

**Cardiac troponin I and electrocardiogram for estimation of
infarct size and for risk stratification in patients with ST-
elevation myocardial infarction treated with primary
percutaneous coronary intervention**

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1. BRIEF SUMMARY

Background: Cardiac troponin and the electrocardiogram (ECG) are essential diagnostic tools in acute coronary syndrome. They are also important prognostic markers. Cardiac troponin reflects myocardial necrosis. Following reperfusion therapy for ST-elevation myocardial infarction (STEMI), ECG-derived measures of ST-segment recovery (STR) reflect tissue perfusion and are associated with clinical outcome.

Aims: In a population of STEMI patients receiving primary percutaneous coronary intervention (pPCI), to study the association of cardiac troponin I (TnI) with infarct size (IS), left-ventricular (LV) function and volumes and microvascular obstruction (MVO); and to study the association of 3 measures of STR with IS and LV function.

Methods: Post hoc analyses of the 227 patients enrolled in the overall negative FIRE (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) trial. cTnI sampled at 24 and 48 hours. STR calculated immediately following pPCI and at 90 minutes. The outcome measures (IS, MVO and LV function and volumes) were obtained by (late gadolinium enhanced) cardiac magnetic resonance (CMR) at 5-7 days and 4 months following the index event.

Results: cTnI was significantly correlated with IS, LV ejection fraction (LVEF) and LV volumes at both 5-7 days and 4 months. The associations between cTnI and these outcome measures at 4 months remained after adjustment for the early CMR evaluation. cTnI was associated with MVO independent of IS. All 3 STR algorithms were significantly associated with IS and LVEF at 4 months. The simple metric of worst-lead residual ST-segment deviation evaluated at 90 minutes post-intervention was comparable to the more complex STR algorithms.

Interpretation and conclusion: Both measurement of cTnI and calculation of STR provide useful information on IS and LV function and remodeling in STEMI patients treated with pPCI, and may allow for early and simple risk stratification of this patient population. The clinical utility of these findings for prognostic assessment awaits further prospective studies.

2. ACKNOWLEDGEMENTS

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I am greatly indebted to my mentor and main supervisor Professor Dan Atar who introduced me to cardiovascular research and academic medicine. His scientific insights, skillful guidance, generosity and support have been absolutely critical to the realization of this thesis. Dan has been available literally every day for the better part of 4 years for consultations and immediate feedback. I also much enjoy our continued collaboration. Many thanks are also due to my co-supervisor Professor Peter Clemmensen and his research fellow Maria Sejersten at Rigshospitalet in Copenhagen. They helped me get started with the ECG analyses and provided scientific guidance and support throughout.

Dan introduced me to Dr. Rainer Henning, CEO of Fibrex Medical GmbH (Vienna, Austria). Rainer kindly made available the database from the FIRE trial to me. Without his support this project would not have been possible. I would also like to thank the rest of the Fibrex team and the external collaborators, especially statistician Marcos Marin-Galliano at IFE (Essen, Germany) and Professor Peter Buser at Basel University Hospital, Switzerland.

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3. ABBREVIATIONS

ACS	Acute Coronary Syndrome
AUC	Area Under the Curve
CK- (MB)	Creatine Kinase – (Myocardial Band)
CMR	Cardiac Magnetic Resonance
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
ECG	Electrocardiogram
EDVI	End Diastolic Volume Index
ESVI	End Systolic Volume Index
LDH	Lactic Dehydrogenase isoenzymes 1 and 2
LV	Left-Ventricular
LVEF	Left-Ventricular Ejection Fraction
(A)MI	(Acute) Myocardial Infarction
MVO	Microvascular Obstruction
pPCI	Primary Percutaneous Coronary Intervention
SPECT	Single-Photon Emission Computed Tomography
STEMI	ST-segment Elevation Myocardial Infarction

4. LIST OF PAPERS

Paper I

Hallén J, Buser P, Schwitter J, Petzelbauer P, Geudelin B, Fagerland MW, Jaffe AS, Atar D. Relation of cardiac troponin I measurements at 24 and 48 hours to magnetic resonance-determined infarct size in patients with ST-elevation myocardial infarction. *Am J Cardiol.* 2009;104(11):1472-7.

Paper II

Hallén J, Jensen JK, Fagerland MW, Jaffe AS, Atar D. Cardiac troponin I for the prediction of functional recovery and left ventricular remodeling following primary percutaneous coronary intervention for ST-elevation myocardial infarction. *Heart.* 2010; 96(23):1892-7.

Paper III

Hallén J, Jensen JK, Buser P, Jaffe AS, Atar D. Relation of cardiac troponin I and microvascular obstruction following ST-elevation myocardial infarction. *Acute Cardiac Care.* 2011; 13(1):48-51

Paper IV

Hallén J, Sejersten M, Johanson P, Atar D, Clemmensen PM. Influence of ST-segment recovery on infarct size and ejection fraction in patients with ST-segment elevation myocardial infarction receiving primary percutaneous coronary intervention. *Am J Cardiol.* 2010;105(9):1223-8.

5. INTRODUCTION

5.1 A Short History of Acute Myocardial Infarction

Coronary artery disease is the leading cause of death worldwide (1). The global burden of cardiovascular disease is expected to increase in the coming years, as falling mortality rates from coronary artery disease in the Western world (2, 3) are more than offset by the continuing epidemiological transition in developing countries away from nutritional deficiencies and infectious disease towards chronic and degenerative pathologies, cardiovascular disease being the most prominent (4-6).

Acute MI is a serious and potentially lethal manifestation of coronary artery disease, afflicting more than 7 million people worldwide each year (7). James Herrick established MI as a distinct clinical entity in 1912 and also installed the mainstay management strategy - which prevailed for the next 50 years - in stressing the importance of “absolute bed rest” (8). Thanks to a remarkable scientific journey throughout the last 60 years – spanning epidemiology, basic science, and clinical trials – such long-held beliefs are now considered obsolete; and a comprehensive, continually evolving evidence base has been generated, from which contemporary preventive and therapeutic strategies have been developed.

Beginning in the late 1940s, prospective studies were designed to define lifestyle, environmental and other factors contributing to the incidence of MI (9-12). In the 1960s, designated coronary care units were established in many hospitals to monitor AMI patients and ensure prompt resuscitation in the event of life-threatening arrhythmias (13); a development called the “single most important advance in the treatment of acute MI” (14). These significant progressions were then augmented by basic science studies elucidating many of the key underlying mechanisms of AMI, which paved the way for landmark clinical trials spanning from acute interventions such as reperfusion therapy to long-term pharmacological therapies that are now cornerstones in the management of AMI patients (15-22). The combined impact of these

preventive and therapeutic measures has resulted in large reductions in mortality following AMI in the developed world (23).

In STEMI, the last innovation to provide clear-cut incremental benefit has been the introduction of pPCI. When delivered in a timely fashion pPCI reduces early death, re-infarction and stroke compared to pharmacological reperfusion by fibrinolysis (24). Further therapeutic innovations and effective preventive strategies are still being pursued (25, 26). Among these are efforts to protect the ischemic myocardium against reperfusion injury. Reperfusion injury refers to tissue damage occurring as a consequence of blood supply being reestablished after a period of ischemia (27). Mitigation of reperfusion injury has been demonstrated in animal models, but proved difficult to replicate in clinical studies (28). Several approaches have been tested, but none have yet demonstrated efficacy in pivotal clinical trials (29, 30). The FIRE (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) trial, from which database this thesis is based, also failed to replicate experimental findings in a clinical setting (31, 32). The concept of post-conditioning has emerged as one of the most promising strategies to confer cardioprotection in the setting of reperfusion (33, 34). It is currently being evaluated in multiple randomized trials. So far, results have been encouraging (35, 36), but benefits may be restricted to patients with large infarctions (37).

Despite the significant improvements in prognosis, a gradient of risk still exist following AMI, and risk stratification remains crucial to allocate resources efficiently, optimize patient outcomes and limit adverse events. Particularly, early identification of high-risk patients with extensive myocardial injury is important to ensure appropriate administration of pharmacotherapies and prophylactic interventions (22, 38, 39).

5.2 Brief overview of the thesis

The studies upon which this thesis is built aimed to gain specific insight into the prognostic applications of two well-established diagnostic and risk stratification tools for evaluation of STEMI patients: Cardiac troponin measurements and the ECG. The methodological approach is simple: Essentially, measurements of cTnI levels and assessment of ST-segment recovery following pPCI are analyzed in relation to the extent and degree of myocardial necrosis and cardiac function and volumes, all determined by CMR. The analyses are based on investigations performed on a population of STEMI patients treated with pPCI within 6 hours from onset of symptoms. The study population participated in a randomized, placebo-controlled trial (FIRE) designed to characterize the safety and potential cardioprotective properties of a novel compound called FX06 in the setting of reperfusion therapy for STEMI. The FIRE trial was negative regarding the primary endpoint of CMR determined infarct size at 5-7 days.

The first section will place the subject in context. An overview of the cardiac troponins and their contemporary applications in the management of AMI will be followed by an introduction to the concept and history of estimating myocardial necrosis by blood-borne biomarkers. Last, the use of ST-segment analysis for assessment of reperfusion status and prognosis will be summarized. To facilitate interpretation of the rationale of my own investigations, the “knowledge gap” that this project sought to fill will be highlighted in the relevant sections, before the specific aims of this thesis will be articulated in the next chapter.

The subsequent parts will cover the methods used and the findings of our investigations. A discussion of the main results of each study will be followed by a consideration of the methodological approach, before the findings of the project as a whole are interpreted in concert and a tentative, integrated understanding of the clinical implications is proposed.

5.3 Acute Coronary Syndromes

ACS is a unifying term for a set of signs and symptoms reflecting reduced myocardial perfusion resulting in myocardial ischemia. If the myocardial ischemia is severe enough to cause myocardial cell death, the acute coronary event is classified as an AMI, while ACS without evidence of necrosis is called unstable angina. AMI is then further subdivided according to whether persistent electrocardiographic ST-segment elevations are present (ST-segment elevation MI and non-ST-segment elevation MI). Three variables are at the core of the operational definition of AMI: 1) symptoms of ischemia; 2) ECG changes indicative of new ischemia; and 3) the detection of a rise and/or fall in biochemical markers of myocardial necrosis (40). In response to the continuing diagnostic advances, especially with regard to the measurement of cardiac biomarkers, the European Society of Cardiology and the American College of Cardiology convened in 1999 to formulate joint recommendations for a Universal Definition of Myocardial Infarction (41). The consensus document was updated in 2007 (40).

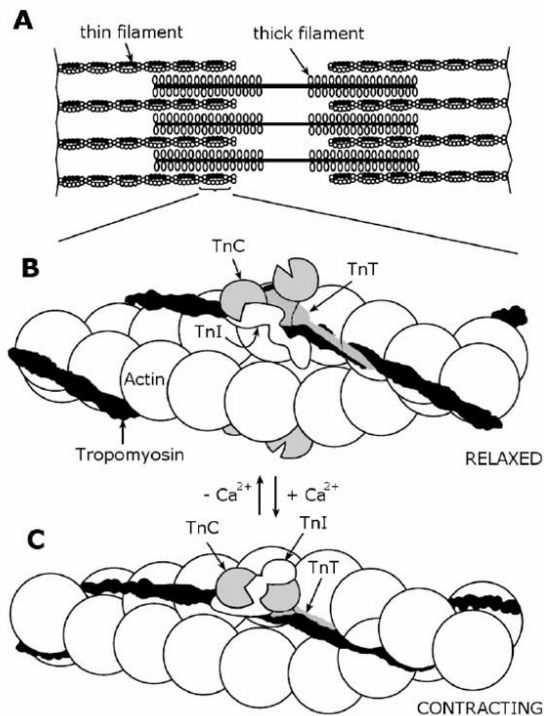
5.4 Cardiac Troponin in Acute Myocardial Infarction

5.4.1 *The biology of troponin*

Troponin is a protein complex of three subunits (I, C and T) that modulate the calcium-mediated interaction between actin and myosin in skeletal and cardiac muscle tissue (figure 1) (42, 43). Subunits I and T exist in 3 different isoforms: in fast and slow skeletal muscle and in myocardial cells. Each isoform is the product of a separate gene (44-46). The unique myocardial isoforms (cTnI and cTnT) can be detected by assays of monoclonal antibodies directed against cardiac-specific epitopes (47). In the myocardial cells, the majority of the cardiac troponins are bound to the contractile apparatus, while a small fraction (3-8 %) is free in the cytoplasm (48-50). In the event of myonecrosis, cardiac troponins are released and can be detected in the bloodstream only a few hours afterwards, as the cytosolic form is released; and then for a prolonged period of up to

2 weeks, as the structural pool is slowly liberated (49, 51). The cardio-specificity coupled with the fact that cTnT and cTnI do not circulate in measurable levels among healthy individuals¹ has given rise to their use in clinical cardiology replacing by and large older markers of myocardial injury. Whether cardiac troponin may also be released in response to pure myocyte ischemia without necrosis remains controversial (54).

Figure 1. The cardiac troponin subunits and their role in muscle contraction. Adapted from Collinson et al (55).



¹ With the new highly sensitive assays now being developed this is no longer true, as minute amounts of circulating troponin are detectable also in a substantial fraction of a healthy reference population (52). Cut-offs and consideration of the temporal dynamics of cardiac troponin values are required to differentiate acute events from chronic conditions (53).

5.4.2 *Cardiac troponin assays*

Assays for both cTnI and cTnT have progressively improved since their introduction in the late 1980s (56-59), and current assays are much more sensitive than previous generations of older assays. There are a number of different cTnI assays in clinical use which exhibit a large variability in their sensitivities (60, 61). These differences are caused by the extensive degradation of cTnI which results in various fragments of circulating cTnI and that antibodies used in the different assays detect these fragments differently (62-65). Although cTnT is also subject to extensive degradation before and after release from necrotic myocytes (66), standardization is not usually a problem as there is only one commercially available cTnT assay, although in 5 different generation of assays (52, 57, 67-69). cTnT and cTnI assays provide comparable diagnostic and prognostic information, except in patients with renal failure (63, 70).

5.4.3 *Current applications in AMI – diagnosis*

Measurement of cardiac troponin is the “gold standard” test for myocardial necrosis and was established as the standard serologic biomarker for diagnosis of AMI by the joint committee of the European Society of Cardiology/American College of Cardiology in 2000 and in the 2007 update (40, 41). The cut-off value recommended was at the 99th percentile of a healthy population. Compared to the previous biomarker of choice CK-MB, measurement of cardiac troponin improves both the specificity and sensitivity for the detection of cardiac injury (71, 72). Cardiac troponin is not able to effectively exclude AMI at presentation. Serial measurements are necessary and current recommendations advocate a time-interval of at least 6 hours to rule out AMI (53). Further improvements of the analytical performance of cardiac troponin assays may allow for a slightly earlier diagnosis or rule-out of AMI (73, 74), and adoption of these newer assays has been strongly supported by clinical experts (75-77).

5.4.4 *Current applications in AMI - prognosis*

The prognostic assets of cardiac troponins in non-STEMI were established soon after indications of their diagnostic properties emerged (72, 78). A meta-analysis from 2001 of 21 studies involving > 18,000 non-STEMI patients found that cardiac troponin elevations were associated with a 3.4-fold increase in risk of death and AMI at 30 days (79). The prognostic value of cardiac troponin in this setting reflects its correlation with myocardial necrosis. Accordingly, quantitative analyses of levels of cardiac troponin – as opposed to a dichotomized categorization of positive/negative enhances the predictive value (80). Cardiac troponin release may also be a marker of underlying severe coronary stenosis and complex lesions, culprit lesion thrombosis and downstream embolization with microinfarction (81-83), although these findings are also linked to the total burden of myocardial necrosis.

In keeping with the mechanistic studies indicating that cardiac troponin elevations signal more severe disease, it has been found that cardiac troponin measurements help identify non-ST elevation ACS patients who will benefit from various therapeutic interventions. This was initially demonstrated for anti-thrombotic treatments such as low-molecular weight heparins and glycoprotein IIb/IIIa inhibitors (84-89). It was found that additional antithrombotic therapies reduced the risk of adverse events in the cardiac troponin-positive patients, in effect mitigating the increased hazard associated with troponin elevations; while no benefit of the drugs was observed in troponin-negative patients. Subsequent studies have extended this concept to include early coronary intervention (90, 91).

In STEMI, the prognostic properties of cardiac troponins are influenced by the sampling time-point. A positive cardiac troponin on admission is probably reflective of longer ischemic time and more extensive myocardial necrosis, and consequently predictive of clinical outcomes (92-96). Cardiac troponin levels following reperfusion therapy reflect the extent of myocardial necrosis which is the subject of the next section.

5.5 Estimation of Infarct Size by Biochemical Markers

5.5.1 Historical perspective – the initial rationale behind infarct size estimation

As the establishment of coronary care units in the 1960s reduced mortality from ventricular arrhythmias, most deaths following AMI were related to cardiogenic shock and progressive heart failure due to extensive myocardial damage (97). At the same time emerging experimental evidence suggested that the evolution of AMI was a dynamic process susceptible to mitigation by therapeutic interventions (98, 99). Thus, the initial attempts at estimating infarct size by use of biochemical markers arose from the need to define surrogate parameters to evaluate experimental and clinical trials aimed at cardioprotection and infarct size reduction².

5.5.2 Historical perspective – the basic principles of enzymatic indices of infarct size

Elevations of transaminases in peripheral blood in patients with a very recent MI was described for the first time by Karmen and Wroblewski in 1954 (101), and the diagnostic utility of determination of serum enzyme levels in patients suspected of an acute coronary event was established in several studies published in the early 1960s (102, 103). Historically, the three enzymatic markers mainly used for infarct size estimation have been CK, its more cardiospecific isoenzyme CK-MB, and LDH (also known as alpha-hydroxybutyrate dehydrogenase). CK and CK-MB are present in the cytoplasm of myocardial muscle cells and the release kinetics are characterized by an early peak (< 24 hours) and a rapid return to normal levels (< 72 hours) (104). LDH peaks later (~ 36 hours) and remains elevated for a much longer time (> 100 hours) (104).

² The spirit of those times has been vividly captured by one of the early pioneers of that era, Robert Roberts, in the following citation: *Infarct size was central to the major thrust of cardiac research in the 1970s and as such occupied center stage as the culprit to be conquered by interventions designed to cardioprotect and limit myocardial damage (infarct size). Myocardial infarction, with its consequent necrosis, was the leading cause of death and came under intense attack with infarct size posing as the surrogate villain. Investigators were unified in their attack on infarct size, namely, that its course must be charted, the extent of damage quantified, and ultimately therapy designed for its elimination* (100).

Quantitative models for the estimation of infarct size derived from measuring biochemical markers in the systemic circulation were introduced by two different research groups in the early 1970s (Witteveen and Sobel/Shell) (105, 106). In short, these models aimed to account for the cumulative release of the biomarker in question and then relate this directly to the amount of necrotic myocardium³. The model by Sobel/Shell was designed on the basis of rigorously designed experimental studies of CK and then later for CK-MB (106-110). The Witteveen algorithm was a two-compartment model and could be applied for multiple enzymes, but has mostly been used with measurement of LDH (104, 105). In the absence of confirmation from pathologically determined infarct size, surrogate measures related to cardiac function, electrocardiographic findings or clinical outcomes were used to validate the enzyme measurements. Critics of both the Sobel and the Witteveen approach claimed that the models were sensitive to changes in the underlying assumptions and that the estimates were only useful for small infarctions (111-113). Nevertheless, a multicenter, randomized study in which enzyme release was correlated directly to quantitative histological measurements of infarct size in patients who died, seemed to confirm the accuracy of the enzymatic models (114).

5.5.3 *Historical perspective – the influence of reperfusion*

The emergence of reperfusion therapy introduced a new source of uncertainty for enzyme-based infarct size estimation as it was found that early recanalization of an infarct-related artery accelerated enzyme release. This was initially documented in experimental animal studies and then confirmed in clinical trials (115-117). The difference in the kinetics of enzyme liberation was not a problem *per se*, but it was uncertain whether the more rapid rise in enzyme levels also

³ The following parameters were needed: 1) The release ratio, which is the fraction of the marker depleted from the myocardium that is released into the circulation; 2) The clearance rate and distribution volume. This was needed to calculate the total amount of enzyme released into the blood; 3) The amount (activity or concentration) of the marker that represented a given weight of infarcted tissue, so that the size of the infarct could be calculated. This has conventionally been expressed as gram-equivalents. 4) Last, the sampling interval necessary to determine a time activity (or concentration) curve so that the total amount released could be calculated accurately.

signaled a larger total release of enzymes caused by an increased release ratio. If this was the case then possibly the cardioprotective potential of reperfusion therapy would not be captured by enzyme based infarct size estimation. Although no definite consensus was established, several studies found that myocardial LDH release was consistent regardless of whether thrombolytic therapy was administered or not (118, 119). Results of much later investigations have since supported the validity also of measuring CK AUC or CK-MB AUC for comparison of different reperfusion regimens (120). In the 1980s, enzymatic estimates of infarct size were used in several trials assessing new treatment regimens (118, 121-123). Some studies used the Witteveen model (118, 122) to calculate the total enzyme release and others used the integrated AUC as a surrogate for infarct size without calculating the total amount released (121, 123).

5.6 Cardiac troponin for infarct size estimation

5.6.1 Pathophysiological principles and methodological approach

The release kinetics of the cardiac troponins in the context of myocardial necrosis are characterized by an initial cytosolic liberation with a peak in the case of reperfusion, and then a slowly abating plateau phase reflecting degradation of the structural pool (56-59). This makes the marker uniquely suited to both early diagnosis of infarction and also, at a later time point, for assessing the extent of the myocardial necrosis (49).

In the absence of reperfusion the release pattern is characterized by a slow increase in plasma concentration which peaks at day 3 or 4 (49, 124). Reperfusion therapy induces an early peak of both markers as the cytosolic pool is rapidly washed out, but there is no evidence that cumulative troponin release is impacted by reperfusion therapy (49, 124, 125). Evidence suggests that plasma levels are independent of reperfusion status from the first day and forward, largely reflecting the slow degradation and liberation of the structural pool (49, 124). In reperfused patients it has been shown that while both cTnT and cTnI peak early (< 12 hours), the

disappearance of cTnI is somewhat accelerated compared to cTnT, although both are still significantly elevated at 72 hours (126, 127).

Most studies on cardiac troponin differ from previous investigations on LDH, CK and CK-MB in that they do not employ mathematical models to account for the cumulative release of the biomarker from necrotic tissue. Several explanations for this simplification exist: First, the kinetics and release ratios of the troponins are not very well defined (128). Second, the fact that troponins can remain elevated for several weeks makes it impractical to account for the total amount released from the myocardium. Last, with the emergence of SPECT and CMR it is possible to directly relate cardiac troponin values at specific time points, or as derived variables such as peak or AUC, to infarct size determinations from cardiac imaging.

5.6.2 *The empirical evidence*

In assessing the subsequent studies on the association between cardiac troponin and infarct size and other measures such as LVEF, it is important to recognize the differences in design. The most important variables to consider are: 1) the population studied: STEMI vs. non-STEMI, whether reperfusion was given, and the reperfusion modality; 2) which standard was used for comparison: infarct size determined by SPECT, CMR, or other biomarkers, and at what time was this examination performed; 3) the cardiac troponin assay employed; 4) the time-points used for sampling; and 5), which variables were used in the correlation analyses: peak troponin, AUC troponin or at a specified time-point.

The first report describing the correlation between cardiac troponin and infarct size was presented by Hugo Katus and colleagues at a congress in 1991⁴, and the first articles detailing the relationship between cardiac troponin and infarct size was published in 1993 (129, 130). Table 1

⁴ Katus HA et al: *Serum troponin T levels on day 4 after acute myocardial infarction are correlated with infarct size.* 9th European Congress of Clinical Chemistry, Cracow, Poland, September 8 – 14, 1991

summarizes the published articles reporting correlations between infarct size and cardiac troponin.

Table 1. Overview of published studies on the relation of cardiac troponin and infarct size. Those studies listed in *Italic* at the end, were performed or published after the present project was commenced.

Author (year) [reference]	Population studied (n)	Design	Assay used	Sampling intervals	Variables used	Comparator (infarct size)	Other outcome measures	Correlation coefficients
Omura (1993) (129)	AMI, q-wave (n=34), Thrombolysis	Prospective	cTnT 1 st generation	Every 3 h (0-24 h), 6 h (24-72 h)	Peak	SPECT 4 weeks	-	0.77
Wagner (1995) (130)	AMI, q-wave (n=21), Thrombolysis	Randomized trial	cTnT 1 st generation	Every 4 h (0-24 h), 8 h (24-48 h), daily until discharge	Peak AUC	SPECT 5 weeks	-	0.73 (peak) 0.54(AUC)
Mair (1995) (131)	AMI, q-wave (n=21), Thrombolysis	Randomized Trial	cTnI (ERIA Diagnostics, F)	Every 4 h (0-24 h), 8 h (24-48 h), daily until discharge	AUC	SPECT 5 weeks	-	0.53
Tanaka (1997) (132)	STEMI (n=42), Thrombolysis (n=10) and pPCI (n=32)	Prospective	cTnI (Stratus, Dade, Behring, US) cTnT 1 st generation	Every 3 h (0-24 h)	Peak	-	Regional hypokinesia, (ventriculogram)	0.84 (cTnI) 0.85 (cTnT)
Apple (1998) (133)	AMI (n=39), Thrombolysis (n=12) and pPCI (n=1)	Prospective	cTnI (Stratus, Dade Int., US)	6, 12, 24 and 36 h	Peak	-	LVEF (echocardiogram)	0.46
Rao (1998) (134)	STEMI (n=50) Thrombolysis (n=32) No thrombolysis (n=18)	Retrospective	cTnT 1 st generation	1 sample between 12-48 h	Single-point (12-48 h)	-	LVEF (ventriculogram) 2 days – 32 weeks	0.72
Kanna (2001) (135)	AMI (n=121) Thrombolysis (n=71) pPCI (n=3)	Prospective	cTnT 1 st generation	1 sample on day 3 or 4	Single-point (day 3-4)	-	LVEF (95, ventriculogram; 7, echo; 5 SPECT)	0.48 (first AMI, n=88)
Licka (2002) (136)	AMI (n=37) Thrombolysis/pPCI (n=23) and no/failed reperfusion (n=14)	Prospective	cTnT 2nd generation	Every 4 h (0-24h), 8h (24-72h), once daily until day 10	Single-point (72 h value)	SPECT 10 – 18 days	-	0.72 (no-reperfusion) 0.78 (reperfusion)
Pantaghini (2002) (137)	AMI (n=65) Thrombolysis/pPCI (n=55);No revasc (n=10)	Prospective	cTnT 3rd generation	Every 6h (0-48h) and at discharge (40 – 160 h)	Discharge value	SPECT at discharge and at 3 months (n=58)	LVEF (SPECT)	0.62 (at discharge) 0.56 and 0.70 (LVEF at discharge and 3 months)
Rao (2003) (138)	STEMI (n=201)	Prospective	cTnT 2nd generation	1 sample 12-24 h	Single-point (12-24h)	-	LVEF (echo)	No correlation ROC: 0.91 for LVEF <40 %
Ohlmann (2003) (127)	STEM (n=87) pPCI (n=73)	Prospective	cTnI (Stratus II, Dade, Behring, US)	3,6,9,12,24,48,72 h	Single-point, peak, AUC	QLDH	LVEF (SPECT)	>0.8 (all time-points from 6h, AUC, peak) -0.5* (LVEF)
Ingkanisom (2004) (139)	AMI (n=33) Thrombolysis, n=23	Prospective	cTnI (assay not reported)	4 and 8 h	Peak	CMR (1-2 days)	-	0.83 (for revasc patients, n=23)

Panteghini (2005) (140)	STEMI (n=63) pPCI (n=29 + 11) Thrombolysis (n=25) No revasc (n=9)	Prospective	cTnI (Accu-cTnI, Beckman Coulter, US)	12 and 48 h	Single-point	SPECT (first week)	LVEF (SPECT, first week, 3 months)	0.55/0.61 (12/48 h) LVEF, 1 week: 0.45/0.57 (12/48h) LVEF, 3 months 0.51/0.69 (12/48h)
Steen (2006) (141)	AMI (STEMI:n=23; non-STEMI, n=21)	Prospective	cTnT 3 rd generation	96 h	Single-point	CMR (4 days)	-	0.91 (STEMI) 0.58 (non-STEMI)
Younger (2007) (142)	AMI (n=93) Thrombolysis, n=71	Prospective	cTnI (Accu-cTnI, Beckman Coulter, US)	12 h and 72 h	Single-point	CMR (2-5 days)	MVO	0.56/0.62 (12/72h)
Giannitsis (2008) (143)	AMI (n=61 and STEMI:n=30)	Prospective	cTnT 3 rd generation	Day 1,2,3,4	Single-point, peak and AUC	CMR (4 days)	-	0.64/0.66/0.65/0.66/0.65/0.7 (D1/2/3/4/peak/AUC)
Tzivoni (2008) (144)	STEMI (n=378), pPCI	Randomized trial (CASTEMI)	cTnT (assay not reported)	2,4,12,24,48,72 h	Peak, AUC	SPECT (7 and 30 days)	LVEF	Day7: 0.71/0.72 (peak/AUC) Day 30: 0.66/0.67 (peak/AUC)
Vasile (2008) (145)	STEMI (n=28)	Retrospective	cTnI (Accu-cTnI, Beckman Coulter, US)	Day 1,2,3,4	Single-point	CMR (4 days)		0.75/0.78/0.71/0.71/0.76 (D1/2/3/4/peak/AUC)
Chia (2008) (126)	STEMI (n=378), pPCI	Randomized trial (EVOLVE)	cTnI (AsSym Troponin I ADV, Abbot Labs, US) cTnT 3 rd generation	2,4,12,24,48,72 h	Single-point, AUC	SPECT (5 days, 30 days)	LVEF	cTnT at 72h 0.65/0.63 (5/30 days) cTnI at 72h 0.73/0.71 (5/30 days)
Bohmer (2009) (146)	STEMI (n=103), pPCI	Randomized trial (NORDSTEMI)	cTnT 3 rd generation	67 h	Single-point	CMR (3 months)	LVEF	0.84 0.63 (LVEF)
Hassan (2009) (147)	STEMI (n=168), pPCI	Randomized trial (Mission)	cTnT 3 rd generation	Every 6 h (0-48h)	Peak	CK (cumulative)	LVEF (SPECT, 3 months)	0.73 (CK and cTnT) 0.5 (LVEF)
Byrne (2010) (148)	STEMI (n=1237), pPCI	Prospective	cTnT 2 nd generation	8,16, 24 h, then daily	Peak	SPECT		0.45
Kling (2011) (149)	STEMI (n=103), pPCI	Prospective	cTnT 4th generation	Admission, 8, 16 h, Day 1,2,3,4	Peak, AUC, single-point	CMR (< 8 days)	LVEF, EDV, ESV	0.56/0.60/0.58/0.62/0.64/0.68 (D1/2/3/4/peak/AUC)
Mavr (2011) (150)	STEMI (n=80), pPCI	Prospective	cTnT (assay not reported)	Admission, 8,16 h, Day 1,2,3,4	Peak, single-point	CMR (2-4 days and 4 months)	LVEF, EDV, ESV	Day 2-0.58/0.65/0.62 4 month: 0.65/0.75/0.67 (D1/2/3)

AMI, acute myocardial infarction; SPECT, single-photon emission computed tomography; AUC, area-under-the-curve; LVEF, left-ventricular ejection fraction; STEMI, ST-elevation AMI; CMR, cardiac magnetic resonance. References for the different cTnT assays: 1st generation (57), 2nd generation (67), 3rd generation (68)

5.6.3 *Data on cardiac troponin for infarct size estimation and prognostic assessment – the situation in 2008*

In total, 18 studies had been performed until the middle of 2008 that investigated the relation of cardiac troponin and infarct size or cardiac function. As is evident from table 1 they differed widely in size and design. For many crucial questions, the empirical evidence was weak or non-existent:

Few studies had employed a cTnI assay, and of those, with a few exceptions from the 90s, only the Accu-cTnI assay (Beckman-Coulter, US) had been used. The performance of other widely used cTnI assays was not known. Only a handful of small investigations had employed CMR as the imaging endpoint. CMR has emerged as the preferred imaging assessment of infarcted tissue, because of its precision, high spatial resolution, and ability to delineate even small infarctions. The correlation between cTnI measured at early time points and infarct size was not well defined. Moreover, most studies had measured infarct size in the immediate aftermath of the acute event and the association between cardiac troponins and infarct size evaluated in the chronic phase was not well understood.

Several studies had demonstrated the association of cardiac troponins with LV function and volumes following MI (134, 137, 138, 140, 144). However, both LV function and volumes evaluated in the sub-acute phase are poor indicators of chronic cardiac status because these parameters may change over time due to reversible factors like stunning and hibernation and as a result of chronic LV remodeling. No study had investigated whether cardiac troponin may complement pre-discharge assessment of LV function and volumes for prediction of LV remodeling and chronic cardiac status. Such data are of importance to better define the proper role of cardiac troponin measurements in risk stratification.

A third area where more data were needed concerned the association of cardiac troponin with MVO. MVO following reperfusion therapy for STEMI reflects persistent compromised perfusion

at the tissue-level. It was initially described angiographically in experimental studies (151). In clinical settings it can be diagnosed by various angiographic measures (152, 153), contrast echocardiography (154), electrocardiography (155), or CMR (156). MVO is the result of complex pathophysiological mechanisms related to ischemia-reperfusion injury and distal embolization of atherosclerotic debris (157). Presence of MVO post-MI is a marker of adverse LV remodeling and may be associated with poor prognosis independently of infarct size (158, 159). Data also suggest that MVO diagnosed by CMR is a stronger predictor of LV remodeling than MVO identified by other modalities (156). In this perspective it would be of interest to understand whether cTnI release in MI also reflects MVO, independent of infarct size. Only one study has looked at the relation of MVO and cTnI (142). This study found that cTnI release at 72 hours after admission was related to MVO, but did not address whether this relation was independent of infarct size.

This thesis' papers I, II and III were designed to address these specific questions mentioned in the preceding paragraphs.

5.7 ST-segment recovery for evaluation of reperfusion and prognosis

5.7.1 ST-segment deviation as a marker of ischemia and reperfusion

An electrocardiogram is the recording (*gram*) of the electrical activity (*electro*) generated by the cells of the heart (*cardio*) that reaches the body surface. A.D. Waller recorded the first electrocardiogram in 1887 (160) and in 1903 Einthoven invented the first practical electrocardiograph. Einthoven received the Nobel Prize for his “*discovery of the mechanism of the electrocardiogram*” in 1924. The ECG remains a pivotal investigational and diagnostic tool in cardiology today.

ST-segment elevations in response to coronary ligation-induced myocardial ischemia were shown in dogs by Smith as early as in 1918 (161). Deviations in the ST-segments are the result of

an altered transmembrane potential in the ischemic myocardium compared with adjacent regions of non-ischemic myocardial cells. In dogs with occluded coronary arteries, the magnitude of ST-segment elevation correlated with the extent of myocardial necrosis on histological examination (99, 162, 163), and myocardial reperfusion induced normalization of ST-segment elevation (164). Later, when fibrinolytic treatment was evaluated in clinical trials, the observation of ST-segment recovery in response to reperfusion was extended to humans (165).

Evaluation of ST-segment recovery (or resolution) entails quantifying the amount of ST-segment deviations after reperfusion therapy in one or several leads. Conventionally, this is related to the ST-segment deviations at “baseline” that is recorded before intervention, with ST-segment recovery being expressed in relative terms as percent of ST-segment recovery. Throughout the years, numerous measures of ST-segment recovery have been employed, such as relative ST-segment elevation (or deviation) resolution in all affected leads, in the worst affected leads, or residual ST-segment elevation (or deviation) in all or one leads (166).

5.7.2 ST-segment recovery following reperfusion therapy by thrombolytics or pPCI

For the last 20 years, assessment of ST-segment recovery after thrombolytic therapy has been used in multiple clinical trials and in patient management and has been shown to provide information on epicardial reperfusion status, microvascular function and tissue-level reperfusion and prognosis (155, 162, 167). Guidelines recommend evaluation of ST-segments after thrombolytic therapy to identify patients with failed coronary reperfusion who are candidates for rescue PCI (168).

In STEMI patients treated with pPCI, where most patients are successfully recanalized, ST-segment recovery is largely reflective of tissue perfusion and microvascular function. Many studies have demonstrated that ST-segment recovery remains a powerful prognostic marker in pPCI populations (169-172). These investigations, however, were all retrospective, lacked

standardized timing of post-PCI ECGs and preceded the routine use of stents, GP IIb/IIIa inhibitors and thienopyridines (173). Additionally, the published reports used very different methods for quantifying and categorizing ST-segment recovery. To address these shortcomings in the empirical evidence, the investigators of the APEX-AMI (Assessment of Pexelizumab in Acute Myocardial Infarction) trial, pre-specified an ECG substudy with core lab analysis of protocol-specified postprocedural ECGs, to evaluate the relationship between 6 different measures of ST-segment recovery and clinical outcomes in about 5000 STEMI patients (173). In this study published in 2008, the APEX-AMI investigators reported that a simple ST-segment recovery measure of residual elevation in the most affected lead on the post-PCI ECG performed at least as well as more complex methods that required comparison of pre- and post-procedural ECGs or calculation of summed ST-segment deviation (173). The results reported from the APEX-AMI trial replicated the findings of a post hoc study 4 years earlier by McLaughlin et al (172). Furthermore, De Luca et al reached essentially the same conclusions in a prospective observational study also published in 2008 (174).

Several studies have investigated the relation between ST-segment recovery and infarct size (175-177). Only one study, however, extended from a pure pPCI population, and this article did not compare different algorithms of determining ST-segment recovery (177). In the FIRE trial, a core laboratory analysis of protocol-specified ECGs obtained before the intervention, immediately following and 90 minutes post-procedure had been performed. Thus, it was possible to explore the relation of different measures of ST-segment recovery and infarct size as determined by CMR in a contemporary STEMI population all receiving pPCI. This thesis' paper IV investigates the relation of infarct size and ST-segment recovery in the FIRE trial.

6. AIMS

This work was performed to develop more insight into the prognostic applications of cTnI measurements at 24 and 48 hours after admission and ST-segment recovery for early risk stratification of acute STEMI patients receiving pPCI. The specific objectives can be summarized as follows:

- 1) to characterize the association between single-point measurements of cTnI and infarct size determined by CMR at both < 1 week and 4 months after the index event
- 2) to determine the prognostic value of cTnI for prediction of LV dysfunction and changes in LV function and volumes from < 1 week after the index event to 4 months
- 3) to describe the relation between cTnI and MVO determined by CMR
- 4) to characterize the association of 3 measures of ST-segment recovery with infarct size and LV function

7. METHODS

7.1 Study design and population

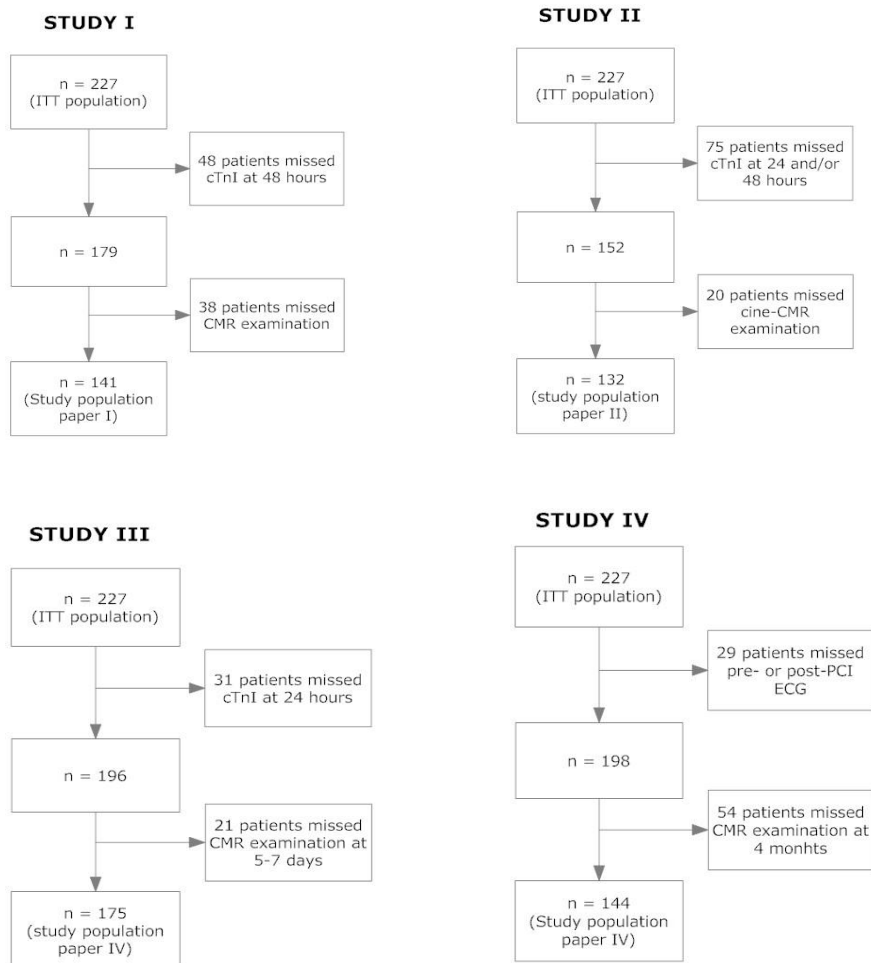
All the published articles of the present thesis extend from data gathered in the FIRE trial, which was designed to evaluate the cardioprotective efficacy of FX06 as an adjunct to pPCI in patients with acute STEMI (31, 32). The study was conducted between October 2006 and March 2008, in a randomized, double blind, placebo-controlled fashion.

Patients with a first STEMI from a single culprit lesion and no other serious comorbidities undergoing pPCI as indicated per standard of care were included. Additional inclusion criteria were presentation within 6 h of onset of symptoms, > 2 mm ST-segment elevation in at least 3 ECG leads, and a single culprit lesion with TIMI flow grade 0/1 in the infarct-related artery. Patients with prolonged ischemic symptoms, cardiogenic shock, peripheral vascular disease, and history of kidney (serum creatinine > 250 mol/l) or liver dysfunction were excluded. Eligible

patients were randomly assigned to active drug or placebo. FX06 was administered in 2 intravenous bolus injections of 200 mg each during PCI, the first immediately before the guidewire passed the occlusion and the second 10 min (\pm 5 min) later. Concomitant therapies were allowed except for thrombolytics and adenosine. Figure 2 outlines the selection of patients in the 4 substudies.

Written informed consent was obtained from all patients and the study was approved by all local ethic committees. All patients were followed up clinically for 4 months.

Figure 2. Flow charts illustrating selection of patients to each substudy.



7.2 Cardiac troponin I measurements

cTnI was protocol-specified to be sampled in all patients at 24 and 48 hours after admission. All samples were analyzed in a blinded core laboratory (Spranger Laboratories, Ingolstadt, Germany). cTnI was measured on the Abbott AxSym System (Abbott Diagnostics, Abbott Park, Illinois, US) using the second-generation AxSYM Troponin-I ADV assay. The analytical sensitivity of the assay is 0.02 ng/ml with a 10% coefficient of variation at 0.16 ng/ml, and the 99th percentile of a reference population is 0.04 ng/ml.

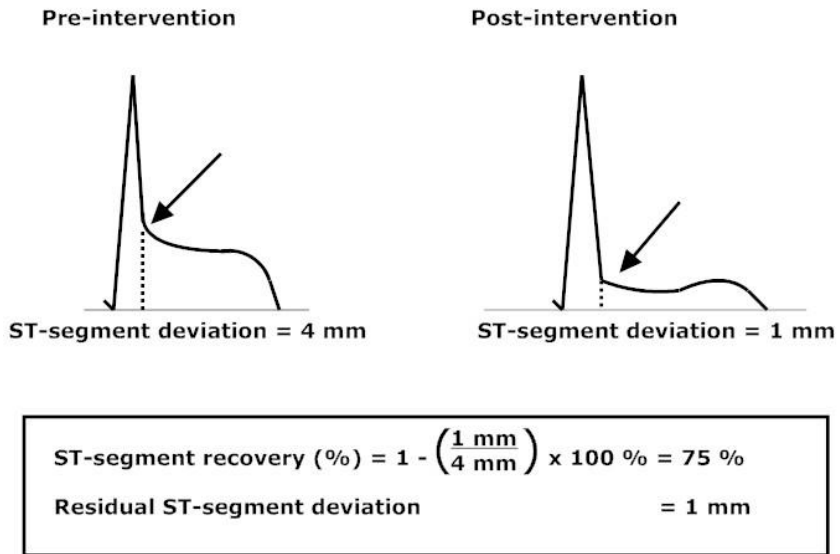
7.3 Electrocardiographic methodologies

A standard 12-lead ECG was recorded in all patients at randomization (baseline ECG). Additional ECGs were obtained immediately after the intervention as clinically feasible and at 90 minutes after pPCI. The ECGs were analyzed blinded by the core laboratory at Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. ST-segment deviation was measured manually at the J-point to the nearest 0.5 mm in all leads. The sum ST-segment deviation was calculated as follows: for anterior infarction, the sum of the ST-segment elevation in V₁ to V₆, I, and aVL; for nonanterior infarction, the sum of ST-segment elevation in leads II, III, aVF, V₅, and V₆ and ST-segment depression in leads V₁ to V₄.

Three methods for calculating and categorizing ST-segment recovery were used: (1) sum ST-segment deviation resolution (percentage of resolution of sum ST-segment deviation from baseline to after pPCI) analyzed in 3 categories ($\geq 70\%$, $\geq 30\%$ to $< 70\%$, and $< 30\%$); (2) single-lead ST-segment deviation resolution (percentage of resolution comparing the lead with the most prominent ST-segment deviation at baseline and after PCI, irrespective of the electrocardiographic lead in which ST-segment deviation was measured at baseline) analyzed in 3 categories ($\geq 70\%$, $\geq 30\%$ to $< 70\%$, and $< 30\%$); and (3) worst-lead residual ST-segment deviation (the absolute magnitude of residual deviation in the most affected lead on the post-PCI

ECG, without reference to the baseline ECG) analyzed in 3 categories (<1 mm, 1 to <2 mm, and ≥ 2 mm). An illustration of the calculation of relative ST-segment recovery and residual ST-segment deviation is shown in figure 3.

Figure 3. The calculation of relative ST-segment recovery and residual ST-segment deviation.



7.4 Cardiac magnetic resonance imaging

The protocol specified for all patients to undergo CMR examination at 5 days (range 5 to 7 days) and at 4 months. The CMR examination involved an ECG-triggered acquisition of a stack of short-axis slices covering the entire left ventricle from the base to the apex using steady-state, free-precession pulse sequence. The imaging parameters were as follows: slice thickness of 8 mm, no gap between slices, temporal resolution > 50 ms, matrix 224 to 256 x 224 to 256 using magnetic resonance scanners from all major vendors operating at 1.5 Tesla. In addition, long-axis 2-, 3-, and 4-chamber views were acquired with the same parameter settings. Late gadolinium enhanced images were acquired in the same slice orientations 20 minutes after administration of

a gadolinium-based contrast agent (0.25 mmol/kg). Inversion time was adjusted in each patient to null the signal of normal myocardium. The gadolinium enhanced images were obtained in mid to late diastole to minimize motion by adjusting the trigger delay (~ 450 ms). Other imaging parameters were as follows: slice thickness of 8 mm without gap, matrix 192 to 256 x 192 to 256, and flip angle 20°.

Using short-axis cine loops of the left ventricle from base to apex, endocardial and epicardial contours were traced manually in each slice at end systole and end diastole to measure areas. LVEF, ESVI and EDVI, and myocardial mass were calculated in a standard fashion. All short-axis slices were assessed for areas of signal enhancement. Total volumes of signal enhancement were obtained by summing compartments of all slices covering the entire left ventricle and expressed as a percentage of LV myocardial mass. MVO was defined as any region of hypoenhancement within the hyperenhanced area and was included in the calculation of total infarct size.

All images were analyzed at the central site by a single, blinded, experienced CMR reader followed by blinded review by a level III CMR expert (Peter Buser or Jens Bremerich, University Hospital, Basel, Switzerland). In the case of discordance between the primary and expert reviewer, consensus was reached. Intraobserver variability was assessed in a subset of 40 randomly chosen studies and the intraclass correlation was 0.85 for 26 studies from day 5.

7.5 Statistical methodologies

The data were approached statistically by a limited set of analyses. Variables are presented as mean (standard deviation) or median (interquartile range) depending on the distribution.

Associations between variables are reported as Pearson correlations or Spearman rank correlations. Differences between groups were assessed by Student's t-test or Wilcoxon rank sum (Mann-Whitney U) test depending on the distribution of the variables. Linear regression and

binary logistic regression models were used to explore relations between variables. For multivariable analyses, variables were either entered into the model in block or following univariable analysis (with a p-value of 0.2 as the criterion for inclusion). Receiver operator characteristics curves and c-statistics were generated to show the ability of cTnI alone or prognostic models to discriminate between binary outcomes (i.e. small vs. large infarct size or LVEF < 40 % vs. \geq 40 %).

8. RESULTS

8.1 Paper I

The association between infarct size and single-point sampling of cTnI was investigated. There was a highly significant correlation between late gadolinium enhanced CMR measured infarct size and cTnI sampled at 24 ($r = 0.66$ (5 days) and $r = 0.63$ (4 months)) and 48 hours ($r = 0.67$ (5 days) and $r = 0.65$ (4 months)). In a multiple regression analysis for predicting infarct size at 4 months ($n = 141$), cTnI and infarct location retained an independent association with late infarct size even taking into account early infarct size.

8.2 Paper II

The ability of cTnI to provide prognostic information on chronic LV dysfunction and LV remodeling following STEMI was explored. In linear regression models adjusted for early (5 days) assessment of LVEF, ESVI and EDVI, single-point cTnI at either 24 or 48 h were independent and strong predictors of changes in LVEF ($p < 0.01$), EDVI ($p < 0.01$) and ESVI ($p < 0.01$) during the follow-up period. In a logistic regression analysis for prediction of an LVEF below 40% at 4 months, single-point cTnI significantly improved the prognostic strength of the model (AUC = 0.94, $p < 0.01$) in comparison with the combination of clinical variables and LVEF at 5 days.

8.3 Paper III

The paper explored the association of cTnI and MVO in STEMI patients receiving pPCI. The presence of MVO following STEMI was associated with larger infarct size and higher values of cTnI at 24 and 48 h. For any given infarct size or cTnI value, there was a greater risk of MVO development in non-anterior infarctions. cTnI was strongly associated with MVO in both anterior and non-anterior infarctions ($p < 0.01$) after adjustment for covariates including infarct size.

8.4 Paper IV

The paper aimed to define the association between infarct size and cardiac function as determined by CMR imaging and different metrics of ST-segment recovery. All 3 ST-segment recovery algorithms were associated with the final infarct size and cardiac function. Worst-lead residual ST-segment deviation performed the same as, or better than, the more complex methods and identified large subgroups at either end of the risk spectrum (median infarct size from the lowest to highest risk category (percentage of left ventricle: 7.7% [interquartile range 10.8], 13.1% [interquartile range 13.6]; 24.6% [interquartile range 21.1]); with adjusted odds ratios for infarct size greater than the median (reference < 1 mm): 1 to < 2 mm, odds ratio 2.3 (95% confidence interval 0.8 to 5.9); ≥ 2 mm, odds ratio 6.3 (95% confidence interval 1.7 to 23.7; c-index 0.781).

9. DISCUSSION

Our results suggest that both cTnI and the ECG convey significant prognostic information in the early phase following pPCI. Swift initial triage and timely reperfusion therapy have significantly improved survival for STEMI patients, and the advent of pPCI as compared to fibrinolytic therapy has further impacted clinical outcome. Even so, a wide spectrum of risk still exists for STEMI patients and effective risk stratification remains important, especially to identify high-

risk patients. Thus, our findings are of clinical relevance as they suggest that simple and accessible markers are available that may facilitate identification of high- and low-risk patients.

9.1 The association of cTnI with infarct size and measures of LV function and volumes

In paper I we found that a single-point determination of cTnI at 24 or 48 hours after admission was associated with infarct size. These results confirm previous studies on the relation of infarct size and cTnI in STEMI patients, but also expand current knowledge along a number of dimensions:

First, our report is the largest assessment to date of cTnI or cTnT and their association with CMR-determined infarct size. Previous studies were smaller and all measured infarct size < 1 week after the index event (139, 141-143). In terms of infarct size measured < 1 week, our data confirmed the findings of these earlier investigations. Our second assessment of infarct size at 4 months showed a marked involution of the infarcted area. This is in keeping with other studies that have assessed infarct size with CMR or SPECT in a serial fashion (178-180). However, we found that this involution did not reduce the strength of the association between cTnI and infarct size. Two recent articles also reported strong correlations between cTnT and infarct size at 4 months in STEMI patients (146, 150). Interestingly, in regression analyses, we demonstrated that cTnI was associated with infarct size at 4 months even when adjustments were made for early infarct size. This suggests that cTnI early after STEMI captures unique information on the extent of irreversible myocardial injury that is complementary to that provided by imaging analysis. This finding is in keeping with cardiac troponin being a marker of cardiomyocyte necrosis.

Second, the present study is only the second to report on cTnI measured with the Abbott AxSym ADV assay. These data thus validate the assay for infarct size estimation following STEMI. After our study was conceived, Stanley Chia and co-workers published their analyses of the EVOLVE (The Evaluation of MCC-135 for Left Ventricular Salvage in Acute MI) trial,

where the same cTnI assay as we employed was correlated with SPECT determined infarct size (126). Their results are in line with our findings, but infarct size was evaluated at 5 and 30 days, and not at 4 months as in our study.

A third novel aspect of our study is that cTnI was determined at 24 and 48 hours. Although some studies have performed sampling of cTnT or cTnI at these time-points previously, our study is the first to demonstrate the utility of this sampling strategy in a population of STEMI patients all receiving pPCI with infarct size estimated at > 3 months. A very recent article published in 2011 has replicated these results for cTnT (150). That a single-point value at 24 hours is comparable to later sampling is consistent with the notion that cTnI values at 24 hours and beyond are reflective of the total infarct burden. However, nearly all patients in the FIRE trial were successfully reperfused, and extrapolation of these results to populations with a less uniform reperfusion status should be done cautiously.

Paper II showed that cTnI determined at 24 or 48 hours after admission is independently associated with chronic LV function and the volumetric changes that take place in the 4 months following the index event. This association persisted when adjustments were made for LV function and volumes measured before discharge.

Previous investigations have demonstrated that cTnT and cTnI are associated with LV function following MI (134, 137, 138, 140, 144). Only one of these analyses was based on a pPCI population and this investigation determined LVEF at 30 days by SPECT (144). Our study replicates these results with a second-generation cTnI assay and with LVEF measured at both < 1 week of the index-event and at 4 months. With the same cTnI assay, Chia et al have recently published results in agreement with our data in a comparable population (126). Our data thus confirms previous findings. The novel aspect of the present study is the analysis of the association of cTnI with the time-dependent changes of LV function and volumes.

Our data confirm that in patients treated with pPCI substantial improvements in LVEF can occur throughout the first months after the acute event (179, 181-184). The principle mechanisms by which improvement in function occurs are thought to be myocardial salvage, stunning and hibernation (184-186). Given the 5-7 day delay in LVEF measurement following the acute event in our population, it is likely that recovery from myocardial stunning and possibly hibernation were the main underlying factors responsible for the marked improvement of LV function observed in a subset of patients. Our data are also consistent with clinical and experimental evidence showing that infarct size is the major determinant of LV remodeling and chronic LV function (179, 180, 182, 187-189). To our knowledge, our study is the first to demonstrate that cTnI, as a surrogate for infarct size, is associated with changes in LV function and volumes, and gives incremental prognostic information on chronic cardiac status to that provided by assessment of LVEF and LV volumes pre-discharge. The major determinants of long-term clinical outcome in both the pre- and post-reperfusion therapy era are chronic LVEF and end-diastolic and end-systolic volumes (190, 191). Thus, our findings strongly suggest that a single-point cardiac troponin value following STEMI may also be associated with clinical outcomes in the long-term. This hypothesis has been investigated in a small prospective study and the results appear to support this notion (192).

Some tentative clinical implications are suggested by these data with the caveat that this is a post hoc analysis. The findings indicate that a single cTnI determination after STEMI is instructive for early assessment of risk despite echocardiographic assessment of function and volumes before discharge. In particular, in patients with an initial LVEF < 40 %, it is likely that cTnI sampling would facilitate identification of a high-risk population with poor prospects for recovery of function, that could be targeted with aggressive and comprehensive anti-remodeling strategies. Likewise, for design of clinical trials aimed at improving post-MI outcomes, cTnI may

be an attractive candidate for logistically simple and reasonably accurate identification of high-risk populations that will benefit the most from novel interventions.

9.2 Microvascular obstruction and cTnI

We found cTnI at 24 or 48 hours following pPCI to be associated with the presence of MVO determined by CMR, independent of early infarct size.

More data has recently been published on the importance of MVO for development of adverse LV remodeling and dysfunction following STEMI (180, 193, 194). In agreement with our results, Neizel et al recently found that cTnT 24 hours after admission was independently associated with presence of MVO adjusted for infarct size (195).

Several interesting results emerged from our analyses. First, the independent association between MVO and cardiac troponins suggested by other investigations is confirmed (180, 195). This is important as it indicates that levels of cardiac troponin in the context of MI are not only reflective of the extent of the initial infarction, but also with presence of MVO. As such, these results help explain and are entirely consistent with the ability of cTnI to predict adverse LV remodeling and dysfunction as demonstrated in paper II.

Second, we observed that the propensity for MVO development, in terms of infarct size or cTnI, is dependent on infarct location. This finding argues that the strong association between larger infarct size and presence of MVO reported in multiple studies is not causal, but rather a result of confounding. In fact, other investigations have also found that the likelihood of MVO development is similar between infarct locations (195, 196). Taken together, the results of paper III reinforce the concept of cTnI as an important risk stratification tool after STEMI, although the direct clinical relevance of these specific findings has yet to be defined.

9.3 ST-segment recovery and infarct size and cardiac function

Our study demonstrated that ST-segment recovery following pPCI for STEMI is associated with chronic infarct size and cardiac function. Furthermore, we found that among the three different metrics of ST-segment recovery we tested, the simple measure of absolute ST-segment deviation in the worst lead at 90 minutes compares favorably with the more complex algorithms of relative ST-segment recovery. The worst-lead residual measure of ST-segment recovery defined a wide spectrum of risk and identified both a high-risk population (infarct size > 24 % of LV) and a large low-risk group (infarct size < 8 % of LV). These results are completely consistent with data on clinical endpoints from the comparable population enrolled in the APEX-AMI trial.

Interestingly, the proportion of patients allocated to each risk category closely mirrored that found in APEX-AMI (173). Our study highlights the probable link (i.e. infarct size) between ST-segment recovery and clinical outcome observed in APEX-AMI.

The present data were the first to link chronic infarct size estimated by CMR with the simple measure of worst-lead residual ST-segment deviation. Since our results were published, several studies have emerged that correlate ST-segment recovery with infarct size determined by CMR (197-199). Two of these studies confirm the association of ST-segment recovery with infarct size, but did not include analysis of the worst lead residual ST-segment deviation (197, 198). The third recent study also included residual ST-segment elevation, but applied the more complex method of summing all ST-segment elevations together (199). They found an association between summed residual ST-segment elevation and LVEF but not infarct size, when controlling for infarct location and number of Q-waves. These findings have yet to be replicated in other populations and methodological concerns with this study have also been expressed regarding selection of the patient population and estimation of infarct size (200).

More data have recently emerged on ST-segment recovery and clinical outcome at 1 and 5 years (201, 202). These studies did not extend from randomized trials but looked at all-comer

STEMI patients receiving pPCI at one center in Holland. The findings suggest that the prognostic power of ST-segment recovery is not restricted to patients enrolled in clinical trials.

The reason why the simple measure of post-PCI residual ST-segment deviation performs equal to, or better than, the more elaborate assessment strategies is not entirely clear; however, the relative measures may be prone to some imprecision owing to their heavy dependence on the preprocedural level of ST-segment deviation. The timing of the post-PCI ECG may also influence results. Across all 3 ST-segment recovery metrics studied, we found that the correlations with infarct size and LVEF were stronger at 90 minutes than immediately following the procedure. Other reports have observed the opposite trend (203, 204). The methodological approach of one of these studies has recently been questioned, however (204, 205). A recent analysis found no difference between early assessment and at 90 minutes (206). In APEX-AMI ECGs were analyzed at 30 minutes and these results appeared very similar to ours. Therefore, analysis of ST-segment recovery in a time-window of 30 and 90 minutes post-PCI seems appropriate.

Considered in the context of the cumulative evidence that our findings support, it is reasonable to draw some tentative clinical inferences from them, although confirmation from prospective studies are needed. It seems clear that analysis of worst-lead residual ST-segment deviation between 30 and 90 minutes following pPCI represents an extremely simple way of identifying high-risk patients. No randomized trials have been conducted to study whether specific interventions are of benefit in the acute phase to mitigate myocardial damage or complications in this high-risk population. Notwithstanding, close monitoring and early initiation and quick up-titration of therapies with well-established cardioprotective properties like renin-angiotensin system inhibitors and beta-blockers seem appropriate.

9.4 Discussion of the methodological approaches

9.4.1 *Substudies of randomized trials - strengths and limitations*

The present thesis is based on a database from a randomized trial. Randomized trials often provide researchers with unique opportunities to develop further insight into the study population beyond addressing the primary research hypothesis. Indeed, it has recently been argued that investigators have an ethical and financial responsibility to design and conduct trials in a fashion that maximizes the scientific knowledge (207). Contemporary, international and carefully monitored randomized trials generate a well-validated database. All of the studies included in this thesis are robust in the sense that the data points were prospectively defined and registered by an independent clinical research organization.

However, it is also important to recognize the potential pitfalls of performing substudies based on clinical trials. Often randomized trials have strict inclusion criteria which may limit the external validity of the results, thus restricting the ability of such investigations to inform clinical practice. The FIRE population obviously represented a select cohort of patients. Patients in cardiogenic shock, with serious comorbidities, or prior MI were excluded. Moreover, inclusion criteria for ST-segment elevations were more stringent than defined in the guidelines, requiring ST-segment elevation > 2 mm in at least 3 leads. Thus, extrapolation of the results to different or more complex clinical scenarios should only be performed with caution.

The present thesis considered the trial population in FIRE combined. Although the trial overall was negative and adjustments were made for randomization in several of the analyses, we cannot totally rule out the possibility that some of the variables we studied were affected by the study drug intervention.

Another point to consider is the “fooled by randomness” effect (208), where spurious, random relationships mistakenly are interpreted as being real. Post hoc analyses increase the probability of such false “discoveries”. Certainly, this possibility cannot be excluded, but the

results of this thesis, while novel, fit very well with previous analyses. Moreover, the findings are biologically plausible and consistent with mechanistic insights. Nevertheless, it is clear that our findings have to be validated in prospective trials, preferably designed to also account for clinical endpoints.

9.4.2 *Sampling of cTnI*

Several issues should be considered. Clearly, it would have been preferable with more sampling time points for comparative purposes. Yet, evidence suggest that there are no large differences between sampling at 24, 48, 72 or 96 hours (145), and our findings are in line with correlation coefficients reported at 72 hours with the same assay (126). In any case, our results demonstrate the clinical utility of an early sample and how it may be integrated with other prognostic variables like LVEF.

Given the multicenter design of the trial and the decentralized collection of samples, some variability in the cTnI measurements would be expected, both in terms of the timing of blood drawing and in pre-analytical conditions (the specimen integrity). Such variability, by increasing the background “noise” level in the data, would weaken the associations with the various outcome measures as reported here. On the other hand, our findings extend from a population where nearly all patients presented within 6 hours from onset of symptoms and had successful pPCI which probably resulted into a more uniform cTnI release pattern than what can reasonably be expected in a real-world setting.

For application of these results in clinical practice, a major drawback is the lack of standardization across cTnI assays (61). Even for the specific assay reported on here, clinical application of the findings would be contingent on knowing roughly what any given cTnI value corresponds to with respect to infarct size or cardiac function, and specific values reported here should be validated in a prospectively designed study.

9.4.3 *Electrocardiogram*

ECG analyses were performed by an independent core laboratory blinded to patient data and study outcomes. ST-segment deviation was measured at the J-point. There is no consensus regarding the exact position along the ST-segment that should be selected for measurement and various studies have employed different positions. In STEMI, the deviated ST-segment may be horizontal, downsloping, or upsloping. Thus, the precise impact of different measuring points would be dependent on ST-segment configuration in each individual patient. However, for internal validity, consistency in selection of the measurement point is paramount. As already mentioned, the timing of the post-PCI ST-segment analysis should also be considered when comparing different studies. In relation to this point, and given the dynamic evolution of ST-segment deviations, it is unquestionable that continuous ST-segment monitoring following reperfusion therapy would have provided additional and more sophisticated information than a snapshot at 90 minutes.

For calculation of ST-segment recovery we also included ST-segment depression in order to account for reciprocal ST-segment elevations. This may potentially complicate comparison with studies only measuring ST-segment elevation, although this impact is likely to be minor.

9.4.4 *Cardiac magnetic resonance*

In the setting of an AMI, CMR can provide a wide range of information, such as infarct size, MVO detection, myocardial edema (area at risk) and also allows for a highly reproducible assessment of cardiac function and volumes (209). It represents a powerful tool for a global evaluation of post-infarction remodeling. However, there are still significant challenges in terms of reproducibility among different laboratories particularly concerning the use of contrast-enhanced CMR for estimation of infarct size (200, 210). Thus, interpretation of our results should

be considered in the context of these remaining challenges and the infarct size measurements found in the FIRE trial may not be directly compared with other reports.

CMR images were analyzed by an experienced imaging core laboratory blinded to patient data and study outcomes. The multicenter and international design of the trial entailed that images were obtained in multiple sites with varying experience with CMR. The quality of image acquisition in each site was evaluated before the study was initiated and found to be acceptable at all participating sites. Despite this, not all images were of optimal quality. On the other hand, thorough and careful analyses by the core laboratory ensured a common standard for interpretation and a low intersubject variability. Because of the multiple sites involved, the CMR protocol specified a somewhat larger dose of gadolinium (0.25 mmol/kg) than is conventionally used for the late-enhanced images, as it has been shown that increasing doses (up to 0.3 mmol/kg) improve sensitivity for detection of myocardial injury (211). Late-enhanced images were protocol-specified to be obtained at 20 minutes after contrast-injection. Estimation of infarct size between 10 to 30 minutes after contrast is highly reproducible (212). The area of the infarction was delineated manually. There is presently no accepted standardized quantitative method for measurement of infarct size (213), although automated or semi-automated approaches have been proposed (214, 215).

Visualization of MVO is highly dependent upon the mode and timing of CMR assessment (216). In the FIRE trial, CMR examination were performed during an interval (5 to 7 days) where earlier studies have suggested that presence and extent of MVO is fairly constant (217). Both first-pass perfusion and late-enhanced imaging can be used to characterize MVO by CMR (216). First-pass perfusion is the most sensitive technique, while late-enhanced imaging detects fewer patients with a worse functional outcome (156). It is not known which modality is the strongest prognosticator, as recent studies have reached opposite conclusions (156, 180, 194). In our study, due to the timing of image acquisition, some areas of MVO may have disappeared because of

contrast wash-in from surrounding regions. If so, this would further have exacerbated a selection bias in favor of more severe MVO. Thus, characterization of MVO by first-pass perfusion would have identified more MVO patients and results may have been different.

9.4.5 *Statistical methodologies*

The statistics applied in these analyses were based on conventional methods. Associations between variables were mostly assessed by Pearson correlations. For continuous data, Pearson correlations are appropriate as long as the relation between the variables is linear, irrespective of whether they are normally distributed or not (218). For the linear regression analyses, the assumption of normally distributed residuals (i.e. difference/distance between observed and predicted values) was met. Another assumption of linear regression is that the variance of the error terms is constant, or in other words that the imprecision of the predicted values of Y is constant for all observed X-values (homoscedasticity). In the linear regression analysis with cTnI (independent variable) and infarct size (dependent variable) the model indicated that the prediction of infarct size is more imprecise for larger infarctions compared to smaller infarction (in other words evidence of heteroscedasticity). Not unexpectedly, a casual inspection of the scatter plots of other reports on cardiac troponins and infarct size suggests that this is not restricted to our data (143, 146, 149). Heteroscedasticity will not bias the relationship between the dependent and independent variables, but may impact the standard errors. We found no evidence of other assumptions not being met in the regression analyses. It should be commented that for estimation of infarct size, since we did not apply forced fits through $(x,y) = (0,0)$, there will be some overestimation of small infarctions at low cTnI levels.

Inclusion in each individual substudy was based on whether the variables under investigations had been collected or not. Thus, in paper I, II and IV patients who did not return for a repeat CMR examination at 4 months were excluded (also if cTnI samples were missing). As reported

in paper I and IV, baseline characteristics of the study populations were representative of the whole FIRE population. Bias may have been introduced, though, if there was a systematic difference between those patients who completed follow-up and those who did not. Even if a significant, systematic bias was present, it is not clear how that would impact the associations between cTnI or ST-segment recovery and infarct size/LV function, but this possibility cannot be discounted. There was some inconsistency in selection of patients for each substudy with regards to the criterion for cTnI samples. In paper I, patients with missing cTnI at 48 hours were excluded, while in paper II patients with missing values at either cTnI at 24 or 48 hours were excluded, and finally in paper III, patients with missing cTnI at 24 hours were excluded. This lack of uniformity is unfortunate.

9.5 The relation of our findings with clinical risk scores and other prognostic tools

Our results should also be considered in the context of the many existing risk scores that have been proposed for AMI patients. In a comprehensive review of post-MI risk stratification in the reperfusion era, Michaels and Goldschlager identified no less than 42 variables independently associated with clinical outcomes (219). Various constellations of these variables have been combined in many different risk indexes over the years. A recent review evaluated the ability of 5 scoring systems based on information available at first medical contact to define high-risk patients for an endpoint of 30 day mortality in STEMI patients treated with pPCI (220). They found a large heterogeneity in the prognostic properties of the various models. Excluded in this analysis were studies also incorporating angiographic variables as evaluated by Halkin et al (221) and De Luca et al (222) in two large datasets of pPCI populations, as well as a recent study extending from the APEX-AMI trial (223). Expectedly, many of the variables found to be predictive of mortality are common among many of the studies like age, Killip class, anterior MI and renal insufficiency.

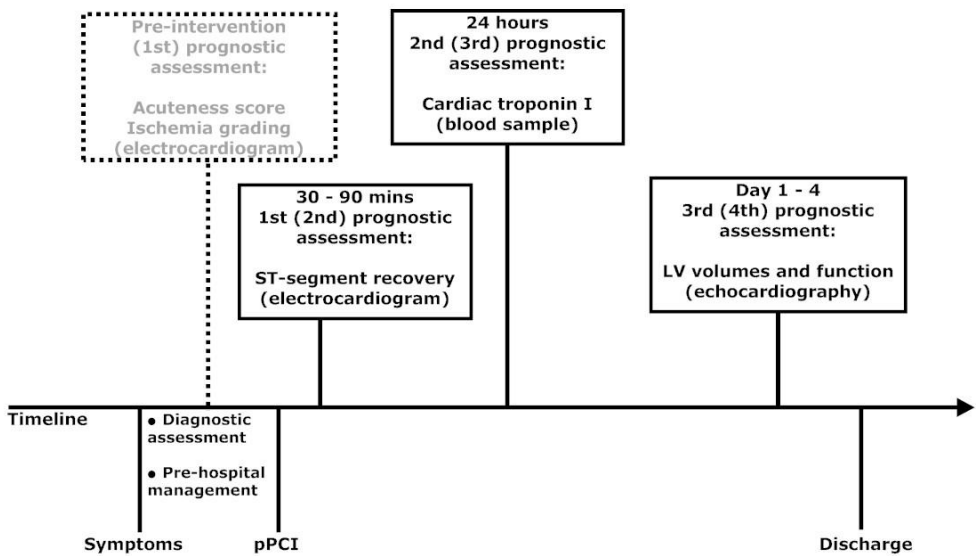
The usefulness of prognostic information is a product of the time at which the information is available and the precision. Clinical risk scores based on information at admission or during acute intervention are imprecise but nonetheless extremely valuable because the early time-point allows for a broad range of therapeutic responses. As indicated by our analyses, both single-point cardiac troponin measurements and indexes of ST-segment recovery add incremental prognostic information to clinical and procedural variables. However, the therapeutic options in response to this information are more limited due to the delayed time at which it is available. Nevertheless, information at 90 minutes post-PCI and then at 24 hours, may still be highly instructive for clinical decision-making regarding monitoring, adjunctive medical therapy and decisions on discharge and follow-up. As such, clinical and procedural risk scores, ST-segment recovery, measurement of cardiac troponin and evaluation of LV function before discharge may all play complementary roles in prognostic assessment of STEMI patients.

9.6 An integrated view of our findings

The concerted application of measures of ST-segment recovery and cTnI for risk stratification in a clinically plausible scenario could look like this (figure 4): Following pPCI, an ECG is obtained at 30 – 90 minutes after the intervention. In this assessment, patients with a residual ST-segment elevation ≥ 2 mm in the worst lead constitute a high-risk population who are likely to develop large infarctions with substantial chronic LV dysfunction. Thus, one could speculate that these patients may benefit from careful monitoring and more aggressive pharmacological strategies already at this early stage. At 24 hours, sampling of cTnI should be performed for further risk stratification. A low value (reference values would need to be defined) would indicate successful reperfusion therapy and little irreversible myocardial injury with a favorable short- and long-term prognosis. A subsequent echocardiographic assessment should be interpreted in light of the cTnI value. For example: A low cTnI value in the context of a severely impaired LVEF suggests a

large potential for recovery of function in the subsequent weeks following discharge. On the other hand, a low LVEF concomitant with a high cTnI value signals a poor prognosis and increased risk of adverse events. These patients in particular may benefit from an aggressive anti-remodeling strategy with renin-angiotensin inhibitors and possibly aldosterone antagonists and close follow-up (22, 39).

Figure 4. Diagrammatic, tentative illustration of different stages in risk stratification of STEMI patients



10. CONCLUSION

The aim of the present thesis was to generate further insight into the prognostic role of the cTnI – a marker of myocardial necrosis – and the ECG in patients with STEMI receiving pPCI.

- 1) In paper I we showed that cTnI measured at a single time-point following pPCI (24 or 48 hours) is associated with early and late infarct size.
- 2) In paper II we found that cTnI complements other conventional risk stratification tools before discharge in prediction of LV remodeling and chronic LV function.

- 3) In paper III we demonstrated that cTnI is associated with MVO determined by CMR and that this association is independent of initial infarct size.
- 4) In paper IV, our analyses showed that ST-segment recovery is associated with final infarct size and LV function and that a very simple algorithm requiring only the post-pPCI ECG is at least comparable to more complex methods for risk stratification.

The clinical utility of these results for prognostic assessment of AMI patients awaits further prospective studies.

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12. PAPERS

