

**Myocardial function by echocardiography for risk stratification in  
patients with heart disease**

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*Series of dissertations submitted to the  
Faculty of Medicine, University of Oslo*

ISBN 978-82-8333-240-7

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Cover: Hanne Baadsgaard Utigard  
Printed in Norway: 07 Media AS – [www.07.no](http://www.07.no)

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## ACKNOWLEDGEMENTS

The present work was carried out at The Intervention Centre and Department of Cardiology, Oslo University Hospital Rikshospitalet, and University of Oslo, in the period of October 2010 – December 2013. It was funded by the South-Eastern Norway Regional Health Authority and the Center for Cardiological Innovation.

First and foremost, I would like to thank my principal supervisor at the Department of Cardiology, professor Thor Edvardsen M.D. Ph.D., for his valuable guidance. When I told my colleagues at Drammen Hospital (my former working place) that I wanted to pursue a Ph.D., they told me to make sure that I got Thor Edvardsen as my supervisor. I was told that he was a true researcher, and that I would be taken good care of. Looking back, they couldn't have been more accurate in their description. Dr Edvardsen's guidance and support has been unmatched. His office door has always been open, and his patience, encouragement, contagious enthusiasm and optimism, and immense scientific knowledge were key motivations throughout my Ph.D. I feel honored to have worked with Dr Edvardsen, and I am indebted to him for my achievements.

I would also like to thank my co-supervisor at The Intervention Centre, professor Erik Fosse M.D. Ph.D., for his excellent guidance and feedback in all my work. Dr Fosse has given me enormous freedom to wander academically, but also to pursue my interests outside the fields of medical research. We've had many interesting conversations, and through his compassion and love for the weak and poor, he has taught me the true meaning of being a humanitarian. I would also like to thank the entire staff at The Intervention Centre, especially Marianne Berg, for always taking such good care of me, and Espen Remme, for valuable collaboration. I will never forget my time there.

During the final phases of my Ph.D., I have been working as a junior doctor at the Department of Cardiology, collecting valuable bedside experience. I would like to thank my clinical supervisor, Dr. Arne Andreassen for precious advice in both clinical and academic matters. There are also several colleagues, without whom this work would not have been possible. I would like to thank Dr Christian Eek and Dr Sebastian Sarvari for priceless support in both acquiring and analyzing echocardiography data, Dr Einar Gude for de-stressing talks and weight lifting in the hospital gym, and Dr Kaspar Broch for useful tips in completing this thesis at our crowded office. I would also like to extend my gratitude towards Dr Deidi Bergestuen and Dr Espen Thiis-Evensen at The Department of Gastroenterology at Oslo University Hospital, for their kind collaboration and help.

Finally, I would like to thank my family. I am forever grateful to my parents, Ghulam Rasool Zahid and Shaukat Zahid, for guiding and inspiring me towards higher education, and for all the sacrifices they made for the well being of their children, at the cost of pursuing their own ambitions. Thanks to my brother and colleague Naeem for valuable support and discussions, and my sisters Sobia and Rabia for their love and encouragement. I would especially like to thank my four children Yousuf, Mikail, Ayla and Rafael, for educating me in how to be a father. I love you more than you'll ever realize, and you are closer to me than the chambers of my heart.

Last, but not least, I would like to thank the most important person in my life, my wonderful wife, Iram. None of this could have been possible without your love, compassion, patience and endless support. You are my mast through the storms of life, and I hope I'm likewise to you, darling.

Wasim Zahid

Oslo, March 2016

## LIST OF PAPERS

1. **Mitral annular displacement by Doppler tissue imaging may identify coronary occlusion and predict mortality in patients with non-ST-elevation myocardial infarction.**

Zahid W, Johnson J, Westholm C, Eek CH, Haugaa KH, Smedsrud MK, Skulstad H, Fosse E, Winter R, Edvardsen T.

*J Am Soc Echocardiogr.* 2013;26:875-84

2. **Early systolic lengthening may identify minimal myocardial damage in patients with non ST-elevation acute coronary syndrome**

Zahid W, Eek CH, Remme EW, Skulstad H, Fosse E, Edvardsen T.

*Eur Heart J Cardiovasc Imaging.* 2014;15:1152-60

3. **Myocardial function by two dimensional speckle tracking echocardiography and Activin A may predict mortality in patients with carcinoid intestinal disease**

Zahid W, Bergestuen D, Haugaa KH, Ueland T, Thiis-Evensen E, Aukrust P, Fosse E, Edvardsen T.

*Cardiology.* 2015 Jun;132:81-90

## **SELECTED ABBREVIATIONS**

2D-STE, two-dimensional speckle tracking echocardiography;

ACS, acute coronary syndrome;

AUC, area under curve;

CAD, coronary artery disease;

CHD, carcinoid heart disease;

DESL, duration of early systolic lengthening;

EF, ejection fraction;

GLS, global longitudinal strain;

IQR, interquartile range;

LV, left ventricle/ventricular;

MAD, mitral annular displacement;

MMP, matrix metalloproteinases;

MRI, magnetic resonance imaging;

NSTE, non ST-elevation;

OPG, osteoprotegerin;

ROC, receiver operating characteristic;

RV, right ventricle/ventricular;

TDI, tissue Doppler imaging;

TGF, transforming growth factor;

TIMP, tissue inhibitors of matrix metalloproteinases

TNF, tumor necrosis factor;

UAP, unstable angina pectoris;

WMSI, wall motion score index;



# INTRODUCTION

## Background

Left ventricular (LV) systolic function is a major predictor of outcomes, and is usually assessed by ejection fraction (EF).<sup>1</sup> Despite the wide spread use of EF for the evaluation of ventricular function, it is associated with several challenging factors concerning image quality, operator experience, and assumptions of ventricular geometry. It is also limited to assessing changes in ventricular cavity, and has a low sensitivity for detecting mild changes in myocardial function. The assessment of global LV function by EF is based on two apical views only, and may therefore overlook more regional changes.

Newer echocardiographic techniques allow the assessment of myocardial function through tissue Doppler imaging and speckle tracking, measuring systolic function more directly, compared to conventional cavity-based parameters.<sup>2</sup>

In this thesis, we tested the ability of these echocardiographic techniques to assess changes in myocardial function, in order to improve diagnostic and prognostic information in patients with heart disease. Coronary artery disease and carcinoid disease with cardiac involvement were used as examples.

## **Ventricular function and cardiac biomarkers in patients with acute coronary syndrome**

The prevalence of coronary artery disease is still high in the Western world, and the main cause of mortality.<sup>3</sup> Quick diagnosis and appropriate treatment are important factors for reduced morbidity and mortality.<sup>4,5</sup> Acute myocardial infarction damages myocardial tissue and leads to impaired systolic function.<sup>6</sup> The extent of the

damage correlates with clinical outcomes.<sup>7-10</sup> Final infarct size measured by contrast enhanced magnetic resonance imaging (CE-MRI) is a powerful predictor of subsequent events in patients with myocardial infarction,<sup>11</sup> and several studies have shown increased risk for mortality after larger infarcts.<sup>12-14</sup> Establishing an optimal cut-off value of infarct size by CE-MRI for identifying patients at risk for future events is challenging, but since both short- and long-term mortality rates have been demonstrated to be increased in patients with infarct size  $\geq 12\%$  of left ventricle mass,<sup>12,13</sup> we opted for this value in our study.

On the basis of electrocardiographic characteristics, patients with acute myocardial infarction are divided in to two main categories; ST-elevation myocardial infarction (STEMI), and non ST-elevation acute coronary syndrome (NSTEMI-ACS). The latter is further divided in to non ST-elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP) on the basis of biochemical changes. In order to reduce infarct size and mortality, patients with STEMI are treated urgently with acute reperfusion therapy.<sup>15,16</sup>

Many NSTEMI-ACS patients also suffer large infarcts, but are not given priority for acute revascularization as they are not identified by traditional diagnostic tools as the ECG.<sup>17</sup> Furthermore, patients with NSTEMI-ACS may also have occluded coronary arteries,<sup>18,19</sup> and probably share many of the pathophysiologic features of patients with STEMI,<sup>20</sup> but miss out on acute revascularization therapy, because the occlusion is not readily identified.

The advent of cardiac specific biomarkers, especially troponins, has revolutionized the diagnostic process in acute coronary syndromes in the last few

decades. And with the increasingly sensitive assays, troponin's ability to diagnose acute coronary syndrome has improved dramatically.

Troponin is a protein complex, and the cardiac isoforms troponin T (cTnT) and troponin I (cTnI) are mostly bound to the contractile apparatus in myocardial cells. In the case of myocardial infarction and subsequent necrosis, troponins are quickly released into the bloodstream, and can stay elevated for several days. In the absence of reperfusion, peak plasma levels are reached after 3 or 4 days, while in reperfused patients tops are reached after 12 hours.<sup>21,22</sup> Their cardiac specificity, and the plasma release pattern, makes troponins ideal for rapidly identifying acute myocardial infarction. Subsequent peak levels can be used for assessing the extent of myocardial damage, i.e. final infarct size, as well as differentiate between NSTEMI-ACS patients with occluded and patent coronary arteries.<sup>23</sup>

Current guidelines for the management of NSTEMI-ACS patients point towards an early invasive strategy and recommend angiography with intention to revascularisation within 24 hours.<sup>24</sup> These recommendations can be challenging to fulfill, and rigorous prioritization may be necessary. In the case of early identification of patients with large infarct sizes or coronary occlusions, the use of troponins is challenged by some person-to-person variability,<sup>25</sup> and the troponin plasma profile: It rises gradually during the hours after the index ischemic event, and reaches peak levels later than the decision making window of 24 hours.<sup>26</sup> Our group has recently shown that two dimensional strain echocardiography (2D STE) and wall motion score index (WMSI) can predict final infarct size in patients with NSTEMI,<sup>27</sup> and that 2D STE can predict coronary occlusion in patients with NSTEMI-ACS.<sup>17,23</sup> But these methods require high quality echocardiography images, with visualization of the

entire ventricular curvature. The techniques may also be time consuming, and dependent on operator experience. A quicker identification of NSTEMI-ACS patients with larger infarcts and/or occluded coronary arteries could lead to better clinical outcomes, potentially increasing survival. We wanted to see whether other, and more easily obtainable, echocardiographic parameters could aid in identifying patients with large infarcts and/or occluded coronary arteries.

Patients with chest pain are frequently admitted to hospital emergency departments, but acute coronary syndrome is often not the underlying cause of symptoms. Even patients with a known history of coronary artery disease experience non-coronary chest pain. Quickly distinguishing these from patients with true NSTEMI-ACS can be difficult in the emergency departments, and most patients are withheld at hospitals awaiting a decision for many hours, in anticipation of blood test results, and correct diagnosis. A more precise distinction could result in a quicker initiation of proper ACS treatment when needed, avoidance of over treating those with non-coronary chest pain, and could also be more reassuring for the patient.

One the other hand, many NSTEMI-ACS patients have minimal myocardial damage and may not warrant immediate coronary intervention. Identifying these patients can be important for decision making when prioritizing patients to revascularization therapy. An experienced cardiologist can easily identify large myocardial infarcts by visual analysis of echocardiograms, but identification of small infarcts might be challenging. Significant changes of left ventricle ejection fraction (LVEF) and WMSI require decreased function in several LV-segments, which might not be present in patients with relatively limited myocardial scar. Both these indices have a weak ability to identify patients with minimal myocardial damage.<sup>28</sup>

Electrocardiography has suboptimal sensitivity for the detection of acute myocardial infarction,<sup>29</sup> identifying acute coronary occlusion,<sup>30</sup> and has a modest correlation with infarct size.<sup>31</sup> There is therefore a need for better risk stratification of NSTEMI-ACS patients.

## **Ventricular function in patients with intestinal carcinoid disease**

Although coronary artery disease is highly prevalent, it is far from the only condition affecting myocardial function. Involvement of the heart is common in many systemic diseases, requiring cardiac assessment, both for diagnostic and prognostic purposes. Intestinal carcinoid disease is one such condition, and in this thesis, we have further explored the role of echocardiography in the management of patients.

Myocardial fibrosis is an important complication of carcinoid intestinal disease, and can affect myocardial function. It is most often associated with small intestine, appendiceal and proximal colonic neuroendocrine tumours (midgut carcinoid tumours).<sup>32,33</sup> Endocardial fibrosis raises special clinical concern since it leads to increased morbidity and mortality in these patients. This phenomenon may occur in 20% to 70% of patients with metastatic carcinoid tumors, and is characterized by plaque-like fibrotic subendothelial lesions resulting from proliferation of myofibroblasts and deposition of extracellular matrix, affecting both mural and valvular endocardium, resulting in retraction and fixation of the heart valves, known as carcinoid heart disease (CHD).<sup>34–38</sup>

However, the damage is not restricted to only the heart valves, and previous research has shown that right ventricular (RV) systolic function by 2D STE is reduced in patients with carcinoid intestinal disease, even in patients without overt CHD.<sup>39</sup> This may occur independently of, or even before, valvular involvement. Left

ventricular (LV) function may also be reduced in patients with carcinoid intestinal disease.<sup>39</sup>

Myocardial fibrosis in carcinoid heart disease is a complex process. Serotonin is an important mediator, but an elaborate interaction between inflammatory cytokines, such as tumour necrosis factor (TNF)- $\alpha$ , and other cytokines and growth factors with fibrogenic properties, such as transforming growth factor (TGF)- $\beta$  and activin A (both members of the TGF- $\beta$  superfamily), as well as osteoprotegerin (OPG) (a member of the TNF receptor superfamily) is also decisive. Matrix degrading enzymes (matrix metalloproteinases [MMP]) and their endogenous inhibitors (tissue inhibitors of MMP [TIMP]) too play central roles in extracellular matrix remodelling. Studies have linked these mediators to various fibrotic diseases, including cardiac fibrosis.<sup>40-45</sup> It has previously been shown that elevated plasma levels of Activin A are associated with the presence of CHD, and that Activin A is expressed in the fibrotic plaques of CHD lesions, suggesting a potential pathogenic link between Activin A and CHD.<sup>46</sup>

Cardiac involvement in carcinoid intestinal disease has traditionally been viewed as mainly a right sided condition, due to a probable inactivation of serotonin in the pulmonary circulation. But several recent studies challenge this view, and have demonstrated involvement of the left side of the heart as well.<sup>47</sup> Our research group has shown that the right ventricular (RV), and the left ventricular (LV) systolic function by 2D speckle tracking echocardiography (2D STE) is significantly reduced in patients with carcinoid intestinal disease.<sup>39</sup> Furthermore, left-sided involvement may occur in up to half of the patients with high urinary 5-hydroxyindoleacetic acid (5-HIAA, the main metabolite of serotonin) with neither a patent foramen ovale, nor bronchial carcinoid,<sup>48</sup> and research shows that serotonin does indeed reach the left

side of the heart, even in the absence deterioration of the heart.<sup>49</sup> This suggests that other factors might play a more central role in the development of cardiac fibrosis in carcinoid heart disease. Cardiac involvement in patients with carcinoid intestinal disease carries high mortality, and there is a need for improved risk stratification of the patients, and to offer better monitoring and follow up.

Among the purposes of this thesis was to identify factors that are associated with mortality in patients with carcinoid intestinal disease, with particular focus on biventricular cardiac involvement as assessed by estimating systolic function by 2D STE in the RV and the LV. Furthermore, the predictive value of inflammatory and fibrinogenic biomarkers towards myocardial function and mortality was evaluated.

## **AIMS OF THE THESIS**

### **General aim**

To investigate whether myocardial function by tissue Doppler imaging and two dimensional strain echocardiography can be used in the assessment of diagnosis and prognosis in patients with heart disease.

### **Specific aims**

- I. To assess whether myocardial function by MAD and GLS can distinguish between small and large infarcts, coronary occlusions and non-occlusions, and predict mortality in patients with NSTEMI.
- II. To assess whether myocardial function by MAD and GLS can distinguish between patients with NSTEMI from stable CAD patients without acute coronary syndrome.
- III. To assess whether DESL by 2D-STE can identify minimal myocardial damage, and can identify acute coronary occlusions, in patients with NSTEMI-ACS.
- IV. To assess whether LV and RV function by 2D-STE, and inflammatory or fibrogenic biomarkers, can predict mortality in patients with intestinal carcinoid disease.



## **MATERIAL**

### **Study population (Paper 1 and 2)**

In study 1, patients were included from Karolinska Institute (KI) (n=106) and Oslo University hospital (OUH) (n=61). The patients from OUH were included particularly for assessing infarct size by contrast enhanced magnetic resonance imaging (CE-MRI).

At OUH, a total of 756 patients were considered for inclusion. Another 143 patients referred for urgent coronary angiography were not considered. After the exclusion of those with previous MI (n=212), delayed referral (n=193), previous CABG (n=193), co-morbidity (n=30), and wide QRS (n=22), the patients included in study 1 were chosen from 150 patients with NSTEMI-ACS. Of these 150 patients, 61 underwent CE-MRI, and were finally included in study 1. The 150 patients from OUH (which were also used for study 2) were originally recruited prospectively for previous studies,<sup>23</sup> and the echo exams were re-analyzed offline in this particular thesis for the additional assessment of MAD (study 1) and DESL (study 2). This should have been stated more clearly in the published papers (1 and 2).

At KI, a total of 227 patients were considered for inclusion. After the exclusion of those with previous MI, CABG and co-morbidity (the investigators at KI could not provide the exact numbers for each of these categories), 117 patients remained. The investigators at KI included only those with NSTEMI (i.e. with elevated serum cardiac biomarkers), and therefore excluded the 11 patients with UAP (no elevation of cardiac biomarkers).

At both centers, patients admitted for NSTEMI-ACS receive standard medical therapy (double-antiplatelet treatment,  $\beta$ -blockers, low-molecular weight heparin, and

statins) if tolerable, according to guidelines.<sup>50</sup> Forty patients with stable angina pectoris and significant coronary disease (>50% stenosis) and no myocardial infarction, as verified by cardiac MRI, were included as controls. This was done to assess whether the different echocardiography markers for LV systolic function could distinguish patients with acute coronary syndromes from those with stable coronary artery disease.

The prevalence of diabetes and the use of cardiovascular medication was higher in the NSTEMI group, than in the control group. The proportion of female patients was considerably higher in the control group. Furthermore, the case control status was also known to the investigator performing offline analysis. The implications of these differences are addressed under “Discussion”. There were no differences in age, body mass index, smoking status and prevalence of hypertension. Patient data, angiography results, echocardiographic films, and MRI results were acquired from the medical records.

The patients in study 2 were included from OUH only, and the population consisted of the entire cohort of 150 patients with NSTEMI-ACS as accounted for above. Study population details are provided below.

Table 1. Clinical baseline characteristics in paper 1.

<b>Characteristics</b>	<b>Patients N=167</b>	<b>Controls N=40</b>	<b>p-value</b>
Age (years)	63.3±11.8	59.8±9.8	0.09
BMI (kg/m <sup>2</sup> )	27.1±3.8	26.0±3.7	0.11
Diabetes	36 (22)	2 (5)	0.015
Hypertension	81 (48)	20 (50)	0.87
Current smoker	45 (31)	8 (20)	0.28
Hypercholesterolemia	155 (93)	30 (75)	<0.001
Male sex	126 (75)	14 (35)	<0.001
B-blocker	144 (86)	26 (65)	0.001
Statins	155 (93)	30 (75)	0.001
ACEI/ARB	92 (55)	12 (30)	0.004
Aspirin	160 (96)	32 (80)	<0.001

Data are presented as mean±SD or n (%). Hypercholesteremia was defined as use of statins or cholesterol>240 mg/dL at the time of angiography. Hypertension was defined as use of antihypertensive medication. BMI indicates body mass index; ACEI, angiotensin-converting enzyme inhibitor, ARB, angiotensin receptor blocker.<sup>51</sup>

**Table 2. Clinical baseline characteristics in paper 2.**

Characteristic	Non-occlusion (117)	Occlusion (n=33)	p-value
Age	57.7±9	58.1±8	0.84
BMI	26.8±4	28.3±3	0.11
Risk factors			
Male gender	81 (69%)	29 (88%)	0.03
Current smoker	41 (35%)	14 (42%)	0.44
Hypercholesterolemia	27 (23%)	12 (36%)	0.12
Hypertension	47 (40%)	13 (39%)	0.94
Diabetes	8 (7%)	4 (12%)	0.47
History of CAD	7 (6%)	1 (3%)	0.69
Medication prior to angiography			
Aspirin	116 (99%)	33 (100%)	1.0
Clopidogrel	113 (97%)	33 (100%)	0.58
LMWH	111 (95%)	33 (100%)	0.34
B-blocker	100 (85%)	20 (67%)	0.01
Statin	106 (91%)	30 (91%)	1.0
Warfarin	0 (0%)	1 (3%)	0.22
ACE-I og ARB	32 (27%)	8 (24%)	0.72
GpIIbIIIa inhibitor	0 (0%)	1 (3%)	0.22

Data are presented as mean ±SD or n (%). BMI, body mass index; CAD, coronary artery disease; LMWH, low molecular weight heparin; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; GpIIbIIIa, glycoprotein IIbIIIa.<sup>52</sup>

### **Study population (Paper 3)**

Ninety-two (92) consecutive patients with histologically verified small intestinal carcinoid tumours were screened for inclusion between 2006 and 2007. The inclusion criterion was a diagnosis of midgut carcinoid disease (by definition carcinoid tumours arising from the jejunum, ileum, appendix, or proximal colon) with histologic confirmation from either the primary tumour, or from a liver metastasis. A diagnosis of midgut carcinoid was confirmed via review of all original biopsy reports.

Since we wanted to assess the influence of neuroendocrine tumours on cardiac function and mortality, patients who had undergone radical surgery (n=18) were not included. Of the 74 remaining patients with residual tumours, echocardiography exams were available in 71, and these were included in the study. No patients with pulmonary carcinoid tumours were included. Carcinoid syndrome was defined as the presence of flushing and/or diarrhea and was present in 81% of the patients.

Abdominal computer tomography (CT) scans were studied to determine the number of liver metastases for each patient. The maximal diameter for each liver metastasis was measured and the average maximal diameter of the liver metastases for each individual patient was calculated and termed liver metastases size (LMS), as a measure for tumour burden.

Patients with other fibrotic diseases, those aged <18 years, and those unable to give informed consent were excluded from the study. None of the patients had suspected coronary artery disease on the basis of medical history and/or electrocardiographic (ECG) changes. The mean time interval from the diagnosis of midgut carcinoid tumour to inclusion was  $5.2 \pm 5.1$  years. Follow-up echocardiography was performed in 46 patients (87% of the survivors), while 7 patients did for various reasons decline follow-up echocardiography. Fifty healthy

age-matched controls were recruited from the hospital staff, and also assessed.

Baseline characteristics are declared in the table below.

**Table 3. Clinical baseline characteristics patients in paper 3.**

	Carcinoid disease (n=71)	Healthy (n=50)	p-value
Age (years)	61.1±12.1	60.7±13.9	0.86
Women	36 (51%)	23 (46%)	0.61
Heart rate (beats per minute)	66±12	64±11	0.58
BMI (kg/m <sup>2</sup> )	25.0±4.3	24.0±3.3	0.23
Time since diagnosis (years)	5.2±5.1	Na	
Ejection fraction (%)	65±7	65±5	0.93
Mitral annular displacement (mm)	12.3±1.5	14.0±1.8	<0.001
e' (m/s)	6.5±1.8	7.6±2.4	0.008
E/e'	10.5±3.2	8.8±3.1	0.009
RV lateral strain (%)	-25.8±4.5	-28.3±3.3	=0.002
LV strain (%)	-19.0±2.5	-21.8±2.1	<0.001

BMI, body mass index; RV, right ventricular; LV, left ventricular; na, not applicable.

Data are expressed as means ± standard deviation. Table adapted from paper 3.

## METHODS

### Echocardiographic measures of left ventricular systolic function

#### Left ventricular ejection fraction

The most widely used measure for left ventricular systolic function is left ventricular ejection fraction (LVEF),<sup>53,54</sup> which represents the stroke volume as a fraction of end diastolic volume:  $LVEF = \text{Stroke volume} / \text{End diastolic volume}$

Despite the widespread use of LVEF, it is associated with several challenging factors concerning image quality, operator experience, and assumptions of ventricular geometry.<sup>55</sup> It is also limited to assessing changes in ventricular cavity, and has a low sensitivity for detecting mild changes in myocardial function. The assessment of global LV function by LVEF is based on two apical views only, and may therefore overlook more regional changes.

#### Wall motion score index

Wall motion score index (WMSI) is another common measure for LV systolic function.<sup>56</sup> It is calculated from a 16-segment model,<sup>57</sup> where each segment is scored on the basis of its motion and systolic thickening as follows: 1 = normal or hyperkinetic, 2 = hypokinetic, 3 = akinetic, and 4 = dyskinetic (or aneurysmatic). WMSI is derived as the sum of all scores divided by the number of segments visualized. WMSI is less affected by regional hyperkinesias compared to LVEF, but it is however limited by the experience and subjectivity of the examiner. It is also time consuming.



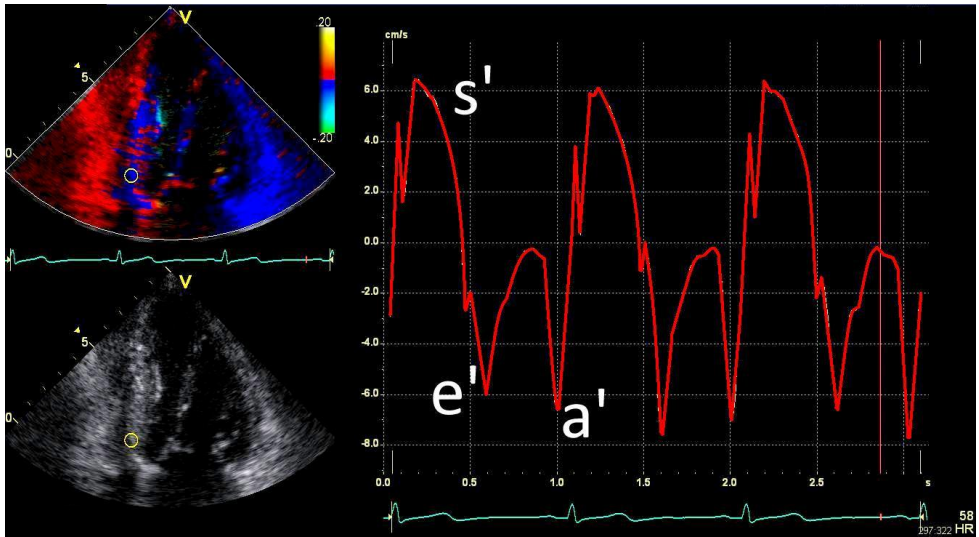
### Tissue Doppler imaging

Tissue Doppler imaging (TDI) depicts myocardial motion by measuring tissue velocity at specific myocardial regions. Through the Doppler equation

$$v = \frac{\Delta f c}{2f_0 \times \cos \theta}$$

velocity ( $v$ ) is calculated based on the shift in frequency between transmitted and received frequency ( $\Delta f$ ). Velocity of sound in tissue ( $c$ ), and the transmitted frequency ( $f_0$ ) are known constants. Conventional Doppler techniques assess the velocity of blood flow by measuring high-frequency, low amplitude signals from small, fast-moving blood cells. In TDI the same Doppler principles are used to quantify the higher amplitude, lower-velocity signals of myocardial tissue motion. TDI can be performed in pulsed wave (PW) mode, or color mode.

Pulsed-wave TDI is used to measure peak myocardial velocities and is particularly well suited for the measurement of long-axis ventricular motion due to the longitudinally oriented endocardial fibers, which are more parallel to the ultrasound beam in the apical views. TDI allows measurement of several indices, which have clinical and prognostic implications. The cardiac cycle is represented by *three waveforms*;  $s'$ : systolic myocardial velocity above the baseline as the annulus descends toward the apex;  $e'$ : early diastolic myocardial relaxation velocity below the baseline as the annulus ascends away from the apex;  $a'$ : myocardial velocity associated with atrial contraction.



**Figure 1.** Tissue Doppler velocity traces acquired from the septal part of the mitral annulus from apical 4-chamber view.  $s'$  denotes the systolic myocardial velocity;  $e'$  the early diastolic myocardial relaxation velocity;  $a'$  the myocardial velocity associated resulting from atrial contraction.

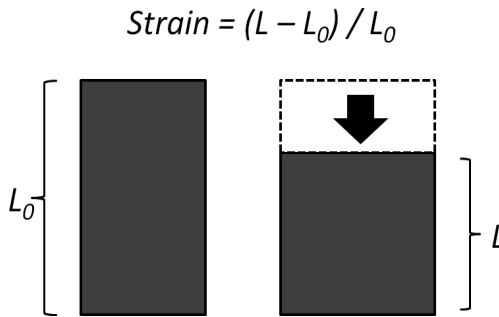
Integration of the velocity curves over time allows us to calculate the displacement of a particular myocardial structure. Since the apex remains relatively stationary throughout the cardiac cycle, mitral annular displacement (MAD) is a good surrogate measure of overall longitudinal left ventricular (LV) contraction and relaxation.<sup>58</sup> To measure longitudinal myocardial velocities, the sample volume is placed in the ventricular myocardium immediately adjacent to the mitral annulus. Pulsed-wave TDI has high temporal resolution but does not permit simultaneous analysis of multiple myocardial segments.

With color TDI, a color-coded representation of myocardial velocities is superimposed on gray-scale 2-dimensional or M-mode images to indicate the direction and velocity of myocardial motion. Color TDI mode has the advantage of

increased spatial resolution and the ability to evaluate multiple structures and segments in a single view.<sup>59</sup>

#### Two dimensional speckle tracking echocardiography

Strain is a clinical measure of myocardial deformation<sup>60</sup> and has been introduced and validated using magnetic resonance imaging (MRI) tagging<sup>61</sup> and sonomicrometry.<sup>62</sup> It is defined as the percentage change of a given myocardial region, either shortening, or stretching, relative to initial length.

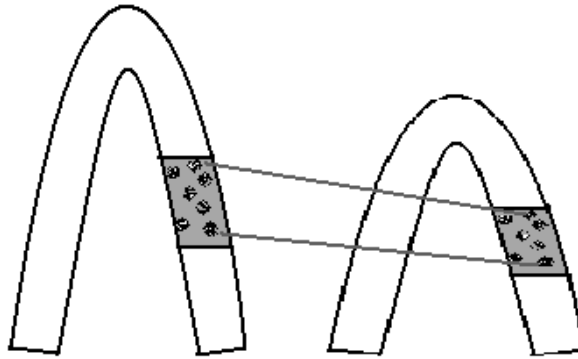


**Figure 2.** In echocardiography, “Strain” is the fractional change of dimension when stress is exerted on the myocardium.  $L$  is the current length, while  $L_0$  denotes the initial length. (Modification of a previously published figure.<sup>63</sup>)

A positive strain value refers to elongations, whereas a negative strain value describes shortening. Normal myocardium shortens along the longitudinal axis during systole, as a result of active force generated by muscle fibers. Quantification of strain is therefore a measure for myocardial function.

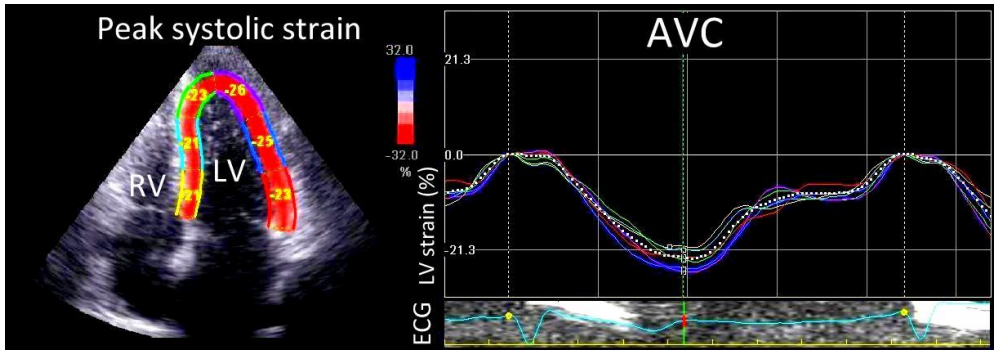
Strain can be measured through two dimensional speckle tracking echocardiography (2D-STE). This method bypasses the problems of angle-dependency of the tissue Doppler technique. 2D-STE is based on 2D gray-scale images, and strain is evaluated by tracking acoustic markers (speckles) during the

cardiac cycle. Strain is calculated for each LV segment as the average relative deformation in longitudinal, circumferential and radial directions.



**Figure 3.** A schematic depiction of the left ventricle from the apical four chamber view, in end-diastole (left) and end-systole (right). Speckle patterns (here shown as black dots), are automatically tracked frame by frame, throughout the cardiac cycle.

As a measure for LV function, peak systolic longitudinal strain, defined as maximum systolic longitudinal shortening, is assessed in 16 longitudinal LV segments and averaged to global longitudinal strain (GLS). Feasibility and reproducibility of GLS has been shown to be excellent in patients with coronary artery disease.<sup>64</sup>



**Figure 4.** A normal left ventricle (LV) viewed from the apical 4-chamber view (left panel). Corresponding segmental longitudinal strain curve (right panel). Aortic valve closure (AVC) defines end-systole. RV, right ventricle.

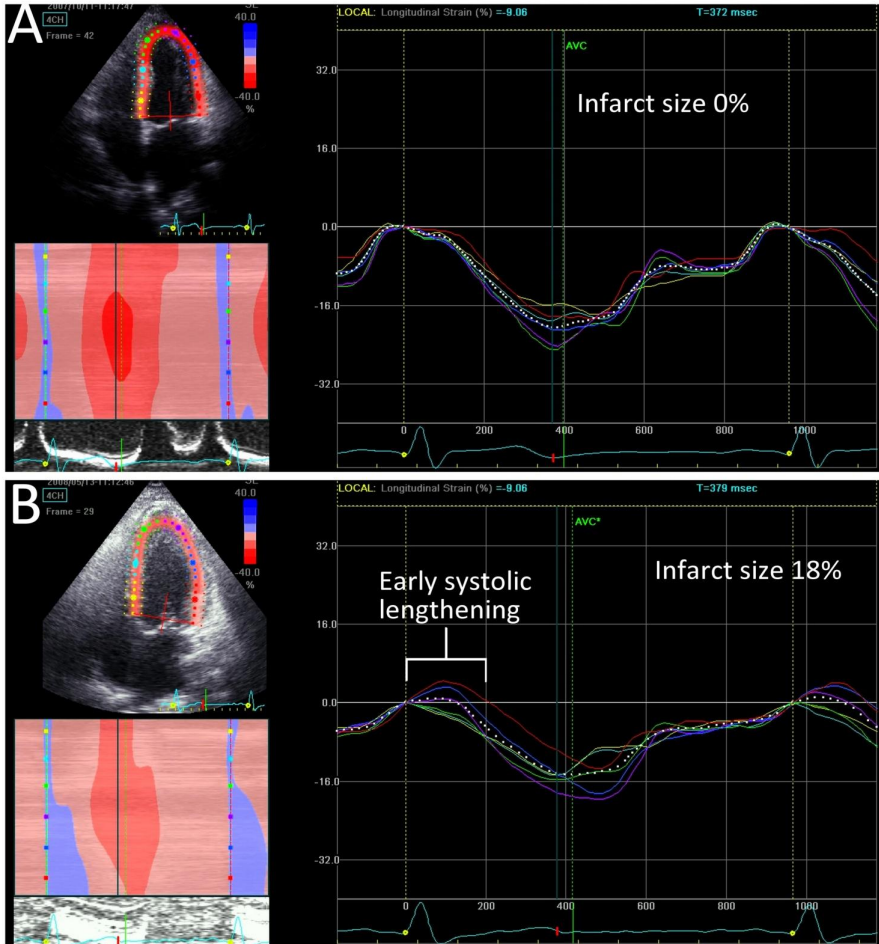
For the assessment of RV function, peak systolic longitudinal strain values can be obtained from the three lateral RV segments, defined as the lateral basal, lateral mid, and lateral apical segments from the apical four-chamber view (Paper 3).

Artifacts resembling speckles will influence the quality of speckle tracking. The method is sensitive to acoustic shadowing or reverberations, which may result in the underestimation of deformation. Reduced signal quality and suboptimal tracking may also create nonphysiological strain traces.

#### Duration of early systolic lengthening

In this thesis, we introduce a potentially new measure for LV systolic function in patients with NSTEMI-ACS. Recent studies have shown that ischemic myocardium tends to lengthen before the onset of systolic shortening, probably due to its reduced ability to generate adequate active force as the LV pressure rises steeply during the isovolumic contraction phase (IVC).<sup>65,66</sup> The duration of early systolic lengthening (DESL) has been shown to be proportional to the infarct size in STEMI patients,<sup>67</sup> and we wanted to assess the diagnostic strength of this measure in NSTEMI-ACS patients. DESL was defined as the time period in which the corresponding strain curve stayed

positive from onset of a Q-wave (or R-wave if Q was absent). The duration of early systolic lengthening in all 16 segments were averaged to obtain an average DESL value per patient.



**Figure 5.** Examples of longitudinal strains by speckle tracking echocardiography (from the apical 4-chamber view). Panel A shows strain traces from a patient with no visible late enhancement on CE-MRI, and there is no early systolic lengthening. Panel B shows strain traces from a patient with a large infarct, and early systolic lengthening can be seen. The white bracket indicates the duration of early systolic lengthening of basal lateral segment (red curve).<sup>52</sup>

## Two-dimensional echocardiography

In study 1, 75 patients were examined immediately before angiography (on average  $2.3 \pm 0.9$  days after the initial admission for NSTEMI-ACS), and 92 were

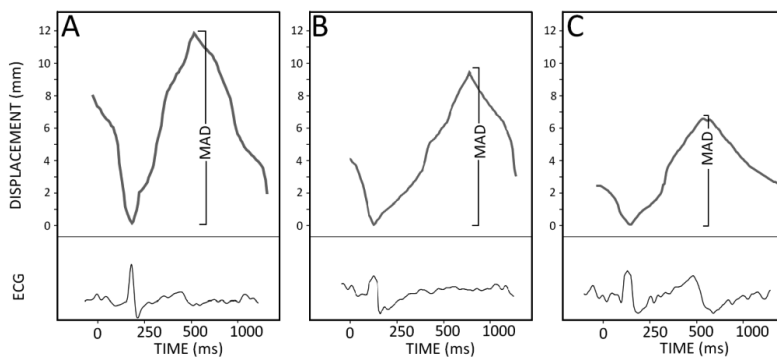
examined shortly after ( $1.7 \pm 1.5$  days) angiography, which was performed within 72 hours of the initial hospital admission. In all three studies, cine-loops from three standard apical views (4-chamber, 2-chamber and apical long axis) were recorded using grey-scale harmonic imaging. In study 1 and 2, WMSI was calculated from a 16-segment model. Each segment was analyzed individually and scored on the basis of its motion and systolic thickening. Each segment's function was confirmed in multiple views. Segments were scored as follows: 1 = normal or hyperkinetic, 2 = hypokinetic, 3 = akinetic, and 4 = dyskinetic (or aneurysmatic). WMSI was derived as the sum of all scores divided by the number of segments visualized. LVEF was calculated using Simpson's method in all three studies.

In study 3, 71 participants underwent complete transthoracic echocardiography at baseline. LVEF was calculated using Simpson's method. CHD was defined as a combination of right-sided valvular dysfunction (i.e., more than mild regurgitation and/or any valvular stenosis) and pathologic morphology (i.e., valve leaflet thickening, shortening, retraction, hypomobility, or incomplete coaptation). Tricuspid valve regurgitation was assessed using current guidelines and graded as none (0), mild (1), moderate (2), or severe (3). Two-dimensional (2D) grayscale images were acquired in the standard parasternal and apical (four-chamber, two-chamber, and long-axis) views at  $67 \pm 26$  frames/sec. Three cardiac cycles were recorded. All images were stored digitally for subsequent offline analysis. Myocardial deformation measurements were performed using STE. In all three studies, echocardiography was performed using Vivid 7 machines (GE Vingmed Ultrasound AS, Horten, Norway). Offline analysis was performed using commercially available software (EchoPAC; GE Vingmed Ultrasound AS).



## **Mitral annular displacement by Tissue Doppler imaging**

In study 1 and study 3, tissue Doppler velocity measurements were obtained from color 2D Doppler images and obtained at the basal part of the left ventricle in the three apical planes in EchoPAC (GE Vingmed Ultrasound AS). The traces were then exported as text files and imported to customized software (GHLab; Gripping Heart AB, Stockholm, Sweden), which calculated the displacement of each of the aforementioned points of the mitral annulus by integrating the velocity curves over time. MAD was defined as the part of the displacement curve corresponding to the interval from the onset of the QRS complex to the end of the T wave on the electrocardiogram. The mean value of these displacements represents the average MAD. Peak  $s'$  and  $e'$  were also acquired at the six basal parts of the left ventricle and averaged. E was acquired from mitral flow Doppler readings from the four-chamber apical views.



**Figure 6.** Longitudinal displacement of the mitral annulus during a complete heart cycle in (A) a patient with stable angina with significant coronary disease without myocardial infarction, (B) a patient with NSTEMI without coronary occlusion, and (C) a patient with NSTEMI with coronary occlusion. MAD is the maximum displacement. ECG, Electrocardiogram.<sup>51</sup>

## LV and RV global longitudinal strain by 2D-STE

The endocardial borders were traced in the end-systolic frames of the 2D images from the three apical views of the LV for longitudinal strain (Papers 1-3), and in the 4-chamber view for RV longitudinal strain (Paper 3). The thickness of the region of interest was adjusted to match the thickness of the myocardial wall. Speckles were tracked throughout the cardiac cycle. Segments that failed to track were manually adjusted by the operator. Any segments that subsequently failed to track were excluded. The software generated 18 longitudinal LV segments from the three apical views which were converted to a standardized 16 LV segment model by averaging the strain values of corresponding apical segments in the apical long-axis and 4-chamber planes. Consequently, peak systolic longitudinal strain, was assessed in 16 longitudinal LV segments and averaged to global longitudinal strain (Papers 1-3). For the assessment of RV function, peak systolic longitudinal strain values were obtained from the three lateral RV segments, defined as the lateral basal, lateral mid,

and lateral apical segments from the apical four-chamber view (Paper 3). End of systole was defined by the timing of the aortic valve closure in apical long axis view.

### **Duration of early systolic lengthening by 2D-STE**

In study 2, all patients were examined immediately before angiography (on average  $2.3 \pm 0.9$  days after the initial admittance for NSTEMI-ACS). The echocardiographic examinations in this population have previously been analyzed, and the results published in earlier papers.<sup>23,27</sup> For study 2 in this thesis, the exams were re-analyzed in order to acquire DESL. The reason for re-analysis was that in the previous work, strain curves were acquired by placing the left cursor in the middle of the QRS-complex. But this strategy largely missed the early systolic lengthening. Therefore all exams were re-analyzed with the cursor placed at the onset of a Q-wave. As described above, longitudinal strain was assessed by speckle tracking echocardiography (STE) in a 16 segment model. For each segment, peak negative systolic strain and duration of early systolic lengthening were recorded (DESL). DESL was defined as the time period in which the corresponding strain curve stayed positive from onset of a Q-wave (or R-wave if Q was absent). As a measure of global systolic function, peak negative strain values of all segments were averaged to obtain GLS. The re-analysis resulted in slightly different GLS values than those published from the same cohort earlier.

The duration of early systolic lengthening in all 16 segments were averaged to obtain an average DESL value per patient. End-systole was defined by aortic valve closure in the apical long-axis view.

## **Magnetic resonance imaging**

In study 1 and 2, the magnitude of the final infarct size was determined by MRI 9±3 months after inclusion in 61 patients, using a 1.5-T unit (Magnetom Sonata, Siemens) on 29 patients and a 3-T unit (Philips Medical Systems) on 32 patients. Late-enhancement images were acquired 10 to 20 minutes after intravenous injection of 0.1 to 0.2 mmol/kg gadopentetate dimeglumine (Magnevist, Schering) in short-axis slices covering the entire LV. Image parameters were slice thickness: 8 mm, gap: 2 mm, and inversion time: 270 ms. Total myocardial area and area of infarcted myocardium was manually drawn (PACS, Sectra, Sweden) on each short axis image. Infarct size was calculated as infarct volume as a percentage of total myocardial volume. The patients had no known coronary disease before inclusion, and had not undergone coronary interventions previously. Between revascularization and MRI at follow-up, 1 patient had re-infarction with minimal enzyme release (troponin T, 0.18 µg/L) in the same vessel as was initially treated.

The distinction between a large and a small infarct was made by setting a cut-off at infarct size of 12% of total LV mass. Minimal myocardial damage was defined as the absence of late enhancement on MRI images (infarct size = 0%). The MRI cohort was therefore dichotomized by infarct size, using 0% as cut-off. MRI was performed depending on lab availability at follow up, and although the patients were not strictly randomized to MRI, there was no systematic bias in giving priority to MRI referral.

## **Coronary angiography**

Coronary angiography was performed using standard techniques with digital image acquisition and storage. Coronary occlusion was defined as TIMI flow 0 or 1, and significant stenosis was defined as a coronary stenosis >50%.

### **Biomarkers and blood tests**

In study 3, plasma levels of C-reactive protein (CRP) and OPG were analyzed by enzyme immunoassays (EIAs) using matched antibodies obtained from DakoCytomation (Glostrup, Denmark) and R&D Systems (Minneapolis, Minn., USA), respectively. Plasma levels of soluble TNF receptor 1 (sTNF-R1), MMP-9, and TGF- $\beta$ <sub>1</sub> were analyzed by EIAs from R&D Systems and plasma levels of Activin A by an EIA provided by Serotec (Oxford, UK). Patient blood samples were additionally analyzed for chromogranin A (CgA), a known tumour marker for NETs, using a radioimmunoassay provided by EuroDiagnostica (Malmö, Sweden) (normal <4 nmol/l). Inter- and intra-assay coefficients of variance were <10% for all assays. Biomarkers were available in all patients.

### **Reproducibility and feasibility**

In study 1, mitral velocities (and the corresponding displacement values) were available in 952 of 1002 sites (95%). Measurements from all six mitral sites were available in 143 patients, and in the remaining 24 patients, measurements were available from at least four mitral sites in each, making it possible to calculate an average MAD in all patients. WMS was available in 2616 segments (98%), and longitudinal strain values could be obtained in 2405 (90%) of all LV segments. LVEF was assessable in 161 patients (96.4%).

In study 2 longitudinal strain values could be obtained in 2363 LV segments (98.4%). All these values were included in the calculation of DESL. WMS was assessable in 2395 LV segments (99.8%). LVEF was assessable in 149 patients (99%).

In study 3 mitral velocities (and the corresponding displacement values) were available in only apical 4-chamber and 2-chamber views (four sites in each patient). It was possible to measure velocities at all four sites in 64 patients (256 sites), and at three sites in seven patients (21 sites). Longitudinal strain values could be obtained in 988 of 1136 LV segments (87%), and in 187 of 213 RV lateral segments (88%). LVEF was assessable in 70 of 71 patients.

For all three studies: The excluded strain values either corresponded to segments outside the visual echo field, or had non-physiologic curve appearance due to poor tracking/image quality.

Reproducibility for MAD, GLS, WMSI, LVEF, DESL, infarct size by MRI and RV strain was checked in 15 random cases (ten in MRI) (selected automatically by PASW):

**Table 4. Inter- and intraobserver variability for the different variables.**

	<b>MAD</b>	<b>GLS</b>	<b>WMSI</b>	<b>LVEF</b>	<b>DESL</b>	<b>MRI</b>	<b>RV strain</b>
Interobserver variability coefficient	0.98	0.94	0.92	0.81	0.93	0.93	0.85
Intraobserver variability coefficient	0.95	0.96	0.98	0.84	0.99	0.93	0.88

p<0.001 for all

## Statistical methods

Continuous variables were presented as means  $\pm$  standard deviation (SD), or median (interquartile range (IQR)). Group differences were analyzed with the Student T-test when the assumption of normal distribution in the groups was met. We used the unpaired T-test when the samples were independent of each other, and the paired T-test when comparing measurements from the same individuals at different times. Categorical variables were presented as numbers (%) and differences between groups were analyzed with the Chi square test when the sample sizes were sufficiently large, and Fisher's exact test when the cell counts were  $< 5$ . Linear correlations were presented with Pearson coefficients as the assumptions of linearity and normal distribution were met. The diagnostic accuracy of the different variables was tested with receiver operating characteristics (ROC) analyses. The optimal cut-offs were defined as the values of the ROC curves that were closest to the upper left corner (with the highest possible sensitivity and specificity). The reliability of the optimal cut-off values was validated using bootstrap resampling (1000 iterations), and 95% confidence intervals based on bootstrap percentiles were presented. Area under curve (AUC) was presented with 95% confidence interval (CI). When performing receiver operating characteristic (ROC) curve analyses, we split the patient populations in half (automatically by PASW; IBM, Armonk, NY). Particularly for study 1, we used half of the cohort to derive optimal cutoff values from ROC curves and then applied these values in the second half of the cohort, in which standard reference methods allowed us to determine true-negative or false-negative and positive cases and calculate levels

of sensitivity, specificity, positive predictive value, and negative predictive value. All statistical analyses were performed with PASW version 18 (IBM Corporation), except for bootstrapping and differences between ROC curves, which were analyzed in MedCalc (MedCalc Software). In study 1 and 3, univariate and multivariate Cox proportional-hazards regression analyses were performed to identify predictors of mortality (hazard ratios are given with 95% confidence intervals). The proportionality hazards assumption was tested by the Schoenfeld method.<sup>68</sup> Partial residuals were calculated for each variable, and association with time was tested with linear regression; there was no significant interaction with time. Adjusted hazard ratios were obtained after adjustment for potential confounders. Event-free survival was analyzed using Kaplan-Meier curves. Differences in event rates over time were evaluated using log-rank tests.

The inherent limitations of the chosen statistical methods, and how they may affect the results in the different studies, are discussed in a separate section under “Limitations”.



## SUMMARY OF RESULTS

### Paper 1

During a mean follow-up period of  $48.6 \pm 12.1$  months, 22 patients (13%) died. Fourteen of 22 deaths were of cardiovascular causes, and the causes of death were unclear regarding the remaining eight patients. MAD, GLS, LVEF, and peak  $s'$  were significantly lower and WMSI and E/e' ratio higher in those who died than in those who survived.

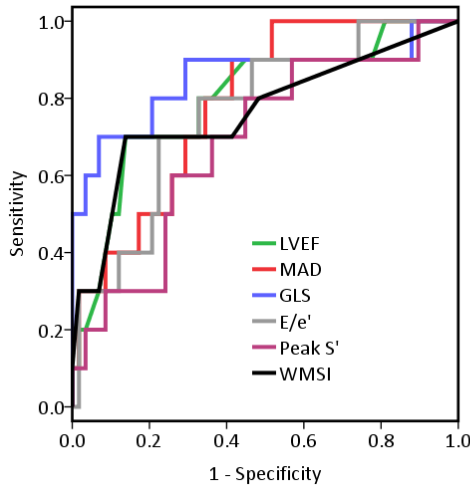
Comparison of areas under ROC curves showed no significant differences between any of the indices for myocardial function. In univariate Cox proportional-hazards analysis, age, diabetes, hypertension, LVEF, MAD, GLS, WMSI, peak  $s'$ , and E/e' ratio were predictors of death. In multivariate analysis including age, diabetes, MAD, and hypertension, MAD, diabetes, and age remained independent predictors of death. Kaplan-Meier analysis showed that patients with MAD values in the first quartile ( $<8.0$  mm) had significantly lower survival probability compared with patients with MAD values in the fourth quartile ( $\geq 11.0$  mm) (log-rank  $P = .004$ ).

Only echocardiographic studies performed before angiography ( $n = 75$ ) were included in the analyses of prediction of coronary occlusions. In this population, 36 of the 75 patients had coronary occlusions. MAD and GLS were significantly lower, and WMSI was significantly higher, in patients with coronary occlusion than in those without coronary occlusion ( $p=0.006$ ,  $p=0.001$ , and  $p=0.02$  respectively), while LVEF, peak  $s'$ , and E/e' ratio did not differ. A cutoff value of 9.5 mm for MAD could identify nine of the 16 patients with occlusion and suggested occlusion in five of the

patients who did not have any. The corresponding numbers for a GLS value of 14.7% were 10 and five patients, respectively.

MAD and LVEF showed moderate correlations ( $r = -0.46$  and  $r = -0.53$ , respectively), while GLS and WMSI showed good correlations ( $r = 0.68$  and  $r = 0.74$ , respectively,  $p < 0.001$  for all) with infarct size. MAD, GLS, and LVEF were significantly lower, and WMSI significantly was higher, in patients with infarct sizes  $\geq 12\%$  than in patients with infarct sizes  $< 12\%$  ( $p < 0.01$  for MAD and LVEF,  $p < 0.001$  for GLS and WMSI).

MAD, GLS, and LVEF were significantly reduced in patients with NSTEMIs compared with those with stable angina with significant coronary artery disease without signs of myocardial infarction (MAD,  $9.6 \pm 2.1$  vs  $12.5 \pm 1.3$  mm; GLS,  $14.5 \pm 3.5\%$  vs  $20.4 \pm 2.3\%$ ; LVEF,  $51.6 \pm 11.0\%$  vs  $57.6 \pm 7.0\%$ ;  $P < .001$  for all). A cutoff value of 10.9 mm for MAD identified 67 of the 88 patients with NSTEMIs and falsely suggested NSTEMI in only two of the 16 patients with significant coronary disease without infarctions. The corresponding numbers for GLS were 73 and one patient.



**Figure 7.** ROC curves of patients with NSTEMI concerning mortality.<sup>51</sup> AUC (95% CI), optimal cut-off values and the calculated levels of sensitivity and specificity are shown in the table below.<sup>51</sup>

	AUC (95%CI)	p-value	Cut-off (95% CI)	Sensitivity	Specificity	PPV	NPV
<b>MAD (mm)</b>	0.79 (0.67 - 0.88)	0.004	9.1 (8.0-10.0)	0.58	0.63	0.20	0.91
<b>GLS (%)</b>	0.85 (0.75 - 0.93)	<0.001	-11.0 (8.4-13.8)	0.42	0.88	0.35	0.91
<b>LVEF (%)</b>	0.80 (0.68 - 0.89)	0.003	44 (44-62)	0.50	0.88	0.40	0.92
<b>WMSI</b>	0.76 (0.65 - 0.86)	0.008	1.3 (1.0-1.3)	0.33	0.79	0.20	0.88
<b>Peak s' (cm/s)</b>	0.69 (0.56 - 0.79)	0.05	4.1 (2.6-5.2)	0.33	0.86	0.27	0.89
<b>E/e'</b>	0.76 (0.65 - 0.86)	0.008	13.4 (8.3-16.9)	0.50	0.72	0.22	0.90

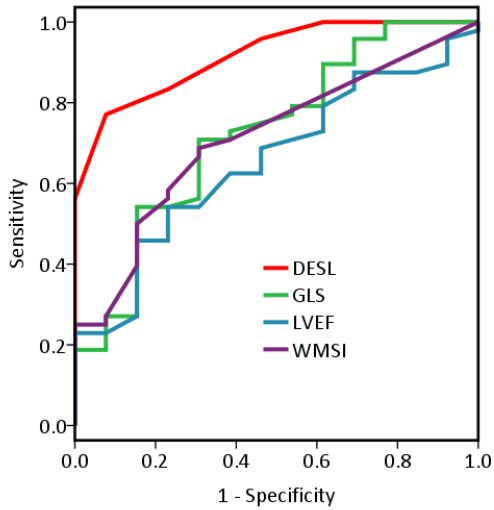
AUC; area under curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; MAD, mitral annulus displacement; GLS, global longitudinal strain; LVEF, left ventricle ejection fraction; WMSI, wall motion score index.

## Paper 2

In the 61 patients investigated with CE-MRI, forty-eight had visible scar, while 13 patients had no visible scarring (infarct size = 0%). LVEF showed moderate correlation ( $r = -0.53$ ), while GLS, DESL and WMSI showed good correlation ( $r = 0.64, 0.67, \text{ and } 0.74$ , respectively,  $p < 0.001$  for all) to infarct size. DESL and WMSI were significantly lower, and GLS significantly more negative (i.e. better shortening) in patients with no visible scar, than in those with infarct size  $> 0\%$  ( $p > 0.001$ ,  $p > 0.01$  and  $p > 0.01$ , respectively).

ROC analyses for the identification of patients with visible vs. no visible infarct revealed that DESL could accurately distinguish between these patients. DESL had the largest area under curve (0.92), and it was significantly better than GLS, WMSI and LVEF ( $p$ -values 0.016, 0.008 and 0.001 respectively). A cut-off value of 50 ms could identify patients with minimal myocardial damage with a sensitivity of 77% and a specificity of 92%.

Thirty-three of 150 patients (22%) had acute coronary occlusions. DESL and WMSI were significantly higher, and GLS and LVEF significantly worse in patients with coronary occlusion, than in those without ( $p = 0.001$ ,  $p < 0.001$ ,  $p < 0.001$  and  $p = 0.009$  respectively). ROC analyses showed that the accuracy was moderate in identifying patients with acute coronary occlusions. DESL had an AUC of 0.65, and a cut-off value of 100 ms could identify a coronary occlusion with a sensitivity level of 33% and a specificity level of 92%.



**Figure 8.** ROC curves based on values from 61 NSTEMI patients undergoing MRI, showing that the duration of early systolic lengthening (DESL) was the most accurate variable in identifying patients with minimal myocardial damage (no visible scarring on CE-MRI).<sup>52</sup> Area under curve (with 95% CI), optimal cut-off values, and the corresponding levels of sensitivity and specificity are shown in the table below.<sup>52</sup>

	AUC *	p-value	Cutoff *†	Sensitivity *	Specificity *
DESL	0.92 (0.82 to 0.97)	<0.0001	>50 ms (10 to 50)	77% (63 to 88)	92% (64 to 100)
GLS	0.72 (0.60 to 0.83)	=0.007	>-18.6% (-22.6 to -17.2)	71% (56 to 83)	69% (39 to 91)
LVEF	0.67 (0.52 to 0.77)	=0.05	<55% (47 to 66)	54% (39 to 67)	77% (46 to 95)
WMSI	0.71 (0.58 to 0.82)	=0.003	>1.06 (1.00 to 1.13)	69% (54 to 81)	69% (39 to 91)

\*) Presented with 95% confidence intervals

†) 95% confidence interval calculated by bootstrap analysis (1000 iterations)

Abbreviations are explained in the text.

### Paper 3

Follow up time from inclusion was  $1274\pm 368$  days. During follow up, 18 patients (25%) died and 53 survived. These patients had comparatively worse myocardial function at baseline, than those who survived. This was demonstrated by significantly worse values for LV strain, RV strain, and MAD ( $p<0.001$ ,  $p=0.003$ , and  $p<0.001$  respectively). Furthermore, there were also significant differences in diastolic function at baseline between the survivors ( $e' 6.9\pm 1.6$  m/s), and the non-survivors ( $e' 5.5\pm 1.9$  m/s,  $p=0.002$ ). LVEF did not differ. Baseline plasma levels of Activin A and sTNF-R1 were significantly higher in patients who died during follow-up than those who survived ( $p=0.001$  and  $p<0.001$  respectively).

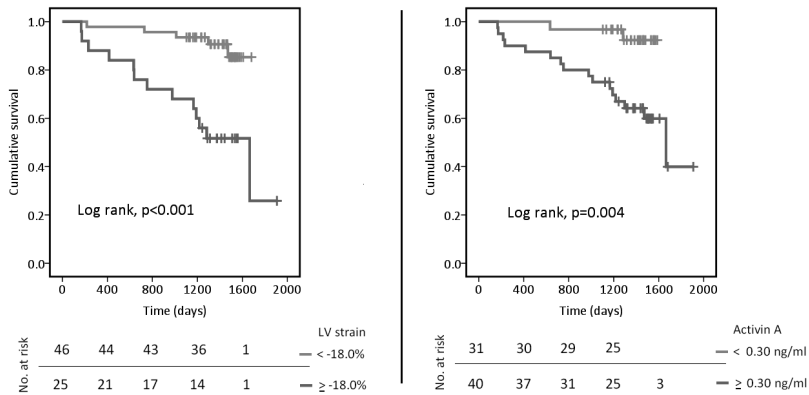
In univariate Cox proportional hazard ratio analyses, baseline Activin A, OPG, sTNF-R1, LV strain, RV strain, liver metastases size (LMS), MAD, and age were predictors of mortality.

In the different multivariate Cox models, LV strain remained an independent predictors of death. Kaplan-Meier analyses for LV strain and Activin A showed that patients with reduced LV myocardial function, or increased levels of Activin A at baseline, had lower survival probability (log rank  $p<0.001$  and  $p=0.004$  respectively).

At baseline, 13 patients were classified as having CHD according to the given definition. During follow-up, six of the 13 patients (40%) with CHD at baseline died, while four new patients had developed echocardiographic signs of CHD. There were no statistically significant differences in any of the clinical variables at baseline between those who went on to develop CHD, and those who didn't.

Compared to baseline, follow up echocardiography revealed a markedly reduced myocardial function by both LV strain ( $-19.7\pm 2.5\%$  to  $-17.9\pm 2.4\%$ ,  $p<0.001$ ) and RV strain ( $-27.2\pm 4.2\%$  to  $-25.2\pm 4.8$ ,  $p=0.027$ ). There was no significant

correlation between Activin A levels, and LV strain values at baseline ( $r=0.20$ ,  $p=0.10$ ), but the patients with Activin A values in the top quartile ( $\geq 0.45$  ng/ml) at baseline, had significantly worse LV strain ( $16.7\pm 2.9\%$ ) at follow-up echocardiography, than those with Activin A values in the bottom quartile ( $\leq 0.23$  ng/ml, LV strain  $18.8\pm 1.9\%$ ,  $p=0.05$ ).



**Figure 9.** Kaplan-Meier analyses comparing survival probability in patients with carcinoid intestinal disease. Left panel: patients with left ventricular (LV) strain < -18.0% compared to those with LV strain  $\geq -18.0\%$ , showing that the latter had significantly higher survival probability than the former (log-rank  $P = < 0.001$ ). Right panel: patients with Activin A < 0.30 ng/ml compared to those with Activin A  $\geq 0.30$  ng/ml, showing that the latter had significantly lower survival probability than the former (log-rank  $P = 0.004$ ). Adapted from paper 3.

## **DISCUSSION**

### **Prediction of mortality in patients with NSTEMI**

Study 1 demonstrated that several measures of myocardial function are accurate tools for diagnostic and prognostic purposes. Specifically, MAD had the ability to predict mortality in patients with NSTEMIs, was able to differentiate between patients with coronary occlusion and non-occlusion, and could distinguish patients with NSTEMI from those with angina with significant coronary artery disease, but without infarctions. Furthermore, our results showed that MAD was inversely correlated with final infarct size measured by MRI and was able to distinguish between small and large infarcts in patients with NSTEMI. The performance of MAD was comparable with that of the other indices of myocardial function (GLS, LVEF, and WMSI). The results imply that decreased MAD is associated with decreased myocardial function, larger infarcts, and higher risk for mortality in patients with NSTEMI.

The systolic excursion of the mitral annulus is a clinically useful measure of LV systolic function.<sup>69,70</sup> The mitral annulus moves in the direction of the apex during systole because of longitudinal LV deformation, and MAD is the average annular motion. The apex has a relatively stationary position throughout the cardiac cycle, and MAD in the longitudinal direction therefore reflects the global shortening deformation of the heart. GLS by 2D speckle-tracking echocardiography has proven to be an excellent marker of myocardial function, but like LVEF and WMSI, it requires good visualization of the ventricular curvature. Furthermore, the analyses require cardiologists or very experienced trainees. Assessment of MAD requires visualization of the mitral annulus only, which is possible in most patients, and the



imaging can be done even by doctors or technicians with limited experience. The MAD method is therefore potentially more robust when parts of the LV wall are poorly visualized.<sup>71</sup> MAD can be obtained easily and quickly, was available in all participants in our studies, and we also demonstrated high reproducibility.

It is well known that reduced LV systolic function after an acute myocardial infarction is associated with increased mortality.<sup>72</sup> Our findings in study 1, were in keeping with this. We found that reduced MAD, GLS, and LVEF, and increased WMSI, were predictors of mortality. To our knowledge, our study is the first to show such an association between MAD and mortality exclusively for patients with NSTEMIs without previously known acute myocardial infarctions undergoing modern revascularization therapy. The patients who died during follow-up were significantly older and had higher prevalence of diabetes and hypertension than the survivors. However, MAD remained an independent predictor of death after adjustment, showing the importance of myocardial function on survival. Furthermore, there were no statistically significant differences between the performance of MAD, and the other indices of myocardial function in distinguishing between patients who died and survived.

### **Prediction of final infarct size**

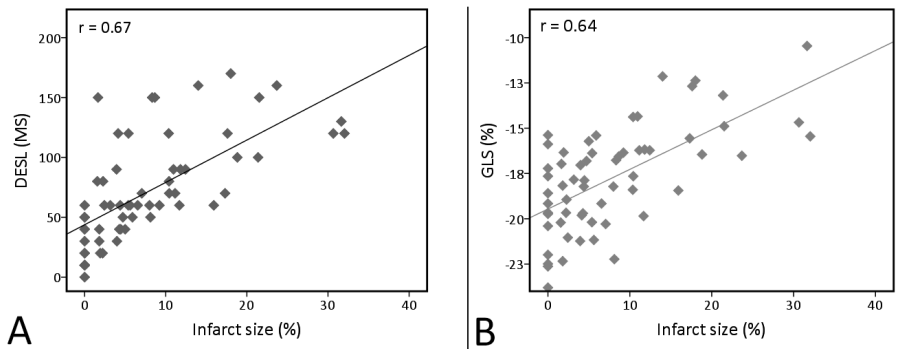
Final infarct size predicts cardiovascular outcomes and mortality,<sup>73</sup> and the gold standard for the quantification, is contrast-enhanced MRI.<sup>74</sup> This modality is expensive, time and resource consuming, and not easily accessible to all patients. GLS by 2D speckle-tracking echocardiography and WMSI are also excellent in predicting final infarct size,<sup>27</sup> but these techniques require time, experience, and good image quality. In study 1, MAD was moderately inversely correlated with final infarct

size and was also able to distinguish between small and large infarcts. GLS and WMSI were superior to MAD, but when image quality is poor, MAD can serve as an alternative. MAD is however less accurate, and has therefore limited value in prediction of infarct size.

In study 2, we assessed the ability of DESL to predict final infarct size, and identify patients with *minimal* myocardial damage (infarct size = 0% on CE-MRI). The ability to distinguish between coronary occlusions and non-occlusions was also tested.

During isovolumic contraction (IVC) there is a substantial "tug-of-war" effect between the different LV segments. Since the volume has to remain fixed, as one segment shortens, another has to lengthen. This is not a necessity when volume can change during ejection. Therefore, during IVC, the non-ischemic segments with preserved contractile force will cause ischemic segments to lengthen as the ischemic segments are incapable of increasing contractile force at a similar rate as the non-ischemic segments. During ejection, though, the constant LV volume constraint is no longer in play, and when the rate of pressure rise ( $dP/dt$ ) decreases, also partly ischemic segments are able to shorten, but the magnitude of the shortening is dependent on the degree of myocardial damage. Therefore our hypothesis was that the patients with the smallest infarcts would have a shorter DESL, and those with the largest infarcts would have a more prolonged DESL.

The results showed that DESL correlated with final infarct size and that its performance was comparable to that of GLS and WMSI. However, DESL was superior to GLS and WMSI in identifying patients with minimal myocardial damage after NSTEMI (infarct size = 0% on CE-MRI). Studying these scatter plots may explain why:



**Figure 10.** Scatter plots showing correlation between duration of early systolic lengthening (DESL) and infarct size (A), and between GLS and infarct size (B).<sup>52</sup>

Most patients with no visible myocardial scarring (infarct size = 0%) had a DESL of less than the proposed cut-off value of 50 ms. GLS, on the other hand, was reduced in several patients even in the event of no visible scar, resulting in a considerable number of false positives. This could be partly explained by the afterload dependency of GLS: reduced shortening in patients with no visible scar could be due to loading factors such as hypertension, rather than ischemia. DESL, on the other hand, may potentially be less dependent on afterload, as it takes place during IVC. This means that the DESL could be useful in identifying patients with minimal myocardial damage. Identifying these patients may be important from both a patient's perspective: it offers comfort, and also from a clinician's point of view: these patients may be suffering from sub-total or unstable lesions, and benefit greatly from PCI, as future infarction could be prevented.

## Identification of acute coronary occlusion in NSTEMI-ACS

Patients with STEMI are treated urgently with acute reperfusion, either mechanically (PCI) or pharmacologically (thrombolysis),<sup>75</sup> as they are believed to have acute coronary occlusion. Mortality increases if such treatment is delayed.<sup>16</sup> Patients with NSTEMI may also have acute coronary occlusions,<sup>76-78</sup> but many of these patients are not selected for urgent PCI because the occlusion is not detected by the ECG. Early identification and treatment of occluded arteries will undoubtedly reduce myocardial damage.

Previous analysis of the OUH cohort in this thesis has shown that 33 of 150 NSTEMI-ACS patients (22%) had acute coronary occlusions,<sup>23</sup> and that the different echocardiographic markers such as WMSI and global strain, could differentiate between patients with occluded, and patent infarct related arteries. However, both WMSI and global strain require good image quality, and experienced examiners. So in paper 1 we wanted to assess whether MAD, which is more easily acquired than WMSI and global strain, could also identify patients with acute coronary occlusion.

As stated above, MAD reflects LV systolic function and larger damage will ultimately lead to more decreased mitral annular movements. Occluded arteries result in greater LV damage, which in turn leads to a shorter systolic movement of the mitral annulus. In study 1, we showed that the subtle changes in myocardial function caused by coronary occlusion in patients with NSTEMI were detectable by MAD and GLS but not by LVEF. There were no significant differences between MAD and GLS in this sense, but none were excellent. However, MAD is easier to obtain and could therefore be a way to increase the likelihood of identifying patients with NSTEMI with coronary occlusions and thereby speed up the treatment of patients at higher risk. WMSI was also significantly different in patients with coronary occlusions ( $1.19 \pm 0.22$ ) and in those without occlusions ( $1.11 \pm 0.20$ ,  $p=0.04$ ), but the ROC curve

was so jagged, that further analysis for checking predictive value seemed futile, and was therefore abandoned.

As discussed earlier, DESL also reflects the extent of myocardial damage. Occluded arteries result in larger LV damage, and should in theory lead to a comparatively longer duration of the lengthening of the ischemic segments than non-occluded arteries. Our study showed that the changes in myocardial function caused by coronary occlusion in NSTEMI-ACS patients were detectable by DESL, GLS, and WMSI, but the performance of these indices were only modest in this context.

There are several potential explanations to why DESL did not perform better in predicting occlusions. Several of the patients without coronary occlusions still had significant stenosis that could lead to prolonged DESL. Furthermore, both echocardiography and angiography was performed a couple of days after the onset of NSTEMI-ACS. During this period, patients received anti-coagulation therapy that could have resolved the occlusions before they could be detected on angiography, but still have existed long enough to cause myocardial damage or stunning, and increased DESL. Both these factors could have resulted in false positives with regards to patients with occlusions. In addition, several patients had short DESL despite of having coronary occlusions. The reason could be that some patients had quite distant occlusions, resulting in lesser myocardial damage. Furthermore, one third of the patients with occlusions had only single vessel disease, also resulting in lesser myocardial damage. Both of these factors would lead to relatively shorter DESL, and hence false negatives.

Given the mentioned limitations, DESL is not really a robust enough marker for identifying coronary occlusions in NSTEMI-ACS patients, especially with peak troponin levels being available (see below).

It is also important to underline that in both study 1 and 2, echocardiography was performed a few days after the initial admission for NSTEMI-ACS. During this period, some of the occlusions might have resolved, either because of medical therapy or spontaneously, and this could have underestimated the true rate of coronary occlusions.

Worth noting is that in previous analyses, peak troponin levels could also differentiate between coronary occlusions and non-occlusions, and identify large infarcts.<sup>23,27</sup> This questions the role of echocardiographic markers at such a late stage of an acute coronary syndrome (as was the case in our study). Acute coronary occlusion is accompanied by very rapid alterations in myocardial function, and infarct expansion and oedema developing during the first hours may affect systolic function. Furthermore, a few days after the initial ischemic event (by the time echocardiography was performed in our studies), peak troponin levels are indeed available to the treating doctors, and can be of great help in identifying patients with large infarcts and occluded coronary arteries. Taking this into account, our findings don't show that echocardiography has incremental diagnostic value at such a late stage, with respect to coronary occlusions. However, the latest guidelines for the management of NSTEMI-ACS patients (which were published at a later time than our articles), recommend an early invasive strategy and angiographic clarification within 24 hours.<sup>24</sup> The role of different diagnostic and prognostic tools (both biochemical and imaging) in such a setting has previously been investigated.<sup>17</sup> In that study there was no significant difference between NSTEMI-ACS patients with or without coronary occlusions with respect to the 12 hour troponin levels, precluding its use as a predictor of coronary occlusion at an early stage. Territorial circumferential strain by two dimensional speckle tracking echocardiography, on the other hand, could predict

coronary occlusion with 90% sensitivity and 83% specificity and might therefore facilitate a more expedite invasive strategy in specific patients and if echocardiographic expertise is present. But as noted earlier, this method is dependent on operator experience. It would be interesting to see whether a simpler parameter as MAD could perform equally well in an acute setting. Given the fact that changes in myocardial velocities are detectable within seconds of the onset of ischemia, MAD could very be a contender to recon with. This, however, remains to be tested. Our findings can therefore not be automatically transferred to an early setting in which patients are first admitted for acute coronary syndrome.

### **Distinction between NSTEMI and stable angina**

Patients with known coronary artery disease are frequently admitted to hospitals with chest pain, but actual myocardial ischemia is often not the case. Still, medical therapy is usually initiated to be on the safe side, in anticipation of biomarker analysis. To mimic this setting, in study 1, we assessed the ability of various echocardiography parameters to differentiate between patients with stable angina and true NSTEMIs. Both MAD and GLS were accurate in making the distinction, while LVEF was not.

However, the clinical benefit of these findings is quite limited. The troponin results are usually available within 2-8 hours after admittance to the emergency room, and NSTEMI can either be confirmed or ruled out. In worst case, the patient is given unnecessary medical therapy for acute coronary syndrome for up to eight hours when decision making relies solely on biomarker analysis, but this very rarely poses any clinical threat. Furthermore, the NSTEMI group and the control group were not fully matched: The prevalence of diabetes and the use of cardiovascular medication were

higher in the NSTEMI group, than in the control group. The higher morbidity in the NSTEMI group could have affected the myocardial function, and exaggerated the differences to the control group. Furthermore, the proportion of female patients was considerably higher in the control group. Studies have shown that women tend to have better (more negative) strain values than men,<sup>79</sup> and this could also have exaggerated the measured differences between the NSTEMI group and the control group. These factors could have disturbed the results, and must be taken into account when interpreting the findings. Unfortunately, this was the population that was available to us at the time of the study.

### **Mechanisms of reduced myocardial function and increased mortality in patients with intestinal carcinoid disease**

Our study in patients with carcinoid intestinal disease, showed that liver metastases size, age, Activin A, LV strain and MAD were independent predictors of mortality in patients with carcinoid intestinal disease. The results also indicated worse baseline diastolic function in those who died, compared to those who survived during follow-up. Follow up echocardiography revealed a reduction in myocardial function by both LV and RV strain, as compared to baseline. The reduced long axis function of the left ventricle, and the disturbed diastolic function seen in our study is in keeping with changes seen in cardiac fibrosis. The patients with the highest levels of Activin A at baseline, had worse LV function at follow-up than those with the lowest Activin A values. Our findings further support a role for activin A in the involvement of cardiac function in CHD, and suggest that this fibrogenic cytokine, in addition to



myocardial systolic and diastolic function, should be evaluated in risk stratification in these patients.

Carcinoid intestinal disease has traditionally been believed to mainly disturb RV function. The reason was believed to be the deleterious effects of serotonin, produced by carcinoid hepatic metastases, and sent to the right side of the heart via the vena cava inferior,<sup>37,38,80,81</sup> thereafter being metabolized to its inactive form, 5-HIAA in the lungs, and not reaching the LV. This idea has been challenged by studies that have shown that serotonin does indeed reach the left side of the heart (even in the absence of valvular involvement),<sup>49</sup> and that many patients with high urinary levels of 5-HIAA have carcinoid involvement of the left side of the heart.<sup>48</sup> Serotonin may therefore not be the only factor required for the development of cardiac involvement, and studies have indeed demonstrated involvement of the left side of the heart.<sup>39,47</sup> Our study further supported these findings, and showed that LV function by strain echocardiography is significantly reduced in patients with carcinoid disease as compared to healthy individuals. More importantly, we also found that LV function at baseline, as assessed by strain echocardiography, but not as assessed by LVEF, was reduced in patients with carcinoid intestinal disease who died during follow-up, as compared to those who survived. This is in accordance with the notion that strain echocardiography is more sensitive than traditional markers of myocardial function.<sup>82-</sup>

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It must however be underlined that, although the changes in myocardial function by LV and RV strain were significantly different with respect to mortality, they were indeed subtle. The 95% confidence intervals of the strain values did not overlap, but the full ranges of values did. The significance of single estimates of

myocardial function may therefore be limited, and several examinations over time will provide a more robust assessment.

Carcinoid tumours produce Activin A, and we suggest that it may be an etiologic factor in the impairment of myocardial function. Activin A has a well documented role in fibrotic processes in various organs, including the heart.<sup>43,44,85,86</sup> It is expressed in some small neuroendocrine tumours,<sup>87,88</sup> and it is possible that these tumours secrete Activin A in to the circulation, where it can exert endocrine effects on a distant organ, such as the heart. However, elevated Activin A levels, both systemically and within the myocardium, have also been documented in patients with heart failure,<sup>89</sup> and a possible source of elevated Activin A in patients with CHD might be increased production in the fibrotic and failing myocardium, with spillover in to the bloodstream. A previous experimental model showed that neonatal rat cardiomyocytes that were exposed to Activin A, significantly increased gene expression of natriuretic peptides as well as TGF- $\beta$ 1, monocyte chemotactic protein (MCP-1), MMP-9, and TIMP-1, mediators all related to myocardial remodelling.<sup>89</sup> In our study, Activin A was

- (i) an independent predictor of death in patients with intestinal carcinoid disease,
- (ii) could identify patients with CHD at baseline, and
- (iii) to some extent predict future LV systolic dysfunction.

Activin A has previously been shown to correlate with disease severity in heart failure, and play an important role in the development of heart failure.<sup>89,90</sup> Even though the patients in our study with the highest Activin A developed more LV dysfunction, we failed to show a linear correlation between myocardial function by

LV strain echocardiography and Activin A levels. We believe that the reason for this is a somewhat small sample size in our study, and hence a type II error. Furthermore, the reduction in myocardial function was rather subtle, and the range of the strain values was quite narrow, making it difficult to demonstrate a linear correlation. One potential way of demonstrating a stronger correlation could be to perform histochemical analyses of cardiac pathology specimens from deceased patients. Such specimens were unavailable to us, but should be studied in forthcoming studies.

Two dimensional STE is a better measure of LV function compared with ejection fraction and wall motion score, and detects more subtle changes.<sup>72</sup> This is the case in patients with diabetes,<sup>91</sup> prediabetes,<sup>92</sup> and certain vasculitis diseases.<sup>93</sup> Microangiopathy is thought to be the cause. A similar mechanism could be partly responsible for the reduced myocardial function in patients with intestinal carcinoid disease, with raised levels of Activin A, and other inflammatory and fibrogenic substances, that have been related to the development of myocardial failure.<sup>94</sup> We suggest that the agents produced as a result of carcinoid disease, may reduce myocardial function both by exerting direct deleterious effect on the myocardium, and by disease of the cardiac microvasculature. This claim warrants further investigation.

We must underline that we do not believe that patients with intestinal carcinoid disease ultimately die of LV failure, but rather that the reduced myocardial function, and the related increased death rate, points towards a more severe underlying carcinoid disease.

In continuation of our group's previous work,<sup>39,46</sup> we wanted to focus especially on LV function by strain and Activin A as prognostic markers. Our study did however show that in univariate analyses, OPG, sTNF-R1, age, MAD and LMS were also good predictors of mortality. Ideally, we should have included and tested

these variables against each other in one single multivariate analysis. But due to the small sample size and few events (deaths), we could only include two variables in a multivariate analysis, and had to construct four different models. This led to an incomplete adjustment and assessment of the independent effect on mortality of the different variables. For example, how MAD performs as a predictor independent of age, LV strain, or LMS, is unanswered. The same can be said about other variables. Furthermore, only bivariate correlations were used for assessing collinearity before including two variables in a multivariate analysis. In posterity, however, we have performed additional multivariate analyses, and we have also calculated variance inflation factors (VIF) for all the variables, for a better assessment of multicollinearity. All VIFs were below 2.5, indicating low collinearity.

Additional multivariate analyses have shown the following: In a model with MAD and age, both remained predictors of mortality with HR of 2.7 (1.8 to 4.0,  $p < 0.001$ ) and 1.4 (1.0 to 1.9,  $p = 0.04$ ) respectively. Similarly with MAD and LMS: HR 2.5 (1.7 to 3.7,  $p < 0.001$ ) and 1.3 (1.1 to 1.5)  $p = 0.003$  respectively. MAD and OPG: HR 2.5 (1.7 to 3.7,  $p < 0.001$ ) and 1.5 (1.1 to 2.0,  $p = 0.004$ ) respectively. When OPG is tested with Activin, the latter no longer stays a predictor of death: HR 1.5 (1.1 to 2.0),  $p = 0.009$ ) and 1.2 (0.9 to 1.4,  $p = 0.156$ ) respectively. OPG with age: HR 1.5 (1.1 to 2.0,  $p = 0.004$ ) and 1.0 (0.8 to 1.5,  $p = 0.387$ ) respectively. MAD and LV strain had high correlation ( $r = 0.60$ ) and were not analyzed together. These results indicate that in this particular study MAD and OPG may be more robust predictors of death than LV strain and Activin A, and this should have been outlined in the paper.

OPG is a member of the tumor necrosis factor receptor superfamily, and is associated with inflammation, vascular calcification, endothelial function, and atherosclerosis.<sup>95</sup> Increased levels of OPG may predict cardiovascular disease and

mortality in both high risk and general populations,<sup>96,97</sup> and reflect the severity of coronary artery disease.<sup>98</sup> Recent studies have also shown that OPG is associated with future risk of myocardial infarction, ischemic stroke, total mortality, cardiovascular mortality, and nonvascular mortality, independent of traditional cardiovascular risk factors.<sup>97</sup> But whether OPG is increased in heart patients due to inflammatory processes in the cardiovascular system, i.e. the heart being the source of increased OPG production in these patients, or if the cardiovascular system is damaged by the high levels of circulating OPG due to other inflammatory processes in the body, remains unclear.<sup>97</sup> Patients with carcinoid intestinal disease carry a high inflammatory burden, and have significantly higher plasma levels of OPG, compared to healthy controls. The patients in our study had no history of cardiovascular disease, and it is therefore unlikely that the increased levels of OPG were a result of coronary disease. It could very well be that the reduced myocardial function observed in our study is a result of the possible deleterious effects of OPG on myocardial tissue, possibly through disease in the microvasculature of the myocardium. This claim warrants further research.

Why MAD seemingly faired better than LV strain in this study is less clear, as the latter is intuitively a more robust measure for LV function. One possible explanation might be the affection of the valvular apparatus in carcinoid disease, which might hamper the mitral excursion (measured by MAD) more than the overall LV function.

There are a few other limitations to our study that should be commented upon. Firstly, the number of patients and events are small, and the results must be verified in larger studies. There is also an issue of missing data: RV strain values were available in fewer individuals, than were other variables. Furthermore, for different reasons,

13% of the patients declined follow-up echocardiography exams. Both these factors can affect the results: A) Fewer RV strain values at baseline makes the assessment of the variable less robust. B) The number of dropouts may have biased the results, as one may opine that the reason for declining follow-up could be a poorer clinical condition in those particular patients. During the study and paper writing process, we opted to go ahead with the analyses of the data as it was; only analyzing cases with available values. But this reduces the statistical power. There are methods for dealing with missing data,<sup>99</sup> like imputing the missing data with replacement values (imputing the mean, regression substitution, imputing an assumed outcome such as worst case), applying single or multiple imputation methods, or using statistical models such as maximum likelihood estimation. Appropriate replacement of the missing data could have made the results more robust, but regretfully we were unfamiliar with such strategies at the time of the publication. In addition to the challenges with RV function assessment (discussed below), this further weakens the RV findings. The number of patients who developed CHD during follow-up was rather low, and the clinical interpretation of this finding must be done with caution. Risk factor data among the healthy controls are incomplete, and comparison with patients must be done with care.

Our findings can have implications for the management of patients with carcinoid intestinal disease. Currently, the main aim of therapy in patients with carcinoid syndrome is control of symptoms, such as diarrhoea and flushing. However, our study suggests that patients who exhibit systolic and/or diastolic dysfunction by echocardiography, or have increased levels of biomarkers, may have higher mortality risk. Identifying such a high risk cohort may help determine which patients may require closer monitoring, or more aggressive therapies.



## LIMITATIONS

### Echocardiography

All diagnostic ultrasound techniques are dependant of image quality and operator experience. Poor images and examinations performed by inexperienced operators may therefore influence the results considerably. Furthermore, traditional measures of myocardial function, LVEF and WMSI, are subjective and often less sensitive to subtle changes. In our studies, the reproducibility of LVEF was moderate, but quite good for WMSI, and comparable to that of MAD, GLS and DESL.

In study 1 and 2, the investigator performing offline analysis of the echocardiograms was blinded to the clinical outcomes as mortality, infarct size and coronary occlusion in order to reduce bias. But this does not remove subjectivity entirely. When an investigator sees an echo exam of a patient with obviously large myocardial damage, it may affect the way he or she performs the analysis. Also, the status of the case control participants (those without an ACS) was known to the analyst, and could have lead him or her to evaluate the myocardial function as better. Furthermore, in study 3, the investigator was *not* blinded to the mortality information, and this may also have affected the offline analysis.

### Limitations of tissue Doppler echocardiography

Doppler tissue imaging detects absolute myocardial velocity and is unable to discriminate passive motion (translation and tethering) from active motion (fiber shortening and lengthening). Therefore, the MAD-values in our studies may not necessarily reflect the true systolic excursion of the mitral annulus plane, but rather a sum of systolic shortening and passive motion of the heart. This affects the precision



of MAD. Furthermore, myocardial deformation is complex and three-dimensional, and longitudinal deformation assessment provides information only on parts of this process. It has previously been shown that MAD is not considerably affected by apical LV dysfunction. A normal MAD-value may therefore ignore apical myocardial damage, and underestimate the patient's actual condition and risk.

In study 3, MAD was based on only three or four mitral sites per patient (as opposed to five or six sites in study 1), potentially making the measurement less accurate. This should be taken in consideration when interpreting the findings of study 3.

### **Limitations of speckle-tracking echocardiography**

Feasibility is often mentioned as a limitation of 2D-STE. The speckles, used in the process of strain assessments, are created by constructive and destructive interferences of ultrasound backscattered from structures smaller than the ultrasound wavelength. Artifacts resembling speckles will influence the quality of speckle tracking. The method is sensitive to acoustic shadowing or reverberations, which may result in the underestimation of deformation. Reduced signal quality and suboptimal tracking may also create non-physiological strain traces. To avoid this, spatial smoothing and previous knowledge of physiologic LV function are used in tracking algorithms. This, however, may incorrectly show regional dysfunction of affect neighboring segmental strain values.

Speckle tracking is especially challenging in structures with thin wall, such as the RV free wall (paper 3). This is reflected in study 3 with weaker reproducibility of RV strain. In this case, the combination of a less robust variable, and the small

differences in mean values (for survivors and non-survivors), makes the findings uncertain. Bearing this in mind, the interpretation must be done with caution.

In the case of too many discarded segmental values, global strain might be inaccurate. The image quality was generally somewhat poorer in study 3, and 13% (LV) and 12% (RV) of the segments had to be excluded. This could have affected the overall results.

Although, more angle independent than tissue Doppler imaging, 2D-STE is not completely angle independent. Ultrasound images typically have better resolution along the ultrasound beam compared with directions at right angles. Therefore, speckle tracking works better for assessments of deformation in the direction along the ultrasound beam than in other directions. This property makes the use of longitudinal strain measurements particularly attractive, since myocardial motion is typically parallel with the ultrasound beam.

Non-physiological strain traces may also arise when morphologic details can't be tracked from one frame to the next due to out of plane motion of speckles. This phenomenon is a natural consequence of the three dimensional nature of myocardial deformation, as speckles move in three, not two dimensions.

Another limitation of 2D-STE is the differences among vendors, as they may have different methods for calculating strain. Our findings can therefore not be automatically transferred to all echocardiography systems. This challenge was avoided in this thesis, as all examinations were performed with GE scanners. We also noticed a slight difference in strain calculations between the different versions of the same software package, demonstrated in study 3. This must also be kept in mind when comparing strain values from different periods of time.

Changes in hemodynamic parameters such as preload, afterload and contractility may influence strain measurements. In study 2, for the assessment of DESL, strain echocardiography was performed during the acute phase of myocardial infarction, and again at follow-up several months later. Even though the patients were clinically stable at inclusion, left ventricular end-diastolic pressure can be higher in the acute setting of myocardial infarction, and affect and hamper strain values. But several months later (as was the case at follow-up), the end-diastolic pressure could be normalized due to revascularization and optimal medical therapy. Furthermore, previously undiagnosed and untreated hypertension (as is often the case at initial myocardial infarction), can also hamper strain values. But at follow-up, hypertension may be well-treated, and not affect myocardial strain in the same way. Both of these factors can potentially affect the observed differences in DESL at baseline and follow-up.

While LVEF and WMSI are common and well-established techniques in most cardiology centers, strain is still relatively new, and not widely used in clinical practice. Strain analysis can be time consuming and difficult to implement in a busy cardiology department, as often is the case in real life practice.

### **Assessment of right ventricular (RV) function**

Although, study 3 was not a specific RV study, a brief discussion on the echocardiographic assessment of the RV function is appropriate. The RV is a complex three-dimensional structure resembling a triangular pillow, making it quite distinct from the LV in both anatomical features and contractility patterns. The RV is very important in cardiopulmonary disease, but due to a strong focus on the left ventricle

and the lack of robust studies establishing normal reference echocardiographic values, a systematic examination of the RV is rarely performed. Current guidelines<sup>100</sup> recommend the use of multiple acoustic windows, and that the report should represent an assessment based on quantitative and qualitative parameters: Right ventricular and atrial sizes, as well as several measures of function, i.e. fractional area change, S', tricuspid annular plane systolic excursion, RV index of myocardial performance and systolic pulmonary artery pressure. Body surface area should also be taken in to account.

In our study, however, the assessment of RV function was based solely on the free wall strain values acquired from the apical four chamber view. This is insufficient, and in addition to the inherent weakness of 2D-STE performance on thin walled structures, the results must be interpreted with caution.

## **Inherent limitations of the statistical methods**

### **ROC curve analysis**

A diagnostic test may have a range of possible values, and one way of determining the cut-off for abnormality/disease is by using a ROC curve. By plotting 1 – specificity against sensitivity, one may compare the specificity and sensitivity for all possible cut-offs, and chose the most appropriate value for the particular clinical context. Sensitivity and specificity are inversely related. Changing the cut-off for sensitivity to improve the performance of the test, specificity is automatically reduced. The area under the ROC curve is often used as a measure of how well a variable predicts a given clinical outcome.

Ideally, a cut-off value should be obtained by ROC analysis in one patient population, and its accuracy be tested in a separate “test cohort” of patients in whom

reference standard results allow you to determine the number of true and false positive, and true and false negative results. By testing the cut-off in the same cohort in which it was derived from, you get a “best case” scenario, and the test may seem more accurate than it is in reality. In study 1, we therefore divided the entire patient population in two (randomly by the statistics software); cut-offs were acquired in one, and the accuracy tested in the second. However, this led to the sample sizes being small, and the end-points few, resulting in coarse and jagged ROC curves. This made it difficult to choose a correct cut-off. Furthermore, had the software selected a different sample of patients, both the curves and cut-offs could be quite different. This makes the findings in study 1 somewhat uncertain, and the results must be interpreted with caution.

In study 2 and study 3 we did not divide the cohorts, as the sample sizes would be too small (especially in study 3), and the accuracy of the different variables are tested within the same population as they were acquired from. This overestimates the performance of the different variables – offering a best case scenario - and interpretation must be done keeping this limitation in mind.

In order to increase the reliability of the optimal cut-offs, we used bootstrap re-sampling (1000 iterations) and presented the values with 95% confidence intervals. Although this method is recognized, it is somewhat controversial: The data of the sample are used to create a large set of new samples by randomly selecting patients from the original sample. In each new sample, some subjects appear twice or more, others not at all. The confidence intervals are therefore theoretically constructed, and not results of actual measurements.

## **Survival analyses**

A rule of thumb states that there should be a minimum of ten outcome events per variable (EPV) in multivariate Cox proportional hazards regression, and that fewer EPVs may worsen the accuracy and precision of the analysis.<sup>101,102</sup> In our studies, the sample sizes were small, and outcome events few. This allowed for a maximum of two variables in each multivariate regression analysis, and prevented us from fully exploring the confounding effects of multiple variables.

### **Study specific limitations**

In the first two studies, echocardiography was not performed immediately after the onset of symptoms but generally a few days after the initial event. By this time peak troponin levels are available in blood tests and can be used for assessing infarct size and distinguish between patients with coronary occlusions and non-occlusions. This questions the incremental diagnostic value of echocardiography at this stage of the acute coronary syndrome. The echo findings can however have an additive value, and influence the decision making, one way or the other. The diagnostic performance of MAD and DESL for identifying patients with occlusions or large infarcts should ideally be tested at an even earlier stage (i.e. immediately after initial admittance), as has been done previously for several other strain echocardiography variables,<sup>17</sup> when they might have incremental value over troponins. Our echo-findings did however predict mortality, and this is of value as it may identify patients with increased future risk, who require closer follow-up.

Also in our studies, medical therapy could have dissolved some of the thrombotic occlusions, and therefore we might have underestimated the true rate of acute coronary occlusions. Since prior myocardial infarction, wide QRS and atrial

fibrillation were exclusion criteria in the first two studies, the methods are not valid for patients with pre-existing systolic dysfunction or arrhythmia.

In study three, population size was small, and events few. Our results and claims must be tested in larger studies. Also due to the small study, the multivariate analyses in the published paper are incomplete, and the independent predictive value of the different variables was not fully explored. Although LV strain and Activin A were emphasized as important predictors, later analyses suggest that MAD and OPG may be more robust.

There is some missing data for RV function that reduces statistical power, and regretfully efforts to impute the missing values were not made. Risk factor data in the healthy controls are also incomplete.

## CONCLUSIONS

### General conclusions

Tissue Doppler imaging and 2D-strain echocardiography are moderately sensitive diagnostic and prognostic tools in patients with coronary artery disease and intestinal carcinoid disease with myocardial affection. They may be helpful in clinical decision making, but certain limitations must be kept in mind.

### Specific conclusions

- I. In NSTEMI patients, MAD and GLS may distinguish between large and small infarcts, are independent predictors of mortality, and may to some extent distinguish between coronary occlusion and non-occlusion. However, the availability of peak troponin levels make the value of echocardiography supportive, rather than incremental.
- II. MAD and GLS may distinguish NSTEMI-patients from those with significant and symptomatic coronary disease, but without infarction. But given the availability of rapid diagnostic cardiac troponins, the clinical value of this is limited.
- III. DESL by 2D-STE shows good correlation to final infarct size and a short DESL can accurately identify NSTEMI patients with minimal myocardial damage, as defined by lack of scarring on cardiac MRI.
- IV. Myocardial function by LV strain and MAD, and the biomarkers Activin A and OPG, are independently associated with mortality in patients with intestinal carcinoid disease. The data also show that these patients have a biventricular deterioration of myocardial function.





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