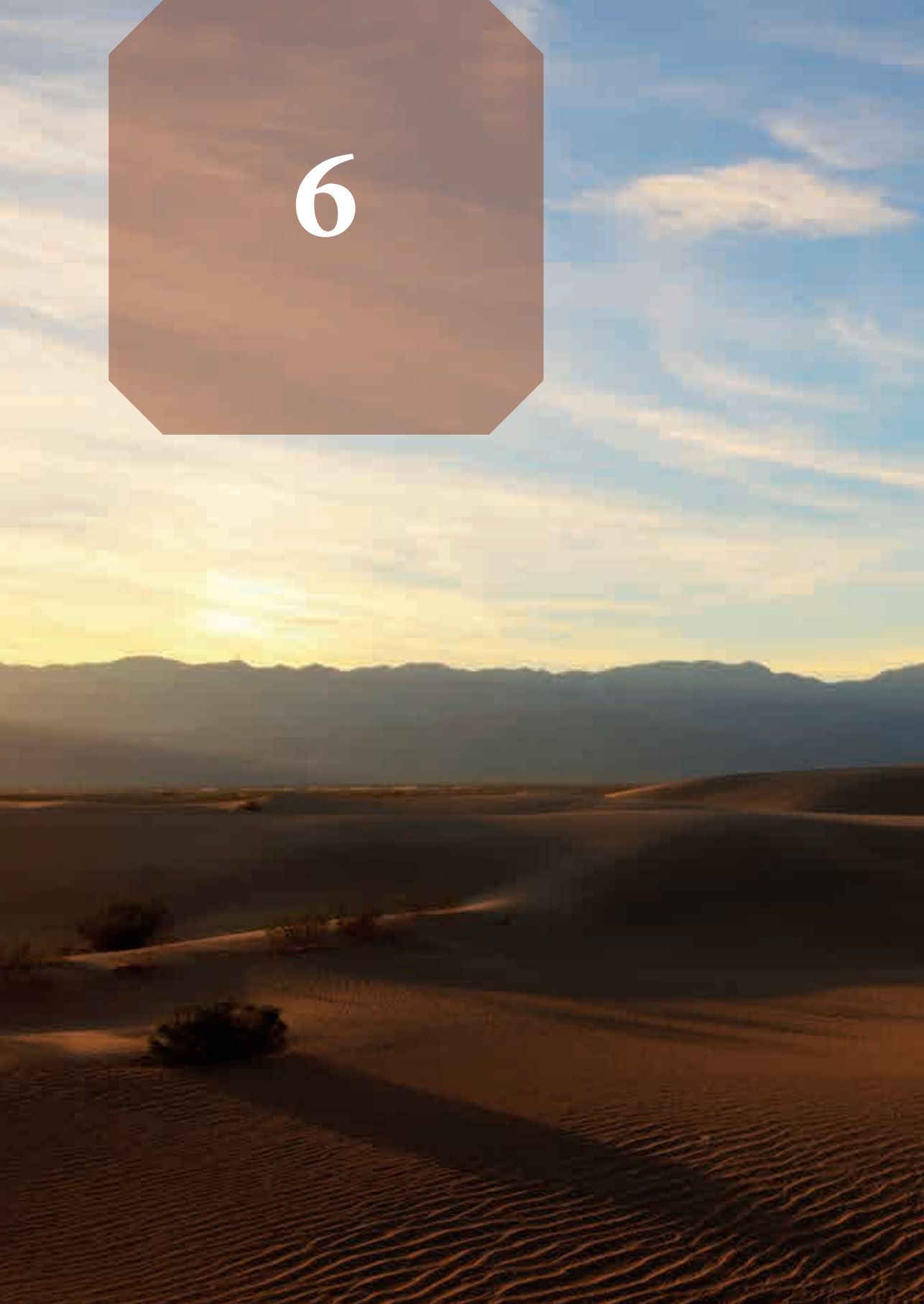
Due to a higher dropout than expected the number of patients that was included in the final analysis was slightly lower than anticipated. However, the final endpoints are all comparable between exenatide and placebo without any trend towards a cardioprotective effect of exenatide. Expansion of the groups is therefore unlikely to change the interpretation of the results. A new power analysis based on the results from this study shows that a sample size of 1161 patients per group would be needed to meet a difference in the primary endpoint. Our exclusion rate in this study is relatively high, due to the fact that we aimed to acquire a population without multiple confounding factors such as multivessel disease, in order to determine the maximum therapeutic effect of exenatide. Also, our initial dropout of 221 patients was mostly due to a high rate of patient refusal. These factors have caused our results to be only moderately applicable to the real world situation.

5.5 Conclusion

In this study, exenatide treatment did not result in reduction of myocardial infarct size as a percentage of the AAR in ST elevated myocardial infarction patients successfully treated with percutaneous coronary intervention. Additional studies are warranted to unravel the reasons for the ambiguous trial results and to identify an optimal treatment protocol.

Part 3:
Therapeutic targets: microvascular obstruction

6



Chapter 6:
Sonothrombolysis in acute stroke and myocardial infarction: a systematic review

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Abstract

Introduction

Current treatment of patients with an acute occlusion of a cranial or coronary artery, in for example ST segment elevation myocardial infarction (STEMI), consists of either thrombolysis or percutaneous intervention. Various thrombolytic agents (tissue plasminogen activators) are used for reperfusion therapy in patients with STEMI. However, their use may be associated with an increased risk of bleeding which is inherent to their action mechanism. Therefore, new methods of coronary clot resolution are being studied in an attempt to potentiate the efficacy and reduce the side effects of thrombolytics. A new method is ultrasound mediated thrombus dissolution, or sonothrombolysis. The current literature exploring sonothrombolysis is diverse in size and quality. In this systematic review of the current literature, we describe cardiovascular applications of sonothrombolysis in patients. A comparison to the neurovascular application in ischemic stroke is made, as more research has been performed on patients suffering stroke.

Methods

A systematic search was performed following the PRISMA guidelines using EMBASE and MEDLINE databases regarding sonothrombolysis in human ischemic stroke and acute myocardial infarction patients.

Results

12 original case-control or randomized controlled trials using a combination of ultrasound and microbubbles were found, 6 trials studied ischemic stroke, 6 trials studied acute myocardial infarction.

Conclusion

This systematic review provides up to date information on the subject of sonothrombolysis.

6.1 Introduction

The primary use of echo-contrast microbubbles is the improvement of echographic images by enhancing the acoustic signal. Microbubbles undergo stable cavitation or oscillation when targeted by a diagnostic ultrasound beam of low intensity. This increases the diagnostic quality of ultrasound images acquired in patients as more sound is returned to the ultrasound probe and thus more information is available to the clinician. However, additional therapeutic effects of microbubbles have been discovered in the last two decades. [169]

In contrast to diagnostic ultrasound, therapeutic ultrasound consists of higher intensity ultrasound. The microbubble not only oscillates but violently bursts and erupts under the intense ultrasound pressure. This violent reaction releases energy in the local environment. [170] Because of this energy release, the application of therapeutic ultrasound as a method of clot destruction in the setting of acute myocardial infarction and acute ischemic stroke has been the topic of intensifying research in human studies. [171–174] If diagnostic and therapeutic ultrasound parameters are combined in the same probe, diagnostic ultrasound with a microbubble infusion can locate the occluded vessel and is capable of visualizing replenishment of microbubbles in the vasculature after application of a therapeutic ultrasound impulse. (Figure 12)

These high intensity impulses are almost always combined with a loading or continuous dose of fibrinolytic agents and this method of treatment is therefore often called sonothrombolysis. [175] Fibrinolytic agents, unfortunately, have the downside of a relatively high occurrence of bleeding as a side-effect. Bleeding is associated with adverse outcome, for example inducing additional haemorrhagic stroke during treatment. Primary goal of investigation into sonothrombolysis is to minimize bleeding risk and maximize treatment efficacy, while possibly reducing treatment delay and achieving early revascularization. When sonothrombolysis is combined with a low dose of antithrombotics, equal efficacy might be achieved while reducing bleeding risk. Increasing the efficacy of ultrasound treatment while decreasing the dose of fibrinolytic agents, is therefore needed. This can be achieved by use of microbubbles [176,177], possibly even without use of a fibrinolytic agent. [178]

An example of an area in which microbubbles and sonothrombolysis are being investigated clinically is in ischemic stroke patients. [155,177] After the discovery of a potential

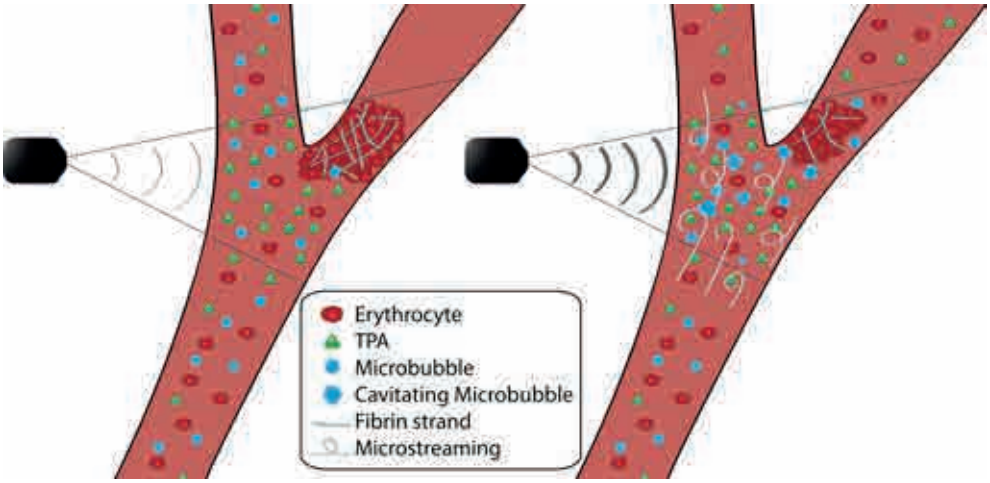

Figure 12: Increasing acoustic pressure using higher duty cycle amount creates cavitating microbubbles. Cavitation creates, amongst other effects, microstreaming which is capable of destroying emboli. These emboli would otherwise not be destroyed using TPA alone.

beneficial effect of pulsed wave Doppler ultrasound combined with recombinant tissue plasminogen activator (rTPA) in patients with acute ischemic stroke [179], several studies have now been performed investigating the effect of ultrasound and rTPA in patients with ischemic stroke. Different ultrasound contrast agents have been utilized in this setting, each with different compositions, and include either lipid or galactose based agents containing either atmospheric air or other gasses. A clinical study compared SonoVue® (Bracco) with Levovist® (Schering) in patients with acute ischemic stroke. A total of 138 patients were included, randomly assigned to treatment with either type of microbubbles. While both agents appeared to improve stroke recanalization rates, there were no clinically significant differences in efficacy and safety found between the two types of microbubbles. [180]

Several in-vivo and in-vitro tests have already been performed to evaluate the efficacy and safety of sonothrombolysis in the setting of acute ST elevation myocardial infarction (STEMI). To date, only one pilot study has been performed in patients who underwent randomized treatment to placebo or sonothrombolysis prior to primary percutaneous coronary intervention (PCI). [181] Other studies in STEMI patients were restricted to ultrasound and (r)TPA; no microbubbles were used except in the previously described pilot study.

PCI is nowadays the favoured method of treatment in STEMI patients. While mar-

kedly decreasing mortality as a result from early recanalization, a significant portion of patients suffers from a phenomenon called no-reflow. While epicardial recanalization is achieved, microvascular perfusion is limited. This microvascular obstruction has a multifactorial origin and is in part thought to consist of microthrombi occurring as a result from the PCI. An additional therapeutic field of interest for the concept of sonothrombolysis is the periprocedural treatment of these microthrombi occurring during PCI. [2,182–184]

Patients in need of quick revascularization, such as in ischemic stroke or myocardial infarction benefit most from a method of treatment in which the clot is dissolved quickly and safely. This systematic review will focus on these patients and elaborate on the current use and level of evidence regarding sonothrombolysis.

6.2 Methods

Search strategies and selection criteria

Literature searches were performed using the Pubmed and Cochrane database. Case-control studies or randomized controlled trials comparing regular thrombolytic therapy and thrombolytic therapy using ultrasound with or without microbubbles were included. In this regard all STEMI studies using this method of treatment were included. Studies regarding ischemic stroke were only included if they randomized treatment for microbubbles. Only studies performed in human patients of adult age written in English, German and Dutch were included in this study. Key words used in the search included sonothrombolysis, thrombolysis, microbubble recanalization, myocardial infarction, microbubble ultrasound thrombolysis, thrombolysis microstreaming, thrombolysis myocardial infarction, microspheres, coronary ultrasound thrombolysis, microbubble ischemic stroke.

Studies using sonothrombolysis with microbubble treatment in patients with ischemic stroke patients are presented in Table 16. The studies concerning human sonothrombolysis in patients with STEMI are summarized in Table 17.

Table 16: Neurology – Clinical studies assessing the effect of ultrasound and microbubbles on thrombolysis in ischemic stroke patients. (r)TPA: (reverse) tissue plasminogen activator, US: ultrasound, MB: microbubbles, PW: Pulsed Wave, CD: Color Doppler, MI: Mechanical Index

Authors	Patients	Randomization factor	Microbubble	US settings	Outcome
Molina et al. 2006 [177]	111	tPA, tPA with US, tPA with US and MB	Levovist	2 MHz	Improvement in short-term clinical outcome and recanalisation rate
Alexandrov et al. 2008 [185]	78	tPA, tPA with US, tPA with US and MB	Perflutren	2 MHz	Improvement in (sustained) recanalisation rate
Perren et al. 2008 [186]	26	r-tPA with US, r-tPA with US and MB	Sonovue	2 MHz PW CD, 189 mW/cm^2	Improvement in clinical outcome after 24 hours when using MB (p=0.05)
Ribo et al. 2009 [187]	18	US, MB rescue treatment	Levovist	2 MHz PW, 750 mW	Safe and feasible
Rubiera et al. 2008 [180]	138	Type of microbubble used	Levovist and Sonovue	1.97 MHz PW, 385 mW/cm^2, MI 0.24	No difference between microbubbles
TUCSON trial 2009 [188]	35	r-TPA, r-TPA with US and 1.4 ml MB, r-TPA with US and 2.8 ml MB	MRX-801	2 MHz, 606 mW/cm^2, MI 0.196	1.4 ml safe, higher dosage associated with severe intracranial haemorrhaging

Table 17: Cardiology – Clinical studies assessing the effect of ultrasound on thrombolysis in STEMI patients. Abbreviations: (r)TPA: (reverse) tissue plasminogen activator, US: ultrasound, PW: Pulsed Wave, MI: Mechanical Index, MB: microbubble, NTG, nitroglycerin, ASA; acetylsalicylic acid, IVUD; Intravascular ultrasound device, TTE; transthoracic echocardiogram. [1] Catheter-based invasive ultrasound therapy

Authors	Patients	Randomization factor	Drugs used	US settings	Outcome
Hamm et al. 1997 [172]	14	No randomization	NTG, ASA, heparin	IVUD, 19.5 kHz [1]	Safe and feasible
Rosenschein et al. 1997 [189]	15	No randomization		IVUD, 45 kHz [1]	Safe and feasible
Singh et al. 2003 [190]	281	Ultrasound / Abciximab	Abciximab	IVUD, parameters unknown	Higher recanalization rate with Abciximab
Cohen et al. 2003 [171]	25	No randomization	Reteplase or tenecteplase	TTE, 27 kHz continuous ultrasound, 0.9 W/cm^2	Safe and feasible
PLUS trial 2010 [173]	360	Ultrasound	ASA, heparin or enoxaparin. Tenecteplase	TTE, PW, 0.12 W/cm^2	Decrease in ST elevation at 90 minutes
Slikkerveer et al. 2012 [155]	10	Microbubble (Luminity ®)	ASA, heparin, alteplase	TTE, 1.6 MHz 3D, MI 1.18, max 26 mW/cm^2	Safe and feasible

6.3 Results

The use of sonothrombolysis in patients with ischemic stroke will be reviewed first. The second part will consist of the use of sonothrombolysis in cardiology. Finally, future applications of microbubble treatment will be discussed.

The clinical trials in ischemic stroke had a wide range of transducer settings and frequencies and most of them compared treatment with rTPA alone, treatment combining rTPA with ultrasound and microbubbles combined with ultrasound and rTPA. (Table 16)

All studies in STEMI patients (Table 17) used either TPA or rTPA but differed in the type of transducer used. Two studies were performed using catheter-based intravascular ultrasound probes, three studies used therapeutic transthoracic ultrasound and one pilot study used diagnostic transthoracic ultrasound. Only one pilot study administered microbubbles in the acute STEMI setting, in a 15 minute infusion prior to PCI. Although microbubbles were safely administered in this setting with no occurrence of MACE even after 6 months, no data on efficacy can be weaned from this study.

Sonothrombolysis in acute ischemic stroke

Alexandrov et al. were the first to describe a beneficial effect of ultrasound on clot dissolution in stroke patients treated with rTPA under transcranial Doppler monitoring. [179] A later publication by the same group found a trend towards better outcome and earlier recanalization when patients were randomized between treatment with TPA and ultrasound combined with TPA. [191] In-vitro studies have demonstrated an enhancement of thrombolysis when microbubbles were added to the treatment regimen. [192,193] Molina et al. compared three different treatment regimens for ischemic stroke. Patients were included if treatment started within 3 hours after onset of ischemic stroke. Treatment with TPA (n=36) was compared with ultrasound and TPA (n=37), and the combination of TPA, ultrasound, and perflutren-lipid microbubbles (n=38). The TPA, ultrasound and microbubbles group achieved the highest amount of complete clot resolution (54.5%) compared to TPA (23.9%) and TPA with ultrasound (40.8%), p=0.038. [177]

Another study performed by Alexandrov et al. [185] used data from the CLOTBUST trial [194] as historic control. Patients in both studies were included if onset of stroke was within 3 hours of presentation. This study administered perflutren-lipid microbubbles, in

combination with transcranial Doppler and TPA, and showed encouraging results. An increase of complete recanalization from 18% (TPA only, n=63) to 50% (TPA, ultrasound and microbubbles, n=12) was observed when ultrasound was combined with microbubbles. Furthermore, recanalization was sustained significantly more often (42%) after two hours with the addition of microbubbles, compared with ultrasound and TPA (38%, n=63) and TPA alone (13%) (p=0.003). The authors found evidence that microbubbles might have permeated beyond the occlusion, further enhancing the sonothrombolytic effect, while ultrasound and TPA alone did not penetrate completely, but this was not clarified in the manuscript.

The same effect was found by Perren et al. comparing the addition of SonoVue® microbubbles (n=11) to a treatment regimen with rTPA and ultrasound (n=15) in acute stroke patients. Patients were only included if treatment with intravenous rTPA and ultrasound monitoring already showed some effect and patients were no longer eligible to receive intra-arterial rTPA. Patients received microbubbles only if a duplex ultrasound signal could be picked up but it was impossible to visualize the entire artery, implicating thrombus obstruction. Outcome was measured using a standardized stroke scale. Flow increase was higher in patients receiving microbubbles after both 30 (p=0.03) and 60 minutes (p=0.03) Patients receiving microbubbles significantly improved compared to controls (p=0.05). [186]

Another study by Ribo et al. used galactose based microbubbles as a backup to regular treatment. However, in this study, microbubbles and TPA were directly injected in the clotted artery through selective catheterization. This intra-arterial technique was used if reperfusion was not achieved within one hour after initiation of TPA and ultrasound therapy. Nine out of 18 patients received this rescue treatment; five showed complete recanalization after 12 hours, and two showed partial recanalization (78%). [187]

Molina et al. used MRX-801, a phospholipid-coated perfluoropropane microbubble similar to perflutren but with a higher concentration of microbubbles, in patients with acute ischemic stroke. In this randomized prospective trial the first cohort treated with ultrasound, TPA and MRX-801 (n=12) showed encouraging results, but there were three cases of intracranial haemorrhage in the second cohort (n=11), treated with a two-fold higher dose of microbubbles. [188] Although these patients had severe strokes and may have had other risk factors for intracranial haemorrhage including hypertension, this study suggested that higher doses of microbubbles may increase the risk of haemorrhagic stroke. Although earlier studies already described an increase in intracranial hemmorhage in some cases, this

was largely attributed to the use of low frequency ultrasound (< 300 kHz). [195]

A meta-analysis, using randomized and nonrandomized clinical studies, performed by Tsivgoulis et al. confirmed the hypothesis that higher frequencies ultrasound are less detrimental with a lower odds ratio in the occurrence of stroke. [196] The occurrence of intracranial haemorrhage might be attributable to the formation of standing waves when using low frequency ultrasound and even disruption of the blood-brain barrier. [197]

A possible higher incidence of intracranial haemorrhage when using high frequency ultrasound as used in the study by Molina et al. might be related to the enhanced effectiveness of TPA when administered in the presence of microbubbles. This supposition was supported by subsequent work of Culp et al., in a rabbit model of stroke, which showed that 1-Mhz ultrasound with TPA and microbubbles increases recanalization rates compared to ultrasound and TPA alone, without an increase in the incidence of intracranial haemorrhage. [178] They also showed effective sonolysis using microbubbles and ultrasound without TPA in a rabbit stroke model. Less intracranial haemorrhage was observed in animals treated with microbubbles and ultrasound alone compared to animals also treated with TPA. [198] This reduction was significant compared to earlier human trials with TPA. [188,195,199] If one assumes that ultrasound and microbubbles alone were effective because they potentiated the effect of endogenous TPA, these results suggest that human studies of microbubbles and ultrasound in stroke patients without tPA, or with low dose TPA, merit consideration.

Sonothrombolysis in Acute Coronary Syndromes

Feasibility, efficacy and safety of transtemporal ultrasound and microbubbles have all been studied in patients with stroke. However, sonothrombolysis is still in its early stages of investigation as a treatment option in STEMI patients. In 1997, two studies were performed using intravascular catheter based sonothrombolysis in patients with STEMI. Both studies used low ultrasound frequencies (19.5 and 45 kHz), applied directly before PCI. PCI was only performed if TIMI grade was below three after ultrasound application. The first study showed intravascular ultrasound improved TIMI flow by at least 1 grade in 13 out of 14 patients. [172] The second study utilizing 45 kHz ultrasound showed TIMI 3 flow prior to PCI was achieved in 86% of patients (total n=15). [189]

The ATLAS study was performed in STEMI patients with an occluded saphenous vein bypass graft (SVBG) and treated with either intravascular therapeutic ultrasound (n=92) or

abciximab (n=91), both followed by PCI. It was shown that ultrasound alone was capable of opening a clotted SVBG with a success rate of 63%, while patients who were under treatment with abciximab alone showed a higher recovery rate of 82% (p=0.008). Also, patients treated with ultrasound alone had a higher incidence of major adverse cardiac events compared to abciximab (p=0.036), but this result is confounded by a higher incidence of Q-wave myocardial infarction in the ultrasound group. The trial was terminated prematurely because of these observations. [190]

Later research focused on the transthoracic application of therapeutic ultrasound. Cohen et al. published results from a transthoracic ultrasound feasibility and safety testing study. A total of 25 patients received reteplase (n=15) or tenecteplase (n=10), and all patients received transcutaneous ultrasound therapy. A low frequency of 27 kHz was used in this trial, combined with a skin cooling system. Therapy started within 30 minutes after arrival in the hospital and continued for 60 minutes. Afterwards, coronary angiography was performed to assess TIMI flow. The results demonstrated that 64% of patients had TIMI 3 flow after ultrasound therapy. Ultrasound parameters were changed after 15 patients, because 3 patients developed skin blistering. These parameters have not been specified by the authors. This study showed the technique to be safe and feasible. [171]

Based on promising results in the previously described and other earlier in-vitro and in-vivo studies [200–203], a large double blind randomized controlled trial was conducted in 391 patients with STEMI. Patients were randomized to either thrombolysis (n=182) or thrombolysis with transthoracic low frequency ultrasound (n=178). All patients were treated with aspirin, unfractionated heparin or enoxaparin, and either reteplase or tenecteplase. The ultrasound group received 60 minutes of low frequency ultrasound (28.3 kHz) with a spatial peak pulse average intensity of 0.38 W/cm2, unless worsening occurred and patients required resuscitation or angiography. Patients in the control group were attached to the same ultrasound generator plus a sham transducer and experienced mild warmth and vibration. Primary end points where achieving TIMI grade 3 flow and > 50% cumulative ST-segment resolution after 60 minutes of thrombolytic administration. This trial was terminated prematurely because no difference was detected in the treatment groups during interim analysis. No significant difference in adverse cardiac events was observed. However, a significant difference in 90 minute ST segment resolution was found in favour of the ultrasound treated group. Part of the population included in this specific statistical analysis had already recei-

ved primary PCI when ST segment resolution was measured. Nonetheless, the authors conclude this might be because of an improvement in myocardial tissue perfusion [173], which was also observed with low frequency ultrasound in vivo. [204] Recently, a pilot study was performed, experimenting on the concept of microbubble enhanced sonothrombolysis in STEMI. A low dose thrombolytic therapy was combined with primary PCI and compared to the addition of microbubble enhanced sonothrombolysis in acute STEMI patients. The pilot study evaluated the effectiveness and feasibility of microbubble treatment in a clinical setting in patients with STEMI. Ten patients were included, equally randomized into treatment or control groups. A 3D echotransducer with a frequency of 1.6 MHz was used. First results show a non-significant difference in TIMI 3 flow rates achieved before PCI between control (1 out of 5 patients) and treatment (3 out of 5 patients) groups. Because of the low number of patients included thus far, no statistical difference in epicardial coronary recanalization rate was observed and no difference in cardiac function was observed during follow-up. No serious adverse events occurred and the study design was deemed feasible and safe. [155]

6.4 Discussion

In ischemic stroke patients, sonothrombolysis without microbubbles have shown a varying degree of success [194,199] when using pulsed wave Doppler transducers. However, in the lower ranges of ultrasound frequencies (< 1 MHz), there is a higher risk of intracranial haemorrhage. [195] When microbubbles are added and higher frequencies were used, the technique proved to be relatively safe and feasible with clinical recovery rates significantly higher when compared to controls. [177,185–188]. Almost all studies thus far have used a sonothrombolysis treatment combined with full dose (recombinant) tPA therapy. Low and no dose tPA experiments are successful in animal models, showing a decrease in systemic side-effects, especially bleeding, while maintaining a local sonothrombolytic effect. [178,198] Studies treating patients with low dose fibrinolytic regimens combined with ultrasound and microbubbles should be designed next to achieve a balance between efficacy and bleeding risk.

Case-control studies evaluating the effect of microbubbles and sonothrombolysis in patients with a myocardial infarction are scarce. A number of clinical trials have been performed comparing sonothrombolysis without microbubbles with standard thrombolytic

therapy, and the results are not consistent. Few clinical studies have been performed in STEMI patients using sonothrombolysis. A large trial was aborted because of lack of effect [173], but two smaller trials showed promising results. [171,205] Three trials used low frequency therapeutic ultrasound (19.5, 27 and 45 kHz) not registered for clinical diagnostic use. Two of these used intracatheter ultrasound devices to achieve thrombolytic effect prior to PCI. While no comparison was made with control patients, clinical safety results and the high percentage of restoration to TIMI 3 flow is encouraging.

All STEMI trials focussed on epicardial recanalization of blood flow. Ultrasound guided cavitation might also target microvascular recanalization. The amount of no-reflow was not measured in any study using sonothrombolysis. No-reflow occurs in up to 40% of patients with acute ST segment elevation myocardial infarction despite successful primary PCI and leads to further impairment of the left ventricle and subsequent complications such as the development of heart failure. [206,207] This has an impact on quality of life, higher use of medication and more hospital admissions. Furthermore, it also leads to an increase in mortality. [208] Pre-clinical trials are already underway examining the effect of microbubble enhanced sonothrombolysis in microthrombi occluding the distal microvascular arteries.

Transthoracic and transcranial echography was performed most often in the studies described in this review. Almost all investigators used 2D transducers where sweeps are necessary to achieve full effect. The relatively new 3D transducers might prove to be of additional benefit, continuously able to target a bigger area in which cavitation occurs. A larger field of effect might be beneficial in a patient population where the culprit lesion is not yet known or found. Future studies should compare both 2D and 3D transducers.

Regarding pulse duration, one experimental in-vivo atherosclerotic pig study compared a 5 second pulse duration with a 20 second pulse duration in a situation of acute myocardial infarction by thrombotic occlusion. An improvement was visible suggesting a benefit to increasing pulse duration. [209] The pilot study in STEMI patients uses a 5 second pulse duration and while we await further results, studies should consider using a longer pulse duration to improve microbubble enhanced sonothrombolytic effect.

In a clinical setting, the diagnostic frequencies used with a transthoracic ultrasound transducer have been utilized effectively during treatment with sonothrombolysis. Their imaging capabilities permit detection of microbubbles within the microvasculature, providing information about replenishment of microbubbles after the application of a therapeutic

high MI impulse and thus allowing optimal application of a new therapeutic impulse. When using simultaneous low MI imaging to guide and optimally time the therapeutic impulses, more microbubbles are available for cavitation, enhancing the effect of sonothrombolysis. Such image guidance may also be critical to the safe and effective application of sonothrombolysis in stroke and treatment of other vascular thrombosis because the ultrasound beam can be aimed directly to the area of interest.

Future clinical application of sonothrombolysis might also encompass a broad scale of medical disciplines, e.g. in vascular atherothrombotic disease or venous thromboses. Pre-hospital treatment with sonothrombolysis in acute coronary syndromes might be used as a neoadjuvant therapy to primary percutaneous coronary interventions. This is especially true for countries that no longer using thrombolytic treatment in STEMI patients due to readily available specialized PCI centres. Sonothrombolysis might also be more effective compared to TPA in a setting of chronic thrombosis, as TPA is optimally used in acute thrombi and rapidly loses efficacy when a thrombus ages. [210]

6.5 Conclusion

Thrombolysis can be enhanced using microbubble accelerated sonothrombolysis. This has been shown extensively in ischemic stroke patients. There is evidence that this treatment option improves outcome in patients with ischemic stroke, dissolving these clots and improving clinical and long-term outcome while possibly reducing bleeding risk.

Few, very heterogeneous, studies exist examining the effect of ultrasound induced cavitation of microbubbles on human patients with STEMI. The technique of sonothrombolysis shows theoretical promise as an adjunctive treatment to PCI but needs to be studied in more detail. Additionally, sonothrombolysis might be used after PCI to lower the incidence of no-reflow in patients. Ultrasound parameters used should be carefully considered when designing a clinical trial. Clearly, improvements in therapeutic transducers are needed, and these therapeutic transducers must be combined with diagnostic imaging to optimize ultrasound induced cavitation events. Randomized prospective trials are needed to further evaluate this new frontier of therapeutic ultrasound, which could then lead to its safe application early in the treatment of acute stroke or acute coronary syndromes.

7

Chapter 7: Sonoreperfusion Therapy Kinetics in Whole Blood using Ultrasound, Microbubbles and tPA

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Abstract

Introduction

Coronary intervention for myocardial infarction often results in microvascular embolization of thrombus. Sonoreperfusion therapy (SRP) using ultrasound and microbubbles restored perfusion in our in vitro flow model of microvascular obstruction.

Methods

In this study, we assessed SRP efficacy using whole blood as the perfusate with and without tissue plasminogen activator (tPA). In a phantom vessel bearing a 40-μm pore mesh to simulate the microvasculature, microthrombi were injected to cause microvascular obstruction and were treated using SRP.

Results

Without tPA, the lytic rate increased from 2.6±1.5 mmHg/min with 1000 cycles to 7.3±3.2 mmHg/min with 5000 cycles ultrasound pulses ($p<0.01$). The lytic index was similar between tPA-only [$(2.0\pm0.5) \times 10^{-3}$ mmHg-1min^{-1}] and 5000 cycles without tPA [$(2.3\pm0.5) \times 10^{-3}$ mmHg^{-1}min^{-1}] ($p=0.5$) but increased [$(3.6\pm0.8) \times 10^{-3}$ mmHg^{-1}min^{-1}] with tPA in conjunction with 5000 cycles ultrasound ($p<0.01$).

Conclusion

SRP restored microvascular perfusion in whole blood and SRP lytic rate in experiments without tPA increased with ultrasound pulse length and efficacy increased with the addition of tPA.

7.1 Introduction

ST elevation myocardial infarction (STEMI) is caused by the acute thrombotic occlusion of an epicardial coronary artery. Contemporary treatment for restoration of epicardial coronary artery patency is primary percutaneous coronary intervention (PCI). However, despite restoration of epicardial coronary artery patency with PCI, adequate microvascular perfusion is often not restored, a phenomenon known as microvascular obstruction (MVO) or no-reflow. This occurs in up to 55% of patients following PCI and portends poor clinical outcome. [2,211,212] MVO, largely caused by obstruction of the microcirculation with atherothrombotic debris, results in local inflammation, platelet aggregation, myocardial edema due to dysfunctional endothelium, and formation of in situ microvascular thrombi. [213,214] While many preventative and curative strategies have been employed [47,215,216], there has been no consistently efficacious therapeutic approach.

Tissue plasminogen activator (tPA) has long been used as part of the treatment regimen in ischemic stroke, ischemic coronary events and peripheral arterial occlusions [217] but while very potent, is prone to cause hemorrhage when administered systemically. [218] Recently, it was found that half-dose tPA was not associated with increased hemorrhage in patients undergoing treatment for submassive pulmonary embolism. [219] This supports the use of lower dose tPA for thrombolysis as a safer alternative. It has also been shown that the therapeutic efficacy of tPA can be enhanced by combining tPA with therapeutic ultrasound (US) and microbubbles (MB).

MB are micron sized (1-5 μm) gaseous spheres encapsulated in a stabilizing shell made of phospholipid, polymer or protein. [170] MB subjected to an ultrasound (US) pulse expand and compress and can lead to non-linear (stable cavitation) oscillations at moderate pressure levels. [220] Increasing the US pressure causes the microbubble to oscillate more violently, known as inertial cavitation. These processes involving stable and inertial cavitation causes microstreaming, fluid jets and a focal temperature increase resulting in bioeffects. [221] Strategies utilizing these potent sources of energy to effectively disrupt thrombi have been mainly focused on recanalyzing large vessels in patients with large thrombi in ischemic stroke and STEMI patients [158] and is known as sonothrombolysis.

Therapy utilizing this lytic effect to restore microvascular perfusion during MVO is

called sonoreperfusion (SRP) therapy. [222] We and others have recently shown that SRP using MB and long tone burst US could restore microvascular perfusion in a rodent model of MVO [222–224], but the optimal US therapeutic conditions for microvascular SRP remain largely unknown. Our in vitro platform offers an opportunity to investigate the mechanisms leading to efficient sonothrombolysis and the kinetics of SRP therapy using US+MB and tPA in whole blood. Whole blood, in contrast to PBS, has higher viscosity and contains endogenous tPA and other blood components, such as RBCs, WBCs, and platelets, all of which could affect MB oscillations and hence SRP efficacy. Accordingly, we examined the hypothesis that US mediated MB cavitation, as quantified by stable and inertial cavitation doses, could cause microvascular clot lysis in our in vitro MVO system in whole blood. We explored whether endogenous tPA in whole blood is sufficient to induce SRP with MB and US in our in vitro model. Finally, we compared the kinetics of MB and US SRP with tPA and a combination therapy of tPA, MB and US.

7.2 Methods

In vitro system of SRP

We used our previously described in vitro model of MVO [225], with a minor modification regarding flow speed (1.5 ml/min previously) to allow for whole blood perfusion. Briefly, the model comprised a phantom vessel containing an intraluminal mesh with 40 μm pores to simulate a cross section of the microcirculation (Figure 13). The system, maintained at 37°C, was perfused with whole bovine blood at a lower constant flow rate of 0.75 ml/min which is an approximation of the physiological flow in small arterioles. [226,227]

Bovine microthrombi were added to increase upstream pressure to 30 mmHg (range 25-35 mmHg) to mimic myocardial MVO. Upstream pressure was monitored as a surrogate marker of thrombus burden. Passive cavitation detection (PCD) was used to quantify MB activity. Depending on the experimental group, MB (2×10^6 MB/ml) were added to the blood perfusate.

US (1 MHz) was delivered with a variable pulse length (1000, 3000 or 5000 cycles) and a peak negative pressure of 1.5 MPa. The US pulses were applied for 20 minutes at a

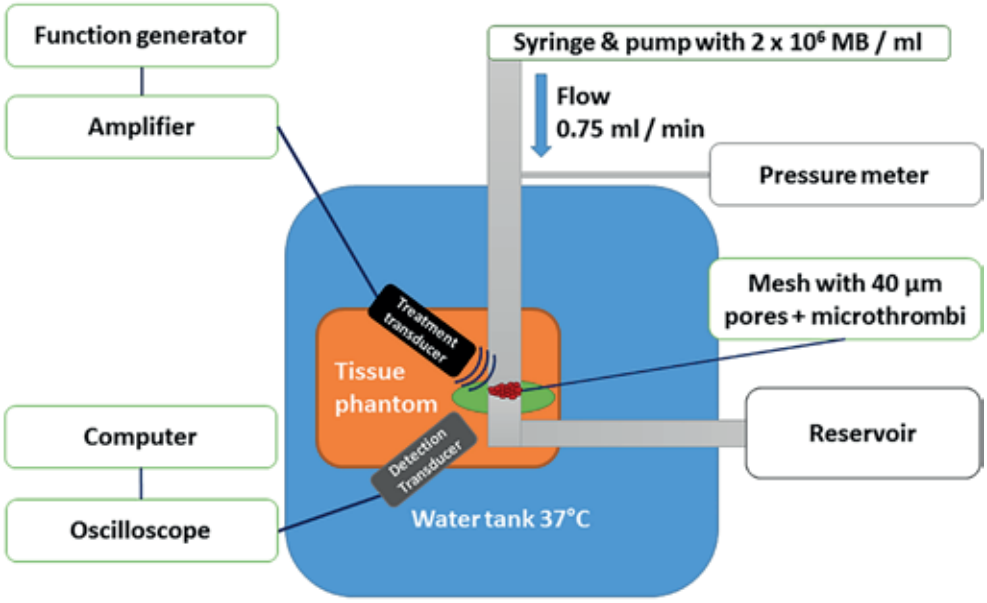


Figure 13: Experimental setup. Whole blood mixed with microbubbles (MBs) was infused at a constant rate through the flow channel. Microthrombi were trapped onto the 40 µm pore mesh, causing upstream pressure to rise. A 1-MHz treatment transducer aimed at the mesh was used to deliver the therapeutic ultrasound. Cavitation activity was detected with a 3.5-MHz transducer and digitized. Upstream pressure was monitored as a surrogate for thrombus burden.

repetition rate of 0.33 Hz to allow MB replenishment to the treatment area between pulses. Experiments without MB or US were performed as control conditions. A new mesh was mounted and exposed to microthrombi as described above for each experiment.

Perfusate

Heparin (1 IU/ml) and acetylsalicylic acid (0.06 mg/ml) were added to fresh citrated bovine blood (LAMPIRE Biological Laboratories, Pipersville, PA, USA) (< 96 h of venipuncture), to simulate the clinical presentation of acute coronary syndrome, during which patients are given heparin (5000 IE) and ASA (300 mg). tPA at 2.5 µg/ml, consistent with the steady state plasma concentration in humans during the infusion phrase [228], was added based on the experimental grouping.

Microbubbles

MB were fabricated by sonicating a mixture of 1,2-distearoyl-sn-glycero-3-phosphocholine (Avanti polar lipids, Alabaster, AL), polyoxyethylene (40) stearate (Sigma-Aldrich, St Louis, MO) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(-polyethyleneglycol)-2000] (Avanti polar lipids, Alabaster, AL) in a 2:1:1 weight ratio in the presence of perfluorobutane gas (FluoroMed, Round Rock, TX). After sonication using a 20 kHz probe (Heat Systems Ultrasonics, Newtown, CT), the MB were washed and resuspended in saline saturated with perfluorobutane and stored at 4°C until use. This procedure produced MB with a mean diameter of 3±1 μm and a concentration of 1-2×10^9 MB/ml, as measured by Multisizer-3 Coulter counter (Beckman Coulter, Brea, CA). [225]

Microthrombi

Thrombi were created by adding $CaCl^2$ (25 mM) to citrated bovine whole blood and incubating at room temperature for 3 h in type 1 borosilicate glass vials. The vial was then shaken for 20 s in a vial mixer (Vialmix, Bristol-Myers Squibb Medical Imaging, New York, NY). The thrombi were filtered through 200 μm mesh pores to produce MT < 200 μm [225]. The bovine blood was chosen for this study as it has been determined previously that ovine clots treated with plasmin most closely resemble the lysis observed with human clots. [229]

Pressure monitoring

A fluid filled pressure transducer (BD DTX plus, Becton Dickinson Co., Franklin Lakes, NJ) was positioned to monitor pressure upstream of the mesh. Baseline upstream pressure during constant flow (0.75 ml/min) and without clot, was calibrated to 0 mmHg.

Ultrasound

US was delivered from a 1 MHz focused single element transducer (A302S-SU-F1.63-PTF, 1 inch/1.67 inch focus, Olympus, Waltham, MA) driven by a pulse generator (33250A, Agilent technologies, Santa Clara CA) and a power amplifier (100A250A, Amplifier Research, Souderton, PA). The US field was calibrated with a 200-μm capsule hydrophone (HGL-0200, Onda Corp, Sunnyvale, CA). The -6 dB beam width was 3.5 mm and therefore covered >90% of the area of the mesh.

Passive cavitation detection

A focused single element broadband transducer with a center frequency of 3.5 MHz (V383-SU-F1.00IN-PTF, 0.375 inch/1 inch focus, Olympus, Waltham, MA) was confocally aligned with the treatment transducer on the mesh for PCD. The detected radio frequency signal was amplified (5073PR, Olympus, Waltham, MA), band-pass filtered (2-20 MHz cutoffs) and digitized on a digital oscilloscope (WaveRunner 6051A, Lecroy, Chestnut Ridge, NY) at 50 MHz sampling rate for off-line processing. Data corresponding to up to 5000 cycles of treatment were analyzed using joint time frequency analysis, with a window size of 250 μs and a time step of 100 μs (60% overlapping) in MATLAB (The MathWorks Inc., Natick, MA) software. The acoustic energy between 3.2-3.8 MHz, but excluding the band between 3.4-3.6 MHz, integrated over the whole tone-burst, was defined as inertial cavitation dose (ICD). The energy in the peak at the ultraharmonic band (3.48-3.52 MHz) above the broadband signal, integrated over the whole tone-burst, was defined as the stable cavitation dose (SCD). [230] The bandwidth chosen for SCD corresponded to the -6 dB bandwidth in the fundamental peak. [176,231]

Statistical and computational analysis

Data were plotted as upstream pressure (normalized to pressure at t=0) as a function of time. The lytic efficacy was quantified by the final pressure drop, the initial lytic rate (rate of pressure drop in the first 4 minutes) and the lytic index (inverse of the area under the pressure-time curve). The lytic rate indicates the initial rate of sonoreperfusion, while the lytic index indicates the overall integrated decrease of clot burden over time. All parameters were tested for significance using analysis of variance (ANOVA) with Bonferroni post hoc correction or student's t-test when applicable. Statistical analysis was performed using SPSS 22 (IBM, USA) and statistical significance was defined as $p<0.05$. Results are expressed as mean ± standard deviation.

7.3 Results

SRP in whole blood without additional tPA

Upstream pressure decreased during SRP therapy, indicating decreased thrombus burden, and SRP efficacy varied by the specific US regime with and without MB (Figure 14). With US alone (1.5 MPa, 5000 cycles, no MB) pressure did not decrease after 20 min of treatment (n=3, $\Delta P=3.9\pm16.9\%$, $p=0.576$). However, sonoreperfusion was achieved when MB were added.

Using 1000 cycles US at 1.5 MPa for 20 minutes with MB, there was a significant reduction in upstream pressure (n=7, $\Delta P=33.7\pm21.7\%$, $p=0.005$). Increasing the pulse length to 3000 cycles yielded a further increase in SRP efficacy (n=5, $\Delta P=57.7\pm13.6\%$ $p<0.001$). A 5000 cycle pulse at 1.5 MPa also produced a significant reduction in pressure (n=5, $\Delta P=59.2\pm13.8\%$, $p=0.013$), but this was not significantly greater than with 3000 cycles ($p=0.906$). In the presence of MB, the lytic rate increased with cycle length: the lytic rate increased from 2.6±1.5 mmHg/min at 1000 cycles to 7.3±3.2 mmHg/min at 5000 cycles ($p<0.01$). Removing MB from the treatment protocol resulted in a very low lytic rate (0.5±0.1 mmHg/min), which was significantly lower than that for 5000 cycle US+MB therapy ($p<0.01$).

SRP in whole blood supplemented with tPA

Compared to previous experiments using PBS perfusate (Leeman et al, 2012), upstream pressure did not fully return to baseline during SRP therapy in whole blood perfusate without tPA. However, a further pressure reduction was achieved with the addition of tPA. When tPA was administered during 1.5 MPa and 5000 cycles, a marked reduction in upstream pressure was observed (n=5, $\Delta P=87.6\pm8.2\%$, $p<0.001$). Using tPA alone (no MB, no US), upstream pressure decrease compared to when tPA and US+MB therapy were applied together was similar ($p=0.655$). The pressure versus time curve for tPA alone was notable for the absence of an initial rapid descent (decreased lytic rate) seen in the US+MB curves (Figure 15). With tPA alone, the lytic rate (1.2±1.3 mmHg/min) was lower than 5000 cycles US+MB ($p<0.01$). With 5000 cycles US+MB therapy, the lytic index was significantly higher with tPA [$(3.5\pm0.8) \times 10^{-3}$ $mmHg^{-1}.min^{-1}$] than without tPA [$(2.3\pm0.6) \times 10^{-3}$ $mmHg^{-1}.min^{-1}$] ($p<0.01$) (Table 18).

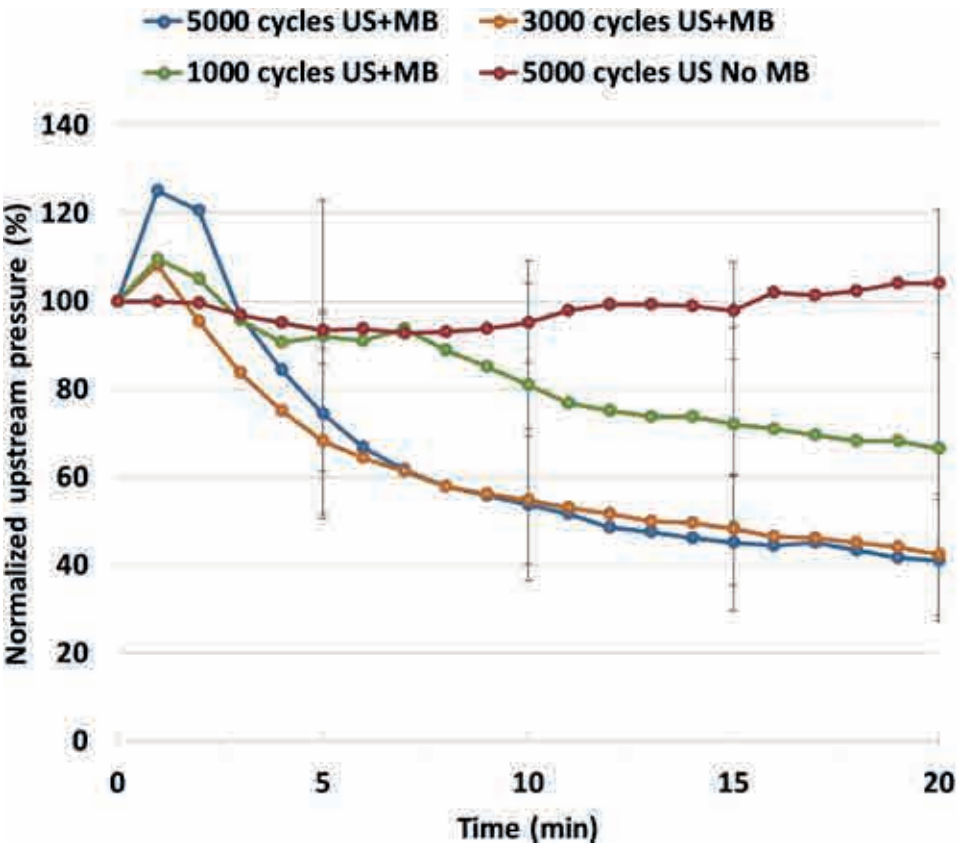


Figure 14: Upstream pressure with pulse lengths of 1000 (n = 7), 3000 (n = 5) and 5000 (n = 5) cycles of ultrasound (US) with microbubbles (MBs) and 5000 cycles without MBs (n = 3) during sonoreperfusion therapy in whole blood. US treatment started at t = 0. All experiments were performed without added tissue plasminogen activator

The lytic rate for US+MB 5000 cycles and US+MB+tPA 5000 cycles was not statistically different (p=NS). In the presence of tPA, the lytic index was significantly higher with 5000 cycles MB+US [$(3.5\pm0.8)\times10^{-3}$ $mmHg^{-1}.min^{-1}$] compared to tPA only [$(2.0\pm0.5)\times10^{-3}$ $mmHg^{-1}.min^{-1}$] ($p<0.05$). Overall, the most effective SRP regime (greatest lytic index and lytic rate) consisted of a combination of tPA and US+MB.

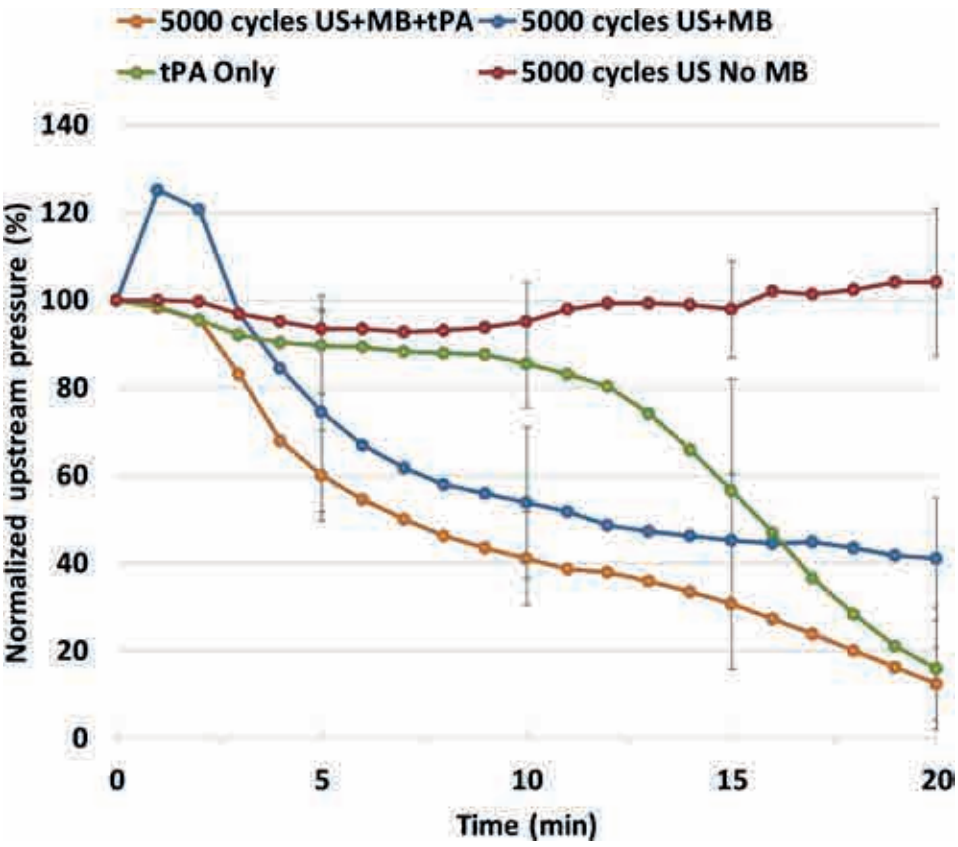


Figure 15: Upstream pressure during sonoreperfusion in whole blood for tissue plasminogen activator (tPA) only (n = 3), 5000-cycle ultrasound (US) 1 microbubbles (MBs) with tPA (n = 5), 5000-cycle US 1 MBs (n = 5) and 5000-cycle US and no MBs (n = 3).

Table 18: Sonoreperfusion efficacy. US=Ultrasound, MB=Microbubbles, tPA= tissue plasminogen activator, $^{+}$ $p<0.05$; * $p<0.05$ vs baseline

	Terminal pressure drop (%)	Lytic rate (mmHg/min)	Lytic index (x10^{-3} $mmHg^{-1}.min^{-1}$)
US+MB 1000 cycles	33.7±21.7^{+}	2.6±1.5	1.9±0.5
US+MB 3000 cycles	57.7±13.6^{+}	4.6±2.5 *	2.1±1.1
US+MB 5000 cycles	59.2±13.8^{+}	7.3±3.2	2.3±0.6 *
US only 5000 cycles	3.9±16.9	0.5±0.1	1.8±0.1
tPA only	84.0±14.1^{+}	1.2±1.3 *	2.0±0.5 *
US+MB+tPA 5000 cycles	87.6±8.2^{+}	4.3±0.8 *	3.5±0.8

Passive cavitation detection

Typical time-frequency analysis and corresponding ICD and SCD calculations are reported in Figure 16. For 1000 and 3000 cycles (Figures. 16a and 16b), cavitation activity was detected throughout the duration of the pulse and cumulative ICD and SCD power plateaued at around 1 ms and 2.5 ms. For the 5000 cycles pulse, cavitation activity persisted up to 5000 cycles as observed on the spectrogram (Figure 16c). The corresponding cumulative ICD plateaued at 3.5 ms but the cumulative SCD continued to increase beyond 3 ms. The corresponding ultraharmonic peaks after 4 ms are clearly visible on the spectrogram. There was no detectable cavitation activity nor ICD or SCD without MB (Figure 16d).

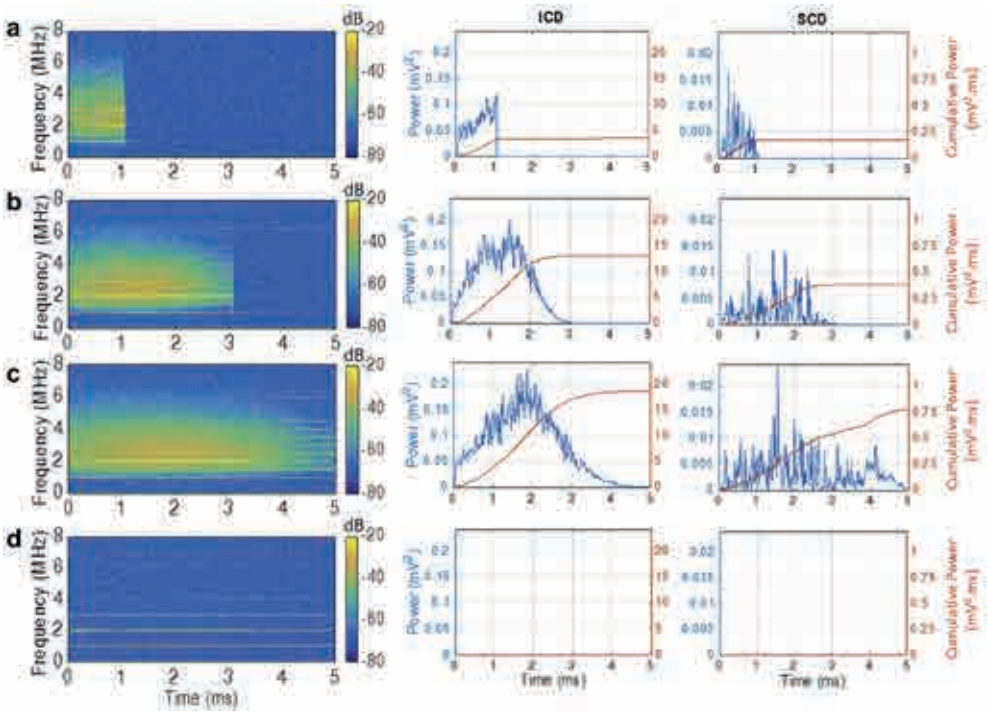


Figure 16: Time–frequency analysis, inertial cavitation dose (ICD) and stable cavitation dose (SCD) for 1MHz, 1.5MPa, and (a) 1000-, (b) 3000- and (c) 5000-cycle pulses during sonoreperfusion and (d) a 5000-cycle pulse but without microbubbles.

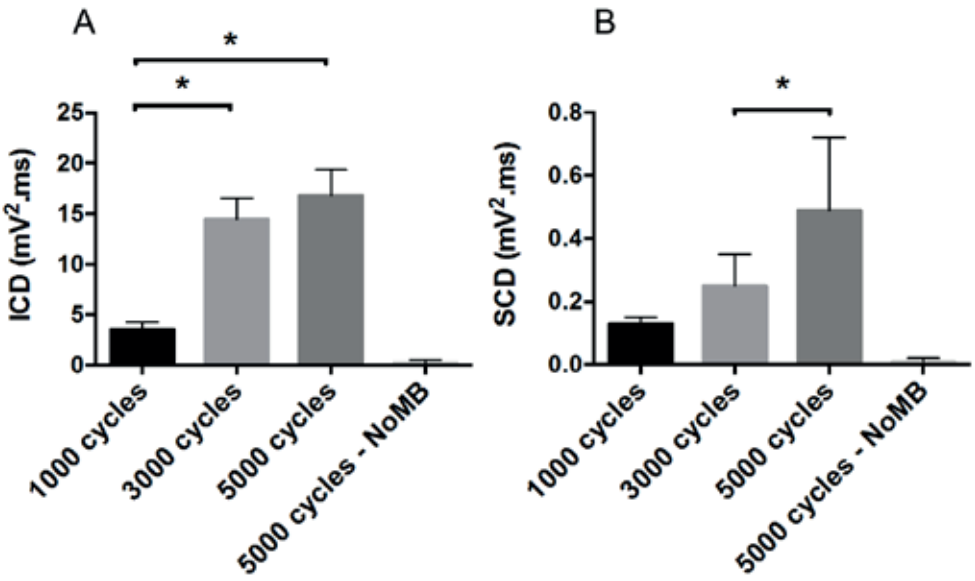


*Figure 17: (a) Inertial cavitation dose (ICD) at 1.5 MPa as a function of pulse length. ICD increased with pulse duration in the presence of microbubbles (MBs) and plateaued at 3000 cycles. (b) Stable cavitation dose (SCD) at 1.5 MPa as a function of pulse length. SCD increased with pulse length in the presence of MBs and was significantly higher at 5000 cycles than at 3000 cycles (n 5 5 per experimental condition, *p , 0.05).*

The aggregate results over repeated experiments are summarized in Figure 17. In the presence of MB, inertial cavitation dose increased with US pulse length up to 3000 cycles (Figure 17a). ICD was significantly higher for 3000 and 5000 cycle (respectively 14.5 ± 2.0 mV^2.ms and 16.8 ± 2.6 mV^2.ms) compared with the 1000 cycle experiments (3.5 ± 0.7 mV^2.ms, $p<0.001$).

ICD did not differ significantly between 3000 and 5000 acoustic cycles regimen. In addition, upstream pressure drop, lytic rate and lytic indices were positively correlated with ICD ($r^2>0.92$, $p<0.05$). The stable cavitation dose also increased with pulse length and reached its highest value with 5000 cycles (0.5 ± 0.2 mV^2.ms), which was significantly higher than for that for 3000 cycles (0.3 ± 0.1 mV^2.ms, $p<0.05$) (Figure 17b). SCD also vanished in the absence of MB. Lytic rate, but not lytic index or pressure drop, correlated with SCD ($r^2=0.99$, $p<0.05$).

7.4 Discussion

There have been numerous in vitro studies using petri dishes, beakers, open or closed loop systems filled with PBS or plasma addressing sonothrombolysis of large clots, generally showing a synergistic effect of US+MB with tPA on clot dissolution. [176,219,224,232–240] Our results indicate that SRP of MVO could be achieved in vitro using whole bovine blood perfusate. In this study, SRP efficacy increased with US pulse length up to 3000 cycles in the presence of MB (Figure 14), similarly to our previous findings using the non-cellular perfusate PBS. [225] In addition, SRP efficacy correlated with ICD, which also increased with pulse length up to 3000 cycles and then reached a plateau (Figure 17a). Interestingly, stable cavitation dose continued to significantly increase beyond 3000 cycles (Figure 17b), but this did not translate into an increase in SRP efficacy. It is not clear what caused SCD to persist while ICD decreased beyond 3000 cycles. The acoustic activity of daughter bubbles and clusters could be in play. [230,241,242] It is unlikely that misalignment between the transmitting and receiving transducers could explain these results as it would affect both ICD and SCD similarly. It is important to point out that the signal level of the SCD was much weaker than that of the ICD, suggesting that the inertial cavitation activity was dominant under high pressure insonation, as would be expected. Our findings therefore suggest that MB inertial cavitation was directly related to the disruption of the microthrombi in our microvascular model with whole blood and without the addition of tPA.

It has been demonstrated previously that without tPA, the macroscopically observed clot size reduction was the result of RBC hemolysis, as the fibrin content of their clots only decreased in the presence of tPA. [238] From this perspective, our results suggest that US+MB disruption of RBC in microthrombi could be sufficient to restore microvascular perfusion, potentially by reducing the size of the microthrombi to less than 40 μm, thus allowing their dislodgement and passage through the mesh.

In humans, the capillary bed has a flow speed of 0.3 mm/s and mean capillary pressure ranging from 19 to 30 mm Hg. [226,243,244] We created a unique in vitro microvascular model that operates with similar parameters, mimicking a situation with clinical MVO. In a previous study, microthrombi were seeded onto a 40-μm pore mesh in this constant flow system, resulting in increased upstream pressure. [225] In that study the kinetics of pressure

drop was measured during US+MB therapy, as a surrogate marker of clot burden reduction during therapy and demonstrated the efficacy of US+MB in achieving SRP within a PBS perfusate.

As earlier studies have already shown that endogenous tPA is present in bovine blood [245], we were interested in determining whether US+MB could achieve more efficacious SRP in our whole blood system compared to PBS. Our current data indicates that SRP efficacy was reduced in whole blood, as evidenced by an incomplete upstream pressure drop at 20 minutes and lower lytic index and lytic rate, compared with previous experiments conducted in PBS. [225] Although we cannot directly compare the two experiments (see limitations below), we surmise that this apparent reduced SRP efficacy is due to the presence of RBCs in the perfusate, which are the major contributors to blood viscosity. [246] This increased viscosity results in damped MB oscillations compared to PBS, as confirmed in a recent study using high speed imaging. [247] Reduced SRP efficacy was also found when plasma viscosity was adjusted to mean blood viscosity of 4 cP for venous and arterial type microthrombi in the same model of MVO. [231]

Our results also support that the addition of low-level tPA was necessary to achieve SRP efficacy similar to that obtained in PBS perfusate as our control experiments without US, MB and without the addition of tPA suggested that endogenous tPA was insufficient to cause effective SRP. This is consistent with results found by Sutton et al, who demonstrated in an ex-vivo artery setup that endogenous endothelial tPA was insufficient to improve sonothrombolysis in the presence of MB and US. [240] Overall, the mitigating effect of blood should be taken into consideration when extrapolating in vitro data using non-blood perfusates to predict in vivo efficacy of a given sonothrombolytic regimen. Additional studies using blood perfusion in this in vitro MVO model while manipulating acoustic and microbubble parameters should help define optimal conditions for maximizing microvascular sonothrombolysis.

Thrombolysis kinetics

One major advantage of our in vitro model is the possibility to quantify reperfusion kinetics, which allows us to compute parameters such as the lytic rate and the lytic index during treatment. Our results clearly indicate that tPA and US+MB thrombolysis individually operate with different kinetic responses. As seen in Figure 15, US+MB mediated reperfusion

is a faster process albeit incomplete in terms of terminal pressure drop, compared to the tPA treatment. Conversely, tPA alone induced a more complete reperfusion at 20 minutes of treatment but had a slower therapeutic onset. This is reflected quantitatively in the lytic rates and indices reported in Table 18. The combination of tPA and US+MB achieved both a fast onset reperfusion and a complete terminal pressure drop. This observation holds a promising potential for in vivo translation of the approach by combining the apparent synergistic tPA and locally targeted MB activity. This synergistic effect may be caused by an enhanced penetration of tPA into the clot, by local MB oscillations creating tunnels in the microthrombi. This allows for a tPA to have an effect locally, circumventing the need for a systemic lytic state with a regular dose of tPA.

In areas where PCI is the gold standard in STEMI treatment, concomitant pharmacotherapy includes anticoagulant/antiplatelet agents such as bivalirudin and glycoprotein IIB/IIIA receptor antagonists, respectively, in place of tPA. [248] While the results in this study do not reflect a situation in which these drugs are used, combined dual antiplatelet therapy and bivalirudin might prove to be beneficial when combined with sonoreperfusion in the treatment of MVO after STEMI.

Safety is also an important consideration. In STEMI, every second of delayed reperfusion causes more tissue damage in the ischemic region of the myocardium. Pre-treatment using therapeutic ultrasound on top of regular treatment might enhance reperfusion prior to coronary intervention, but could also be cause for side-effects. It has already been shown that an increase in pulse duration might be responsible for coronary vasoconstriction in the clinical application of SRP therapy in human STEMI patients. [249] While our experiments showed that 5000 cycles was optimal in vitro, the effects of ultrasound on living tissue, such as possible hemolysis and hemorrhaging, should not be discarded and future studies will have to consider safety as an important trial outcome.

Limitations

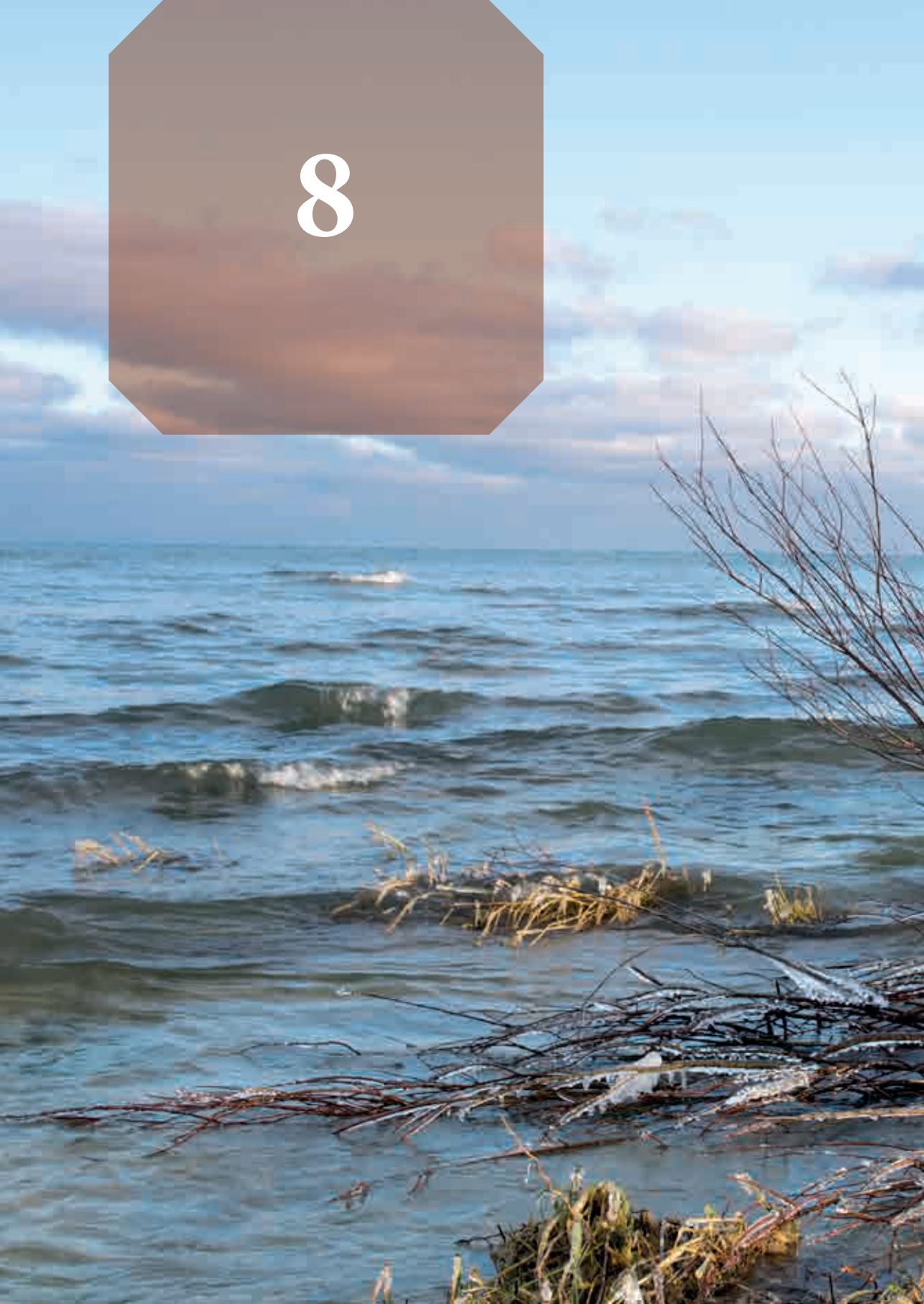
The biggest drawback of our model, like any model for STEMI therapy, is not being able to fully replicate the true process of atherosclerosis, plaque rupture and atherosclerotic thrombus formation. There is a limited number of patho-physiological processes that we can mimic and take into account. Blood was anticoagulated and therefore not all factors of the intrinsic and extrinsic coagulation cascade are present in this in vitro model. Also, our in vitro vascular model does not account for biological effects of SRP on the vascular tone as endothelial and smooth muscle cells are not present. In order to accommodate experiments in whole blood, we had to modify our previously used protocol [225] to correct for viscosity differences between whole blood and PBS. These changes included a reduction in flow rate from 1.5 ml/min to 0.75 ml/min and a decrease in initial upstream pressure from 40 to 30 mmHg, which make direct comparisons between SRP studies in PBS and whole blood imperfect.

Whole bovine blood instead of human blood was used in our experiments as it does not aggregate. [250] This reduced the complexity of our setup, but might also create a bias in results as the formation of RBC aggregates in human blood might further decrease the efficacy of SRP therapy.

7.5 Conclusion

Sonoreperfusion therapy was achieved in our in vitro model of MVO in the presence of whole blood. SRP efficacy without exogenous tPA increased with US pulse length but plateaued at 3000 cycles, consistent with the inertial cavitation dose measurements. tPA in combination with US+MB showed potential for synergistic therapeutic effects, as US+MB favored a rapid therapeutic onset while the addition of low dose tPA was necessary to achieve optimal therapeutic efficacy. Future preclinical studies are needed to validate and build upon these results.

8



Chapter 8:
Unexpected high incidence of coronary vasoconstriction in the "Reduction Of Microvascular Injury Using Sonolysis (ROMIUS)" trial

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Abstract

Introduction

High mechanical ultrasound and intravenous microbubbles might prove beneficial in treating microvascular obstruction due to microthrombi, after primary percutaneous coronary intervention for ST-segment elevated myocardial infarction (STEMI). Animal experiments showed longer pulse duration ultrasound was associated with an improvement in microvascular recovery. This trial tested long pulse duration high mechanical index ultrasound in STEMI patients.

Methods

Non-randomized, non-blinded patients were included in this phase 2 trial. Primary endpoint was any side-effects possibly related to the US treatment.

Results

The study was aborted after 6 patients were included, 3 patients experienced coronary vasoconstriction of the culprit artery, unresponsive to nitroglycerin. Therefore, coronary artery diameters (CAD) were measured in 5 pigs. CADs distal to the injury site decreased following application of US, after balloon injury plus thrombus injection. (1.89±0.24 mm before and 1.78±0.17 after US, p=0.05)

Conclusion

Long pulse duration ultrasound might cause coronary vasoconstriction distal to the culprit vessel location.

8.1 Introduction

Acute occlusion of a coronary artery causes elevation of the ST segment on the electrocardiogram, resulting in ST elevation myocardial infarction (STEMI). Current therapy is focused on immediate restoration of flow of the obstructed epicardial coronary artery. This can be achieved either using thrombolytic therapy or primary percutaneous coronary intervention (PCI), the latter being favoured in situations where trained personnel and specialized equipment is available. [251] Unfortunately, despite successful epicardial reperfusion, myocardial perfusion of the microvasculature is not restored in 5-50% of cases, resulting in adverse clinical outcomes. [66,212,252]

This phenomenon known as no-reflow or microvascular occlusion (MVO), is of multifactorial origin and possibly initiated by microvascular thromboembolization [252,253], as well as intramyocardial haemorrhage [2,214], but platelet and leukocyte aggregation, inflammation, edema and vasoconstriction all play an important role. [254] The relatively sudden reperfusion caused by PCI can also lead to cellular lethal reperfusion injury. [255] This is most likely caused by a combination of factors including high oxidative stress, intracellular calcium overload, (micro)vascular thrombi and inflammation, but the exact mechanism is unknown. [66,215] Detection and treatment of MVO is currently a focus of scientific research, but has led to mixed results in efficacy. [158,256] One potential technique that tries to support PCI in the treatment of patients with acute STEMI is called sonolysis and consists of high mechanical index (MI) therapeutic ultrasound (US) directed at epicardial and microvascular thrombi in order to disrupt them and increase microvascular perfusion. [257] Diagnostic ultrasound has already proven to be a useful tool in the clinical cardiology but normally uses low mechanical intensity US that allows function assessment and myocardial perfusion imaging. Therapeutic US usually applies high intensity US, which by itself causes cavitation in fluids and is therefore not suitable for diagnostic imaging. Combining therapeutic US with intravenous microbubbles significantly increases the amount of cavitation. [170] By using inertial cavitation, a large proportion of cavitating microbubbles release large amounts of energy resulting in microjetting, amongst other effects, capable of destroying thrombi. [158] However, the amount of microbubbles that undergo inertial cavitation is strongly dependent on not only the amplitude of the US, but also on the US frequency and

the mechanical properties of the microbubble used. [258]

Increasing the mechanical index [225] and increasing pulse duration [209] results in increased thrombus destruction in most, but not all studies. In a study by Holland et al, the authors demonstrated that the largest thrombolytic enhancement at 1 MHz was achieved using a 1.0 MPa peak-to-peak pressure amplitude. However, using 120 kHz probes, a frequency that is not used in echocardiography in humans, pressures beyond 0.48 MPa did not result in increased sonothrombolysis. [259,260] The increase of mechanical index and pulse duration might be cause for a reduction of the amount of tissue plasminogen activator treatment needed to achieve thrombolysis in remote areas. [261] A recent in vivo study in rats showed that high mechanical index long pulse tone therapeutic ultrasound was capable of achieving a reduction in micro emboli in the biceps femoris muscle in a thrombotic vascular occlusion model. [222] The current study aims to incorporate these preclinical results in a clinical scenario and is designed to test safety and feasibility of a longer pulse duration (20 μsec) high mechanical index (1.3) US with intravenous microbubble infusion for treatment of microvascular disease in acute STEMI patients using novel software that alternates therapeutic high intensity US and diagnostic low intensity US. This allows for myocardial perfusion imaging as a guide for therapy (theragnostic imaging).

8.2 Methods

Patient population

Consecutive adult patients with acute STEMI were enrolled in the study. Exclusion criteria were cardiogenic shock, known allergy to ultrasound contrast agents, contraindications to MRI and any other reason judged by the investigators to hamper inclusion. After inclusion, patients were treated up to a maximum of 15 minutes with theragnostic ultrasound during the preparation of PCI. US treatment was discontinued immediately upon insertion of the wire through the arterial sheet, or after completion of therapy (15 minutes).

During PCI all patients received bivalirudin. Stent placement was performed on judgement of the interventional cardiologist. After PCI, all patients received an additional 30 minutes of sonolysis therapy. (Figure 18)

The study was approved by the local ethical committee, a Data Safety Monitoring Board (DSMB) was created and the trial was registered at http://trialregister.nl; identifier: NTR4791.

Theragnostic Ultrasound

After consent, all patients received an intravenous infusion of Definity® (Lantheus Medical Imaging, N. Billerica, MA, USA) microspheres of 1.3 ml/min. The dosing protocol and instructions for a continuous IV infusion as specified in the packaging instructions were used to administer Definity to our patients. A Philips S5-1 (Philips Healthcare, Best, the Netherlands) probe on the IE33 system (Philips Healthcare, Best, the Netherlands), placed in the left fourth intercostal space, was used to alternate between diagnostic (contrast imaging only mode, MI 0.18, 1.6 MHz center frequency, 50 Hz frame rate) and therapeutic ultrasound (predefined imaging area similar to the color Doppler box, superimposed on anatomic imaging, MI 1.3, pulse duration 20 µsec, 1.6 MHz center frequency, 50 Hz frame-rate) using myocardial perfusion defects as a guide for therapeutic high mechanical index ultrasound delivery. Diagnostic and therapeutic ultrasound was manually alternated at a rate of 15 seconds per imaging mode, to allow for both optimal microbubble replenishment and treat the entire left ventricle as the 2D probe was manually rotated at 8 rotations per minute, continuously alternating between 0 and 90 degrees during treatment to contain as much of the risk area as possible.

Clinical and imaging data

Extensive blood analysis was performed regularly starting from initial hospital admission and cardiac enzymatic markers were measured every 6 hours until creatine phosp-

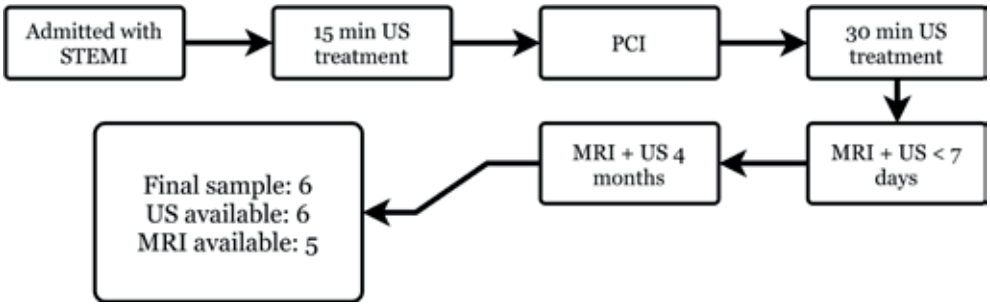

Figure 18: Study flowchart

hokinase MB (CKMB) had peaked.

Magnetic Resonance Imaging (MRI) was performed at 3-7 days after STEMI and at 4 months follow-up. The MRI protocol consisted of multiple imaging modalities including delayed contrast enhanced (DCE) imaging to obtain left ventricular function and infarct size, including area at risk and percentage of MVO. The primary endpoint consisted of the myocardial salvage index. Area at risk (AAR) was measured using the endocardial surface area (ESA) method. [164] DCE was used to determine infarct size and the formula to determine myocardial salvage index was (AAR-DCE) / AAR.

Follow-up DCE MR at 4 months was performed to assess infarct recovery and final scar size. Major adverse cardiac events (MACE), defined as cardiac death, myocardial infarction, coronary bypass grafting or repeat PCI, were registered in this 4 month follow-up period.

Additional pig experiments

In order to determine mechanistically what may be occurring in this setting, we measured serial coronary artery diameters in additional pig experiments using quantitative coronary angiography (CAAS, Pie Medical Imaging, Maastricht, the Netherlands). Measurements were taken proximal to a simulated plaque rupture site, at the site, and distal to the site. Ultrasound was applied in five normal pigs for up to 15 minutes with identical treatment protocol compared with the patients described in Theragnostic Ultrasound. Coronary artery diameters (CAD) of the Left anterior descending artery (LAD) proximal and distal to a 20 mm balloon injury site (as well as the proximal uninjured right circumflex artery [RCA]) were measured in multiple pigs for all three experimental conditions; (1) before and after 10 minute ultrasound application, (2) followed by measurements before and after balloon injury using a balloon catheter inflation to 120% of original vessel diameter, followed by the second round of ultrasound treatment, (3) and finally before and after identical balloon injury combined with a 1 ml infusion of thrombosing arterial blood applied to the site of balloon injury creating a thrombus, again followed by ultrasound treatment, after which the last CAD measurements took place.This ensured that any effect of the balloon injury by itself was accounted for, by repeating coronary artery dimension measurements in all locations again with quantitative angiography after the balloon injury. Balloon injury was performed by progressively inflating a ballon to approximately 120% of its original measured diameter

using a total of three 30 second inflations. The balloon was not moved during inflation. [262]

Approval for these experiments was provided by the animal care and use committee of the University of Nebraska Medical Center.

Sample size and Statistics

A sample of 20 patients was estimated to provide sufficient primary safety and feasibility data. All patients were analysed using intention to treat protocol. Independent sample t-test or Mann-Whitney U test was used as appropriate. Paired t-testing was performed for the proximal and distal LAD diameters prior and after theragnostic US in the pig experiments. Chi square test is used for categorical data. ANOVA with bonferroni post-hoc testing will be used to compare between subgroups of the study population for segmental MR and echocardiographic analysis.

8.3 Results

After inclusion of 6 patients (4 male, 53±11 years old) the study was prematurely halted due to the occurrence of serious unexpected adverse events. (Table 19) During PCI, 3 patients, (50%, 2 female, 1 male) developed severe coronary vasoconstriction of the culprit artery distal to the culprit lesion location not adequately responding to nitroglycerin. This was visible upon initial angiography directly following the first round of US treatment. These patients suffered from RCA, LAD and circumflex artery (RCX) myocardial infarctions.

The first patient had an occlusion in the RCA of segments R1 through R5. Prior to balloon inflation, vasoconstriction was already present and was more apparent after stent placement. (Figure 19, panel E)

The third patient included in this study had vasoconstriction, already apparent on initial angiography, that was less widespread throughout the circumflex artery (Figure 20, panel E) and confined to segments C5, C6 and C7.

Finally, on initial angiography, the sixth patient displayed pronounced coronary vasoconstriction of the left anterior descending artery in segments L3, L4 and L5. (Figure 21, panel E). This patient was the only patient with Thrombolysis In Myocardial Infarction (TIMI) 3 flow on initial angiography. All baseline characteristics can be found in table 19.

Table 19: Patient characteristics. SD = Standard Deviation, BMI = Body Mass Index, M = Male, F = Female, RCA = Right Coronary Artery, RCX = Ramus Circumflex, LAD = Left Anterior Descending, PCI = Percutaneous Coronary Intervention, US = Ultrasound (before [1] and after [2] PCI). HT = hypertension, HC = hypercholesterolemia, DM = diabetes mellitus, COPD = Chronic obstructive pulmonary disease, HIV = human immunodeficiency virus

Patient (age/sex)	BMI	Culprit artery	Symptom - balloon time (min)	Door - balloon time (min)	ST resolution > 50% after:			Side-effects	Comorbidity
					US 1	PCI	US2		
1, 50/M	22.1	RCA	116	33	No	Yes	Yes	Vasoconstriction	HT, HC, 15 PY
2, 36/M	26.8	RCA	87	30	No	Yes	Yes	-	STEMI 1 year prior
3, 68/F	20.4	RCX	169	61	No	Yes	Yes	Vasoconstriction	-
4, 54/M	40.0	RCX	183	57	No	Yes	Yes	-	DM
5, 46/M	27.5	RCX	96	37	No	No	Yes	Urticaria	-
6, 60/F	22.7	LAD	258	73	No	No	Yes	Vasoconstriction	HIV, DM, HC, COPD, HIV
Avg. ± SD	26.5 (± 7.0)		151±65	49±18					

Table 20: MRI parameters. SD = Standard Deviation, M = Male, F = Female, LV = Left ventricle, AAR = Area at risk, MVO = Microvascular Obstruction, MSI = Myocardial Salvage Index, EF = Ejection Fraction, BL = baseline, FU = follow-up

Patient (age/sex)	LV mass (gr/m2)	AAR (Endocardial surface area)	MVO (gr)	Final infarct size (gr)	MSI	EF BL (%)	EF FU (%)
1, 50/M	48.74	10.50	0	6.78	0.65	55	51
2, 36/M	46.03	36.23	0.24	15.24	0.52	56	57
3, 68/F	39.82	27.07	0.48	6.59	0.52	46	56
4, 54/M	75.44	82.32	7.21	24.96	0.61	52	49
5, 46/M	68.2	87.08	9.06	23.02	0.48	43	45
6, 60/F	NA	NA	NA	NA	NA	NA	NA
Avg. ± SD	55.6 ± 15.3	48.6 ± 34.2	3.3 ± 4.4	15.3 ± 8.9	0.56±0.07	48 ± 4	51 ± 6

Another patient, 46 year old male, experienced an allergic reaction with urticaria directly following PCI. Due to refusal of the patient in question, no testing was performed to determine the agent that triggered the allergic response. Overall, average CKMB-max was 233.4±152.0 g/L, complaint-to-balloon time 151±65 minutes. LV mass was 55.6±15.3 gr/m^2 and myocardial salvage index was 0.56±0.07 on average. MVO with intramyocardial haemorrhage was present in 4 patients. For a summary of MRI results, see Table 20.

Follow-up

One female patient with coronary vasoconstriction withdrew consent after the initial hospitalization and was considered lost to follow-up. She was still alive according to the Dutch national mortality registry and was not admitted to the hospital in the 4 month follow-up period. All other patients experienced no long-term side-effects at follow-up and 3D US showed recovery of ejection fraction to 55.1±6.9% on average regardless of the occurrence of coronary vasoconstriction during PCI. This is comparable to other large STEMI trials. [263]

Results from the pig experiments

In the five pigs treated with long pulse duration US, CADs increased in the proximal LAD following the 10 minute ultrasound application prior to balloon injury (p=0.02 for proximal LAD) and did not change significantly in other locations. Following balloon injury there was a 19±16% dilation at the injury site in response to ultrasound, but no significant

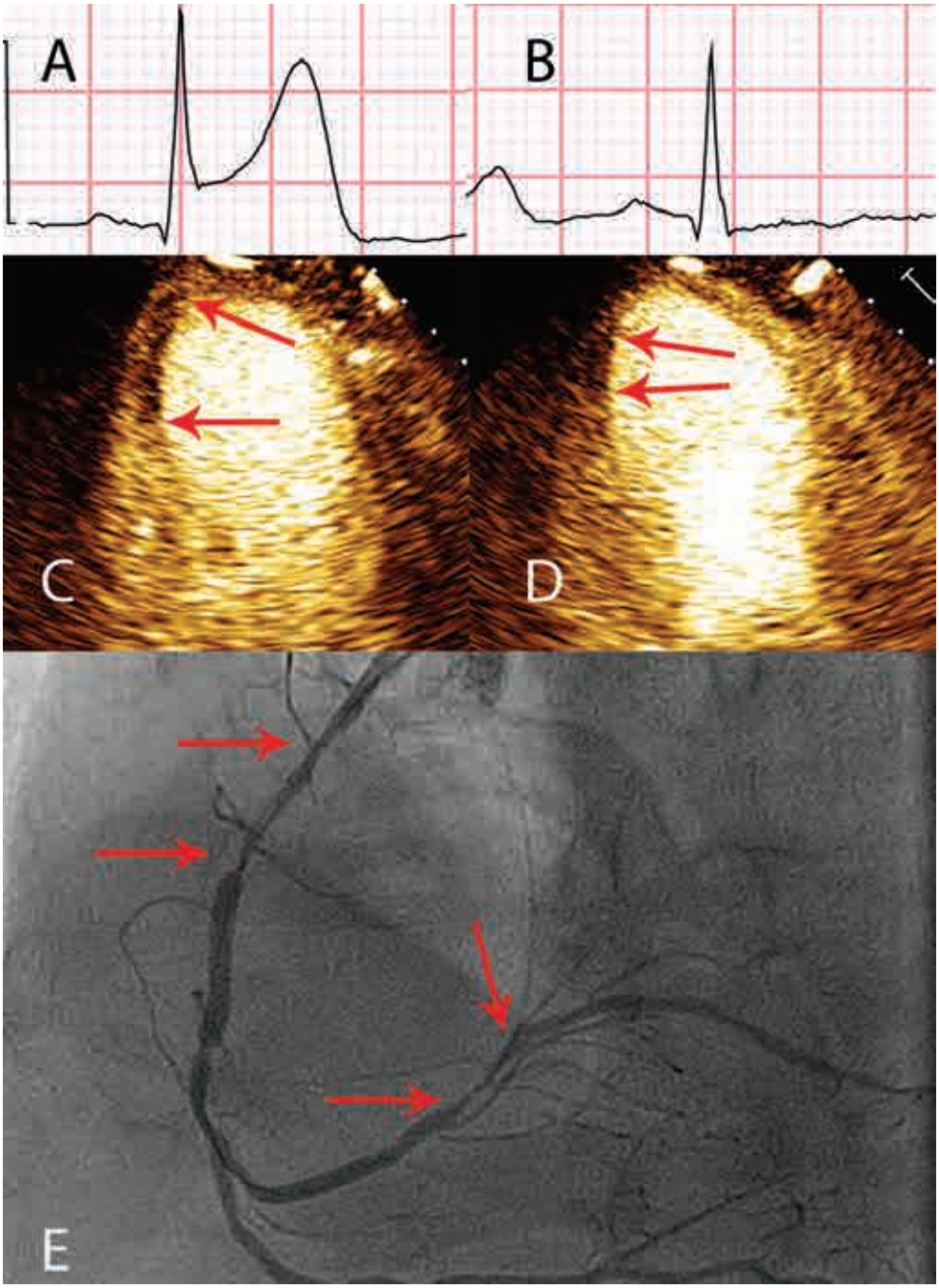

Figure 19: ROMIUS patient 1: Electrocardiogram in lead III before (a) and after (b) percutaneous coronary intervention. Myocardial perfusion defect (between arrows) on initiation of (c) and after (d) ultrasound therapy. An affected right coronary artery (e) with vasoconstriction is indicated (between arrows).

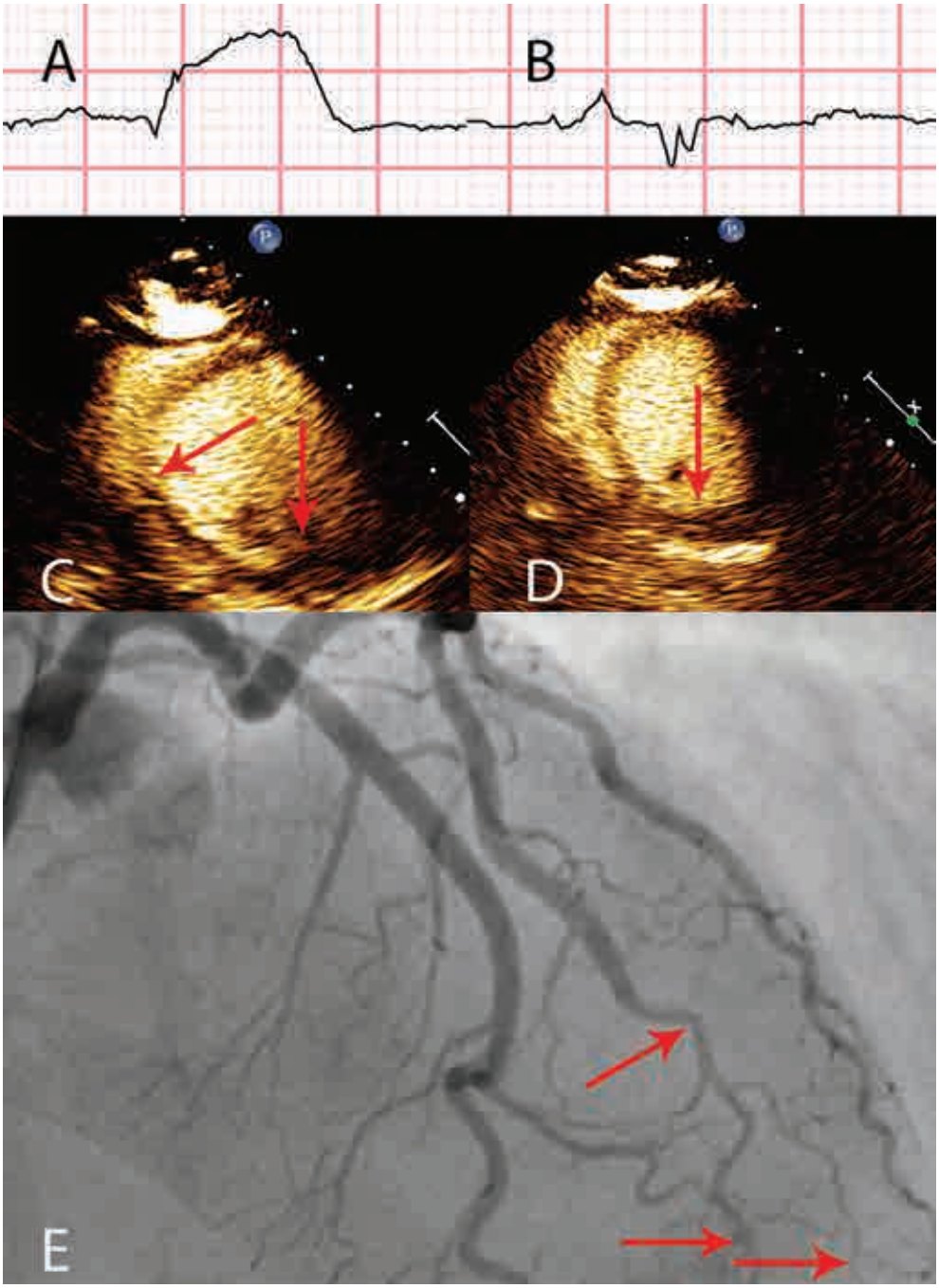

Figure 20: ROMIUS patient 3: Electrocardiogram in lead III before (a) and after (b) percutaneous coronary intervention. Myocardial perfusion defect (between arrows) on initiation (c) and after (d) ultrasound therapy. An affected circumflex coronary artery (e) with vasoconstriction is indicated (between arrows).

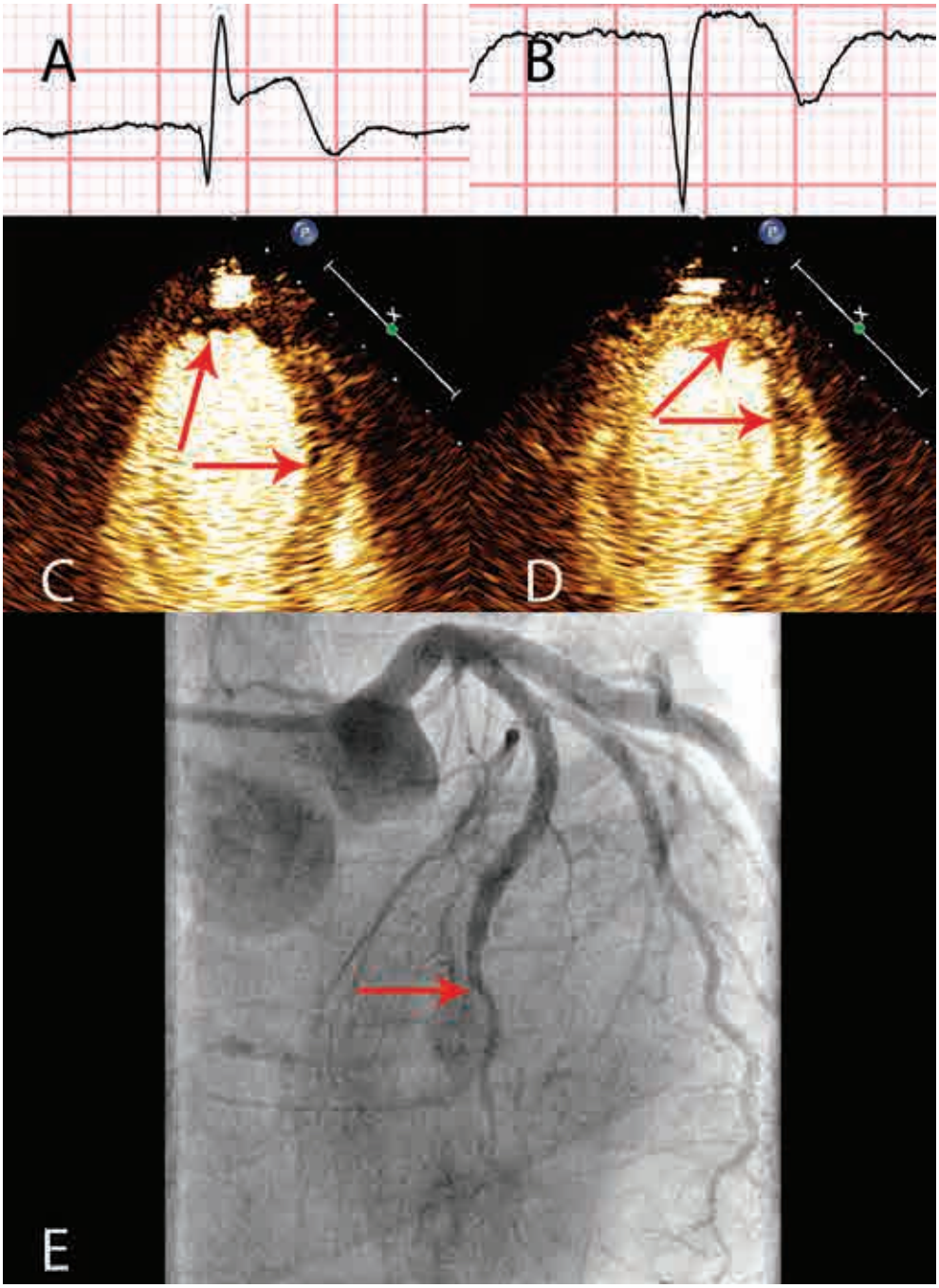

Figure 21: ROMIUS patient 6: Electrocardiogram in lead V2 before (a) and after (b) percutaneous coronary intervention. Myocardial perfusion defect (between arrows) on initiation (c) and after (d) ultrasound therapy. An affected left anterior descending coronary artery (e) with vasoconstriction is indicated (between arrows).

change in proximal LAD or distal LAD.

Following balloon injury and injection of 1.0 millilitre thrombus material, proximal LAD diameters still tended to increase (3.23±0.16 mm before and 3.34±0.15 after US, p=0.119) in response to ultrasound, but there was a significant decrease in distal LAD diameter beyond the balloon injury site, measured after US application (1.89±0.24 mm before and 1.78±0.17 after US, p=0.048). No change in vessel diameter was noted at the injury site. The degree of vessel diameter reduction ranged from 2% to 13%. No changes in vessel diameter occurred in the RCA following any intervention or ultrasound application.

8.4 Discussion

This study was designed to test the hypothesis that the use of a longer pulse duration for sonoreperfusion therapy, would be safe for use in acute STEMI patients scheduled for PCI as a treatment for microvascular obstruction. After inclusion of 6 patients, 3 patients experienced severe coronary vasoconstriction in the culprit coronary artery and 1 patient suffered an acute allergic reaction following PCI. These findings were reported to the DSMB and the trial was aborted to prevent the occurrence of additional myocardial ischemia due to coronary vasoconstriction. All patients were without complaints following PCI and no MACE or other adverse cardiac events occurred during 4 month follow-up.

Despite successful in vivo results from a preclinical trial using an atherosclerotic pig model that reported a beneficial effect of using a longer pulse duration [209], this ultrasound setting might not be feasible for human use. The difference in ultrasound protocol between these two studies lies in the novel longer pulse duration and combination of imaging and therapeutic pulses. The most likely cause for the discrepancy between these two trials is the time point at which the epicardial vasculature was visualized. In the preclinical trial by Wu et al, coronary angiography was performed several minutes after application of US, whereas in the current clinical trial angiography was performed immediately following initial US therapy. Also, previous preclinical trials using a 20 microsecond pulse duration used venous thrombus to create coronary artery occlusion, and not arterial thrombus. Thus, platelet concentration was not typical of acute arterial thrombosis. [261,264]

A previous pilot clinical trial by Slikkerveer et al. using 5 microsecond pulse dura-

tion ultrasound aimed to treat the epicardial coronary artery before PCI. After inclusion of 10 patients, no coronary vasoconstriction had been observed and results indicated that sonolysis might be beneficial in treating epicardial coronary thrombi during STEMI. [155] Both the study by Slikkerveer et al. and the current study used identical timing for the initial application of therapeutic ultrasound and the initial angiography. The ultrasound properties changed in our trial only comprised of an increase in pulse duration, and switch from a 3D to a 2D probe. The coronary artery vasoconstriction that occurred in our patients was visually severe and did not resolve with administration of intracoronary nitroglycerin.

Following these results, we performed additional pig experiments. We measured the coronary artery diameter and we observed that in the absence of prior balloon injury, the intermittent high MI longer pulse durations actually increase coronary artery diameter. This is in concordance with a trial by Belcik et al. that performed experiments in non-ischemic mice and measured an increase in vessel diameter when ultrasound with intermittent high MI pulses were used. [265] Following balloon injury in the proximal LAD in our pig experiments, there is an even greater increase improvement in vessel diameter (19% increase) at the injury site in response to ultrasound. Only in the presence of thrombus does the intermittent high MI application of ultrasound result in a reduction of vessel diameter distal to the injury site. This situation is most similar to what we observed in three out of six patients treated with intermittent high MI ultrasound in our study; the cavitation process in the presence of activated platelets and fibrin, induced vasoconstriction distally. The exact role of platelets or endothelium in inducing the distal vasoconstriction cannot be differentiated here, but the animal studies suggest that both are necessary for any distal vasoconstriction to occur. The animal studies also appear to indicate that the actual plaque rupture sites and sites proximal to the rupture may actually dilate in response to ultrasound in this setting, making the severity of the spasm appear worse distally. While we cannot rule out that the vasoconstriction in our human patients were catheter induced, or caused by STEMI itself, these animal findings indicate that there is a possible causal relationship between these theragnostic ultrasound settings and vasoconstriction.

The question that needs to be answered is how instead of using 5 microseconds pulse duration in the SONOLYIS trial, the use of 20 microseconds in the ROMIUS trial has led to coronary artery vasoconstriction in 3 of our patients. Multiple pathways inducing coronary vasoconstriction exist, one of them is modulated by platelet aggregation. [266] An

increase of platelet aggregation causes the release of thromboxane A2 and serotonin, which are potent vasoconstrictors. [267,268] In the case of bleeding, this is beneficial, as less blood flows through the damaged vessel. In an atherosclerotic state however, platelets also tend to aggregate more thus increasing the amount of locally available vasoconstrictive agents. [266]

Earlier, Bardon et al showed that high MI (1.4) diagnostic pulsed wave ultrasound induced no flow-changes in the middle cerebral artery [269], but they did find distal vasodilation when the radial artery was imaged. [270] These results were obtained using Doppler monitoring without use of microbubbles. The authors hypothesized that the vasodilation occurred due to release of NO. However, in STEMI, ischemia that is present distally to the lesion, causes endothelial dysfunction. This endothelial dysfunction and damage – pre-existing in an atherosclerotic state - causes endothelial cells to produce less prostacyclin and nitric oxide (NO) which in turn are potent vasodilators. [268] Blockage of NO has been shown to remove the vasodilatory effect of cavitating ultrasound in a nonischemic mice model. [265] In ex vivo rat aorta experiments using a high MI (1.9) with a short 2.3 microsecond pulse duration, it was found that endothelial damage, or even destruction, was caused by ultrasound in combination with Optison even without prior atherosclerotic or ischemic vascular damage. This led to a decrease in the ability of the vascular wall to constrict or dilate. [271] Despite these preclinical findings, application of high MI (>1.0) has been found to be safe in humans when used in both stroke and myocardial infarction, when tissue path lengths of around 15 cm are used. [158] It has also been found that ultrasound and microbubbles are capable of creating pores in the endothelial, a process called sonoporation. (Dijkmans et al. 2004) These pores are known to disappear quickly over time (<5 seconds) after a recovery period, depending on the size of the pore created. [272]

While sonoporation is normally a beneficial result if the wanted effect is to increase local drug delivery directly into the cells [273], in case of already pre-existing endothelial damage in STEMI, this might increase endothelial damage. The increase of pulse duration might therefore also increase the amount of sonoporation leading to an increase of damage of the endothelium causing the cell to be destroyed. This enhanced sonoporation could therefore increase the endothelial dysfunction that existed prior to the myocardial infarction, thus increasing the vasoconstriction occurring. The local application of ultrasound might explain why the coronary artery vasoconstriction was only seen in the culprit artery. Also, damaging or destroying the vascular endothelial cells might explain why intracoronary

nitroglycerin is not effective in reversing the coronary vasoconstriction.

Another way through which a longer pulse duration, the destruction of endothelial cells and pore formation might increase vasoconstriction is through a calcium influx into the smooth muscle cells. Normally, calcium channels tightly regulate the influx of calcium through the cellular membrane as the calcium concentration inside the cell is up to a 5000 times lower than the extracellular calcium concentration. [169] During sonoporation there is a fast calcium influx through the transiently induced pores. [274] If the pores do not close in a timely manner, due to the higher pulse duration [275] or large size of the membrane pore [276], these high levels of intracellular calcium may cause side-effects similar to reperfusion injury. [277] If the extent of sonoporation also reaches the smooth muscle cells with a concomitant calcium influx this might cause unwanted vasoconstriction due to activation of the excitation-contraction coupling pathway.

The summative effect of myocardial ischemia, reperfusion damage and long pulse duration sonoporation on endothelial damage, all leading to calcium overload, might be the reason why we observed vasoconstriction in our patients.

Limitations

Due to the premature closure of this study, this trial lacks definitive data on the safety of this new application of ultrasound in humans. New preclinical studies should be designed, focusing on immediate effects of theragnostic ultrasound on cell membranes, sonoporation, calcium influx and coronary vasoconstriction in the setting of activated platelets and endothelial dysfunction.

8.5 Conclusion

Although preclinical and other clinical studies using 5 μsec pulse duration sonolysis in test subjects have not found any safety issues and recently pharmaceutical companies have eased contraindications for their UCAs, this study suggest that using longer pulse duration ultrasound settings might result in distal coronary vasospasm. Further research is needed to determine the exact cause-effect relationship. New clinical trials using similar MI should proceed using short (5 μsec) pulse duration ultrasound.

A

Appendix A: References

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B

Appendix B: English Summary

Advances in health care and advanced therapeutic options in the field of ST segment elevated acute myocardial infarction (STEMI) have drastically reduced mortality. The introduction of primary percutaneous coronary intervention (PCI) as treatment option for STEMI is a major scientific achievement. While being readily available in many countries, the ancient adage 'time is muscle' still holds. Patients will not be in the catheterization laboratory fast enough for PCI to have a complete therapeutic effect. Immediately following occlusion of the artery, intracellular ischemic changes occur triggering a cascade ultimately leading to cellular apoptosis. The two contributors are called lethal reperfusion injury and microvascular obstruction (MVO). Reperfusion injury occurs due to a combination of myocardial edema, endothelial swelling, vasospasm, inflammatory responses and distal thrombus embolization. MVO on the other hand occurs mostly due to wire and balloon manipulation of the occlusion, although it is very likely that there is overlap between these two pathways. The aim of the research bundled in this thesis was to evaluate possible new therapeutic options targeting reperfusion injury and MVO. First, chapter one consists of a general introduction and outline of this thesis.

Of course, being able to determine what patient benefits most from additional therapy is very important. This will not only save money, as novel therapies are often very expensive, but prevents additional side-effects from occurring and (thus) improves patient compliance. The first part discusses possibilities to improve this situation, starting with the second chapter, where a novel non-invasive imaging technique was developed and tested on a population of STEMI patients, in order to determine whether it is possible to predict what patients are more likely to benefit from additional therapy. This technique was called the FLASH (Fluoroscopy Assisted Scoring of Myocardial Hypoperfusion). It consists of the ratio between multiple flow/velocity measurements using frame counts on a coronary angiographic image. Obviously, invasive measurements, e.g. performed during the initial procedure,

are the golden standard when it comes to determining coronary flow. But this requires additional equipment and expertise, which might not always be available. FLASH was found to be able to predict patient mortality in a large population of STEMI patients.

In chapter three an effort was made to predict the long term consequences of STEMI, by using 3D ultrasound to measure myocardial strain. After myocardial infarction, part of the muscle that dies is replaced by connective tissue. The overall function and shape of the heart therefore change, something that can either be good (reverse remodelling) or bad (adverse remodelling). Currently, most clinics use 2D ultrasound to visualize the left ventricle after myocardial infarction, in order to determine infarct size and myocardial function. Furthermore, using ultrasound, it is possible to visually determine the amount of strain, or effort, the myocardium is under when it contracts. In this thesis, it is shown that the 3D obtained global longitudinal strain is predictive of adverse remodelling in patients, while the 3D global circumferential strain at baseline is predictive of the occurrence of reverse remodelling. Determining what patients are most likely to suffer, or benefit, from these phenomena, is critical for long term patient treatment.

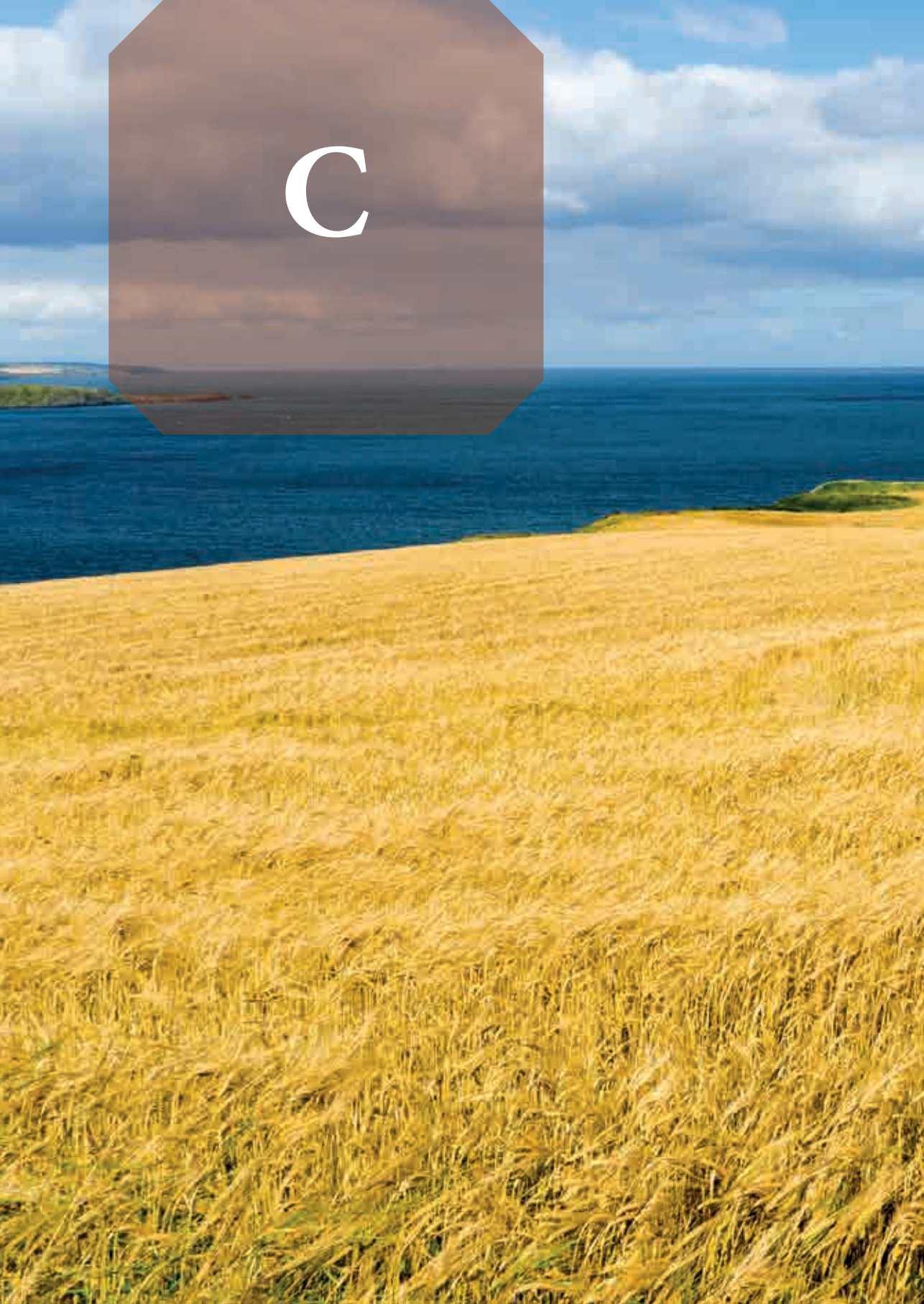
The second part starts in the fourth chapter with a review of the multiple pathways by which reperfusion injury has been targeted by novel therapeutic strategies, such as adenosine and glucose-insulin administration. Of these, recent preclinical trials regarding glucagon-like-peptide-1 receptor stimulation show some promising potential.

One of these GLP1- receptor agonists is called exenatide, which offers a novel therapeutic strategy for reperfusion injury which was researched in human STEMI patients. The results are outlined in chapter five. The infarct size caused by a STEMI occurs due to direct cell death from a lack of oxygen, but is partially dependent on the occurrence of microvascular obstruction. Animal studies have shown that the infarct size can be reduced by administering exenatide, a glucagon like peptide 1 receptor agonist, something confirmed in an initial human trial. While in our study no direct effect was visible on infarct size, it is possible that a dose related effect may have played an important part.

The third part starts with the sixth chapter which focusses on a literature review of another therapeutic field targeting microvascular obstruction, utilizing the combination of ultrasound and microbubbles (or Ultrasound Contrast Agents (UCA)); a technique that is called sonothrombolysis. This therapy utilizes a combination of ultrasound and microbubbles to specifically target and treat localized arterial thrombi. All previously published clinical trials are discussed summarizing currently known data about human trials regarding ischemic cerebrovascular attacks and myocardial infarction. Ideally, treatment of a patient starts as soon as possible after a diagnosis has been made. With STEMI, this usually means administration of pharmaceutical agents in the ambulance and immediate (or with as short of a delay as possible) transport to the catheterization laboratory. Earlier, preclinical studies and early human trials have demonstrated that sonothrombolysis can be a means of treating arterial clots. This is accomplished by a process called cavitation, where UCA are agitated by high intensity ultrasound. When an ultrasound wave hits a microbubble, the sheer force of impact from the sound wave causes UCA to deform (stable cavitation); if the intensity of the ultrasound is high enough, the UCA actually explodes (inertial cavitation). This causes intense stress on the surrounding tissues.

Chapter seven consists of an experiment using an in vitro arteriole mimicking flow model, designed to determine the physical kinetic properties of inertial cavitation. Venous clots were injected into a blood filled flow system, occluding the microvasculature, which was mimicked by means of a small nylon mesh. By changing the ultrasound parameters, as well as adding different combinations of pharmaceutical agents, an optimal therapeutic effect was found.

The final chapter (eight) applied these latest ultrasound settings in a human pilot study, abbreviated as ROMIUS. This study was designed to apply the latest knowledge about ultrasound kinetics in patients with STEMI, in order to determine the therapeutic effect on long term myocardial recovery. Patients admitted to the hospital were randomized to either sham ultrasound without UCA infusion, or ultrasound therapy (sonolysis) with administration of UCA. Unfortunately, this study was prematurely cancelled due to safety concerns; application of ultrasound in patients caused, in contrast with earlier human trials, severe coronary vasoconstriction. Long term side-effects fortunately did not occur, but additional research is necessary in order to fine tune this therapy before widespread use can be considered.


C

Appendix C: Nederlandse Samenvatting

Ontwikkelingen in de algemene gezondheidszorg en geavanceerde therapeutische opties op het gebied van het ST-segment geëleveerd acuut myocardinfarct (STEMI) hebben de sterfte drastisch verminderd. De introductie van primaire percutane coronaire interventie (PCI) als behandelingsoptie voor STEMI is een belangrijke wetenschappelijke prestatie. Hoewel het in vele landen alom beschikbaar is, geldt het oude adagium 'tijd is spierkracht' nog steeds. Patiënten komen niet snel genoeg op het hartcatheterisatielaboratorium om PCI een volledig therapeutisch effect te geven. Onmiddellijk na occlusie van de slagader treden intracellulaire ischemische veranderingen op, welke een cascade teweegbrengen die uiteindelijk leidt tot cellulaire apoptose. De twee bijdragers hieraan worden (letaal) reperfusieletsel en microvasculaire obstructie (MVO) genoemd. Reperfusieletsel treedt op als gevolg van een combinatie van myocardiaal oedeem, zwelling van het endotheel, vasospasme, ontstekingsreacties en distale trombo-embolisatie. MVO ontstaat daarentegen vooral door manipulatie van de occlusie door draad en ballon, hoewel het zeer waarschijnlijk is dat er een overlap is tussen deze twee routes. Het doel van het onderzoek gebundeld in dit proefschrift was om mogelijke nieuwe therapeutische opties gericht op reperfusieletsel en MVO te evalueren. Hoofdstuk één bestaat allereerst uit een algemene inleiding en uiteenzetting van dit proefschrift.

Natuurlijk is het erg belangrijk om te kunnen bepalen welke patiënt het meest baat heeft bij aanvullende therapie. Dit zal niet alleen geld besparen, omdat nieuwe therapieën vaak erg duur zijn, maar voorkomt dat er bijkomende bijwerkingen optreden en verbetert (aldus) de therapietrouw van de patiënt. Het eerste deel bespreekt mogelijkheden hierin. In het tweede hoofdstuk werd een nieuwe niet-invasieve beeldvormingstechniek ontwikkeld en getest op een populatie van STEMI-patiënten, om te bepalen of het mogelijk is om te voorspellen welke patiënten meer baat kunnen hebben bij aanvullende therapie. Deze techniek werd de FLASH (Fluoroscopy Assisted Scoring of Myocardial Hypoperfusion) genoemd.

FLASH bestaat uit de verhouding tussen meerdere flow / snelheidsmetingen met frametellingen op een coronair angiografisch beeld. Het is duidelijk dat invasieve metingen, b.v. uitgevoerd tijdens de initiele PCI, de gouden standaard is als het gaat om het bepalen van de coronaire flow. Maar dit vereist extra apparatuur en expertise, die misschien niet altijd beschikbaar is. FLASH bleek de mortaliteit door patiënten in een grote populatie van STEMI-patiënten te kunnen voorspellen.

In hoofdstuk drie werd een poging gedaan om de langetermijngevolgen van STEMI te voorspellen, door 3D-echografie te gebruiken om de myocardiale spanning (strain) te meten. Na een hartinfarct wordt een deel van de spier die sterft vervangen door bindweefsel. De algehele functie en vorm van het hart veranderen, iets dat goed kan zijn (reverse remodellering) of slecht (ongunstige remodellering). Momenteel gebruiken de meeste klinieken 2D-echografie om de linker hartkamer te visualiseren na een hartinfarct, om de infarctgrootte en de hartfunctie te bepalen. Bovendien is het mogelijk om met behulp van echografie visueel te bepalen hoeveel inspanning of inspanning het myocardium ondergaat wanneer het samentrekt. In dit proefschrift wordt aangetoond dat de 3D-verkregen globale longitudinale strain voorspellend is voor ongunstige remodellering bij patiënten, terwijl de 3D globale circumferentiële stam bij baseline voorspellend is voor het optreden van reverse remodellering. Bepalen welke patiënten de grootste kans hebben op het krijgen van een van deze verschijnselen, is van cruciaal belang voor langdurige behandeling van patiënten.

Het tweede deel begint in het vierde hoofdstuk met een overzicht van meerdere nieuwe therapeutische strategieën welke het reduceren van reperfusieletsel als doel hebben, zoals toediening van adenosine en glucose-insuline. Hiervan laten recente preklinische studies met betrekking tot glucagon-like-peptide-1-receptorstimulatie een veelbelovend potentieel zien.

Een van deze GLP1-receptoragonisten wordt exenatide genoemd, dat een nieuwe therapeutische strategie biedt voor reperfusieletsel, welke werd onderzocht bij menselijke STEMI-patiënten. De resultaten worden beschreven in hoofdstuk vijf. De infarctgrootte veroorzaakt door een STEMI treedt op als gevolg van directe celdood door een gebrek aan zuurstof, maar is gedeeltelijk afhankelijk van het optreden van microvasculaire obstructie.

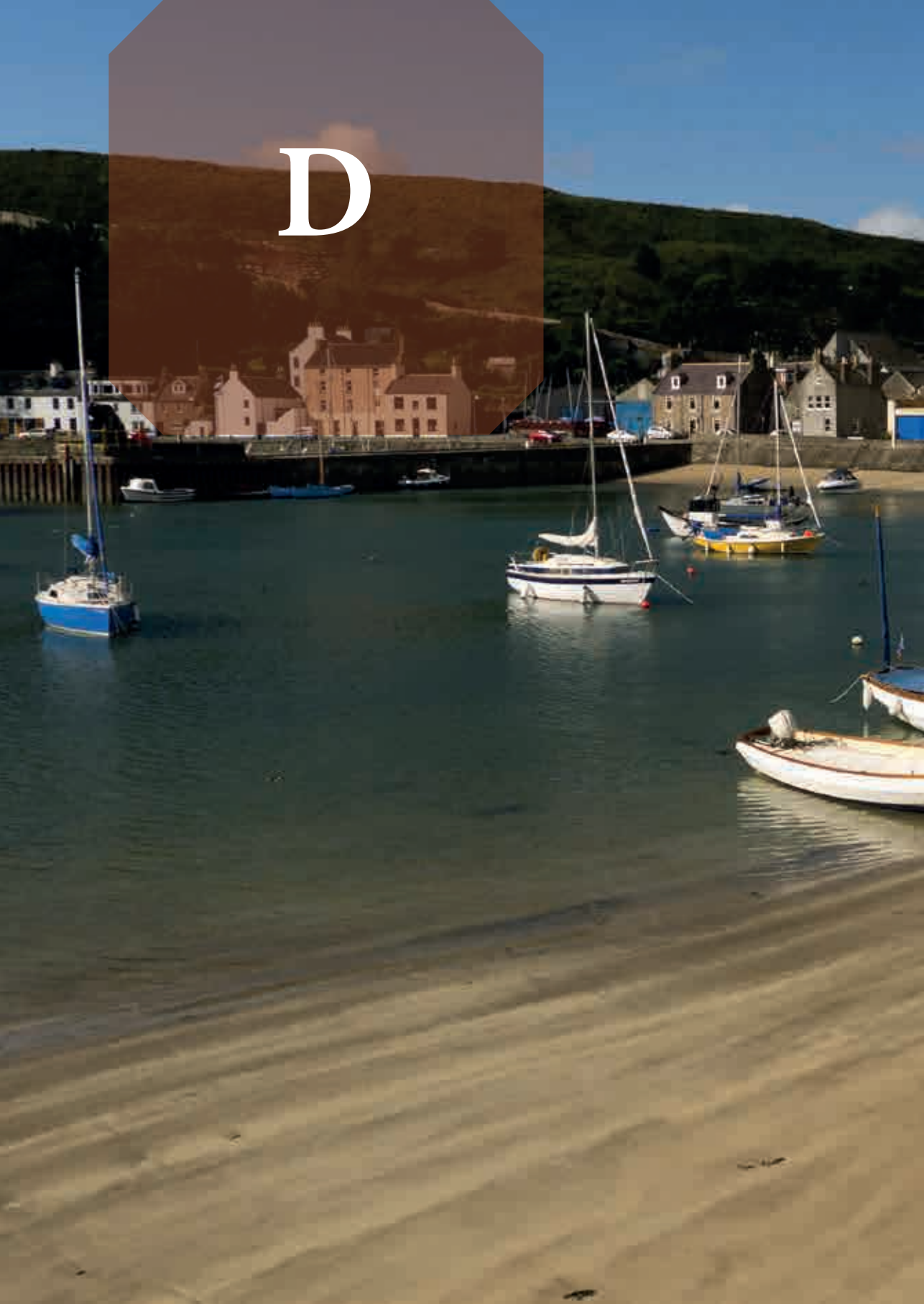
Dierstudies hebben aangetoond dat de grootte van het infarct kan worden verminderd door exenatide toe te dienen, een glucagonachtige peptide 1 receptoragonist, iets dat bevestigd werd in een eerste menselijke proef. Hoewel er in onze studie geen direct effect zichtbaar was op de grootte van het infarct, is het mogelijk dat een dosisafhankelijk effect hierin een belangrijke rol heeft gespeeld.

Het derde deel, begint in het zesde hoofdstuk welke concentreert op een literatuuroverzicht van een ander therapeutisch veld gericht op microvasculaire obstructie, waarbij gebruik wordt gemaakt van een combinatie van echografie en microbellen (of ultrasone contraststoffen (UCA)) wat 'sonothrombolyse' wordt genoemd. Deze therapie maakt gebruik van een combinatie van echografie en microbellen om gelokaliseerde arteriële trombi gericht te behandelen. Alle eerder gepubliceerde klinische studies worden besproken met een samenvatting van de momenteel bekende gegevens over menselijke onderzoeken met betrekking tot het ischemische cerebrovasculair accident en hartinfarcten. Idealiter begint de behandeling van een patiënt zo snel mogelijk nadat een diagnose is gesteld. Met STEMI betekent dit meestal toediening van farmaceutische middelen in de ambulance en onmiddellijk (of met zo min mogelijk vertraging) transport naar het katheterisatielaboratorium. Eerder hebben preklinische studies en vroege proeven bij mensen aangetoond dat sonothrombolyse een middel kan zijn om arteriële stolsels te behandelen. Dit wordt bereikt door een proces genaamd cavitatie, waarbij UCA wordt geagiteerd door ultrasone golven met hoge intensiteit. Wanneer een ultrasone golf een microbel raakt, veroorzaakt de enorme kracht van de impact van de geluidsgolf dat UCA vervormt (stabiele cavitatie); als de intensiteit van de echografie hoog genoeg is, explodeert de UCA eigenlijk (inertiële cavitatie). Dit veroorzaakt intense stress op de omliggende weefsels.

Hoofdstuk zeven bestaat uit een experiment met een in vitro arteriole-nabootsend stromingsmodel, ontworpen om de fysieke kinetische eigenschappen van inertiële cavitatie te bepalen. Veneuze stolsels werden geïnjecteerd in een met bloed gevuld stroomsysteem, waardoor de microvasculatuur werd afgesloten, wat werd nagebootst door middel van een klein nylon gaas. Door de ultrasoundparameters te veranderen, en door verschillende combinaties van farmaceutische middelen toe te voegen, werd een optimaal therapeutisch effect gevonden.

Het laatste hoofdstuk (acht) paste deze laatste echo-instellingen toe in een menselijke pilotstudie, afgekort als ROMIUS. Deze studie was bedoeld om de nieuwste kennis over echografische kinetiek toe te passen bij patiënten met een STEMI, om het therapeutische effect op het myocardiaal herstel op de lange termijn te bepalen. Patiënten die in het ziekenhuis werden opgenomen, werden gerandomiseerd naar ofwel echografie zonder UCA-infusie of ultrasone therapie (sonolyse) met toediening van UCA. Helaas is deze studie voortijdig geannuleerd vanwege bezorgdheid over de veiligheid; toepassing van sonolyse bij patiënten veroorzaakte, in tegenstelling tot eerdere proeven bij mensen, ernstige coronaire vasoconstrictie. Lange termijn bijwerkingen kwamen gelukkig niet voor, maar aanvullend onderzoek is nodig om deze therapie te verfijnen voordat algemeen gebruik kan worden overwogen.

D



Appendix D: Curriculum Vitae

Sebastiaan Theo Roos werd geboren op 26 april 1988 te Utrecht. In 2005 behaalde hij zijn Gymnasium diploma aan het Christelijk College Nassau Veluwe te Harderwijk, waarna hij in Leiden aan de opleiding Geneeskunde begon. In 2007 volgde een oriënterende wetenschappelijke stage bij de kindercardiologie onder dr. ADJ ten Harkel, naar de werking van ICD's bij kinderen in Nederland. In 2009 volgde op dezelfde afdeling een wetenschappelijke stage naar de impact van cardiopulmonaire bypass op systolisch en diastolische hartfunctie, waarbij speckle tracking vergeleken werd met oudere technieken. Verder was hij van 2008 tot 2011 werkzaam op de afdeling Heelkunde, alwaar hij verantwoordelijk was voor ontwikkeling van e-learning en tentamens. Zijn semi-artsstage werd in het Juliana Kinderziekenhuis te Den Haag gevolgd, met specifiek aandacht voor de neonatologie, onder begeleiding van dr. RH Lopes Cardozo. Na het artsexamen is hij in 2011 begonnen met zijn promotieonderzoek, op de afdeling Cardiologie in het VU medisch centrum, waaruit deze thesis is voortgevloeid. Een deel van het onderzoek werd verricht in Pittsburgh, USA onder leiding van prof. F Villanueva. De resultaten van het onderzoek zijn beschreven in dit proefschrift en gepresenteerd op verscheidene nationale en internationale congressen. Extra curriculair was hij van 2013-2015 penningmeester van de Promovendi Vereniging ProVU. Tijdens de afrondende fase van het proefschrift volgden 2 jaar als ANIOS cardiologie in respectievelijk het Spaarne Gasthuis te Haarlem en het VU Medisch Centrum te Amsterdam, gedurende welke de afrondende werkzaamheden aan dit proefschrift zijn uitgevoerd.


E

Appendix E: Lijst van Publicaties

Roos ST, Juffermans LJM, Slikkerveer J, Unger EC, Porter TR, Kamp O. Sonothrombolysis in acute stroke and myocardial infarction: A systematic review. IJC Heart & Vessel 2014;4:1–6

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Roos ST, Yu FT, Kamp O, Chen X, Villanueva FS, Pacella JJ. Sonoreperfusion Therapy Kinetics in Whole Blood Using Ultrasound, Microbubbles and Tissue Plasminogen Activator. Ultrasound Med Biol 2016;42:3001–3009.

Roos ST, Juffermans LJM, van Royen N, van Rossum AC, Xie F, Appelman Y, Porter TR, Kamp O. Unexpected High Incidence of Coronary Vasoconstriction in the Reduction of Microvascular Injury Using Sonolysis (ROMIUS) Trial. Ultrasound Med Biol Elsevier, 2016;42:1919–28.

Roos ST, Labate V, van Rossum AC, Kamp O, Appelman Y. Added value of 3D ultrasound deformation imaging in STEMI patients for early detection of left ventricular remodeling. Submitted

Amier RP, Smulders MW, van der Flier WM, Bekkers SCAM, Zweerink A, Allaart CP, Demirkiran A, Roos ST, Teunissen PFA, Appelman Y, van Royen N, Kim RJ, van Rossum AC, Nijveldt R. Long-Term Prognostic Implications of Previous Silent Myocardial Infarction in Patients Presenting With Acute Myocardial Infarction. JACC Cardiovasc Imaging. 2018 Apr 13.

F

Appendix F: Dankwoord

In de afgelopen 7 jaar hebben veel mensen een grote of kleine bijdrage geleverd aan dit proefschrift. Het eindresultaat heeft weliswaar maar 1 auteur op de voorkant, maar de totstandkoming had niet kunnen plaatsvinden zonder de steun en toewijding van vele tientallen, zo niet honderden, mensen. Derhalve dit dankwoord.

Een belangrijke groep, en daarom ook als eerste vermeld, betreft alle proefpersonen en familieleden, die ondanks de schrik van het plotse hartinfarct, toch mee wilden doen aan experimentele behandelingen en een vaak intensief natraject. Hulde, zonder jullie was dit niet mogelijk geweest.

Otto, toen ik in september 2011 bij jou op gesprek kwam, had ik mij geen enkele voorstelling kunnen maken van het avontuur waar we in zouden duiken; bij en door jou was alles mogelijk, van onderzoek doen in Pittsburgh tot via-via een obscuur flesje microbellen opduikelen. Dank voor alles wat ik door jou geleerd heb.

Yolande, wij kwamen elkaar pas op een later moment tegen, waarbij de mogelijkheid om een studie over te nemen ervoor gezorgd heeft dat ik een heel andere tak van sport heb mogen ervaren. Ik ben blij dat ik deze mogelijkheid heb aangepakt, jullie stijl is dusdanig verschillend dat ik hoop er met het beste van 2 werelden vandoor te gaan!

Bert, via het ICIN ben ik in het VUmc terecht gekomen, dank voor deze mogelijkheid om vele jaren Amsterdam mijn thuisbasis genoemd te mogen hebben.

De leden van mijn leescommissie wil ik via deze weg ook bedanken voor hun tijd in het beoordelen van mijn proefschrift. In het bijzonder bedank ik Klazina, voor de gezellige tijd die we samen in Pittsburgh hebben doorgebracht en Pieter, voor de goede gesprekken onder het genot van lekker eten in Utrecht.

Of course I also want to wholeheartedly thank you, Liza and John, for allowing me to visit Pittsburgh and perform research under your guidance; you have both taught me a lot. I am also grateful for the support and good times with the rest of the team, especially (in random order) François, Rick, Judith, Reagent, Linda and Xucai! I had an amazing experience!

Ook het EXAMI-team uit Utrecht wil ik bedanken, in het bijzonder Leo en Geert, voor al hun ondersteuning tijdens en na het uitvoeren van de studie ter plaatse.

Van de afdeling moleculaire celbiologie wil ik met name Tineke en Josefien bedanken; zonder hun hulp had ik veel lab-vaardigheden niet kunnen opdoen.

In het VUmc zijn ook vele behulpzame mensen welke ik dankbaar ben; allereerst natuurlijk de onvoorwaardelijke steun van de echolaboranten, in het bijzonder Vidya, Marian, Marielle en echo-mama Linda. Ik heb vele technieken geleerd en hoop jullie ooit nog eens te overtreffen.

Verder wil ik alle verpleegkundigen van CCU, de HCK en 5B in de afgelopen jaren bedanken, met name de Exami zal grijze haren hebben bezorgd bij sommigen, toch dank voor jullie doorzettingsvermogen!

Daarbij horen natuurlijk ook de arts-assistenten welke in de loop der jaren heel veel van 'mijn' patiënten ondersteund hebben, maar ook de stafleden en in het bijzonder de interventiecardiologen, welke toch altijd kritisch bleven kijken naar de studies die uitgevoerd werden.

Ook de research verpleegkundigen zijn cruciaal geweest. Mary, dank voor het altijd mogelijk maken van (het plannen van) een MRI. Ook Debbie en Ellen; dank voor de steun, maar ook gezelligheid in de afgelopen jaren.

Mijn medeonderzoekers, en dan vooral mijn 5D kamergenoten Ahmet, Mischa, Paul, Monique en LiNa, en later Lynda, maar natuurlijk ook Wynand, Ibrahim, Lourens, Stefan, Roel, Raquel, Nina, Gladys, Alwin, Henryk -Jan, wil ik via deze weg bedanken voor de gezellige borrels, boottochten, congresavonturen en alles daaromheen.

Naast onderzoek heeft promoveren natuurlijk ook organisatorische aspecten; mijn tijd als penningmeester van promovendivereniging ProVU heeft mij ook waardevolle herinneringen gegeven, waarvoor ik in het bijzonder Ilona en Tom wil bedanken. Ook iedereen bij het PNN dank voor de fijne momenten.

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Het leven bestaat gelukkig niet alleen uit werk en onderzoek, ook daarnaast moet voldoende ontspanning plaatsvinden. Daarom wil ik al mijn vriend(inn)en bedanken voor de gezellige tijd, gezamenlijke vakanties, wijnavondjes, sportmomenten en alles daaromheen. In het bijzonder geldt dat voor de volgende mensen.

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Leonie, al bijna 15 jaar geleden 'erbij' gekomen, maar met meer dan goed resultaat. Dank voor je vriendschap en steun in alles, als ook de fijne herinneringen aan spelletjesavonden!

Anne, vanaf de start van geneeskunde raakten wij bevriend, iets wat hopelijk nog lang zal voortduren. Ook jou wil ik bedanken voor de fijne tijd met vakanties en ontspanning, laten we hopen dat Maud een toevoeging wordt daaraan.

Benjamin, jij kan natuurlijk daarbij niet achter blijven; tenslotte blijf je me op de (race)fiets ruim voor. Hopelijk kan dat binnenkort andersom worden, en racen we nog vele jaren samen over de (afgesloten) wegen!

Martijn, ondanks onze vele verschillende standpunten is er 1 ding wat ons boven alles verbindt; het kunnen genieten van het leven in al zijn (maar vooral culinaire) aspecten. Je hebt mijn ogen destijds in Frankrijk verder geopend; iets waarvoor ik je altijd dankbaar zal zijn. Ik hoop dat ik nog vele jaren van je mag leren; besef dat ik enorm naar je op kijk!

Emile, toen wij op dag 1 van de introductie-week geneeskunde naast elkaar gingen zitten, was ons nog niet bekend hoe diep onze vriendschap zich zou gaan wortelen. Dat jij hier naast mij zou staan was al wél vanaf dag 1 van mijn promotie overduidelijk. Wij hebben elkaar op onze diepste en hoogste momenten kunnen ondersteunen, wat mij betreft zetten we die trend nog vele lange jaren voort!

Lieve Marcel & Nannette, ik kan mij niet voorstellen hoe ik dit zonder jullie had kunnen doen. Ondanks alle kracht die we verloren hebben, bleven jullie een onwankelbaar vertrouwen in mij hebben. Ik voel mij gekoesterd en geliefd en ben dankbaar dat ik in jullie gezin een plaats heb gekregen; mijn gelofte aan jullie dochter in stand houden zal mij nimmer energie kosten.

Lieve Noam, klein (te) stoer broertje van me, wat bewonder ik je kracht en doorzettingsvermogen. Ik ben blij met alle steun die ik van jou heb mogen ontvangen, weet dat ik altijd ook voor jou klaar zal staan. Dank dat je deze גוי in je hart hebt gesloten.

Lieve Stephanie, oh zo trots ben ik op wat jij ondanks alles in je leven hebt weten te bereiken. Ik ben vooral blij dat we elkaar de laatste jaren zo goed hebben leren kennen; weet dat je in de toekomst altijd langs kan komen als Nijkerk je teveel wordt, de high-tea's zijn hier in Leiden zoals je weet ook veel beter! Ik wens je alle liefde en geluk in de toekomst toe.

Lieve Papa, tussen ons zijn woorden vrijwel nooit nodig; als binnenvetters weten we wat we elkaar willen zeggen en wat er in het koppie om gaat. Toch wil ik het hier heel duidelijk maken; ik hou van je, bedankt voor de steun, liefde en onvoorwaardelijke trouw. May the force be forever with us.

Lieve Mama, wat ben je toch een krachtig en mooi persoon. Dank dat je al vele jaren mijn plagerijtjes verdraagt, en dank voor je grote interesse en bezorgdheid in mijn gestel en leven. Ik had mij geen liefdevollere moeder kunnen bedenken en ik hoop dat duidelijk is hoeveel ik ook van jou hou, want het tegeltje blijft waar; 'mijn mama is de beste op de hele wereld'.

Mijn lieve Ilanit, lief klein meisje, wat hadden wij het ongelooflijk mooi samen. Ik weet zeker dat je trots op me neer kijkt vandaag, maar ook in de verdere toekomst. Weet dat ik probeer om jouw herinnering in al mijn handelingen duidelijk te maken, zodat jouw naam nooit in de vergetelheid zal raken. Ik draag de liefde die wij voor elkaar hadden de rest van mijn leven met me mee en mocht er ooit een moment komen dat wij elkaar weerzien, hoop ik dat we kunnen voortzetten wat ons veel te vroeg is ontnomen. Ik hou van je en heb je innig lief.