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**THIS THESIS IS BASED ON THE FOLLOWING PAPERS:**


**BACKGROUND**

**Acute myocardial infarction**

Coronary artery disease (CAD) is a major public health concern and contributes significantly to mortality and morbidity in all countries (1). CAD represents the progressive narrowing of coronary arteries from the build up of intramural plaque material. Luminal narrowing leads to decreased oxygen delivery to the cardiomyocytes which can result in angina symptoms and/or reduced cardiomyocyte contractility leading to overall depressed left ventricular function, potentially resulting in heart failure (HF)(2).

Acute coronary syndrome represents the sudden development of critical luminal narrowing or total occlusion in an epicardial coronary artery, due to the aggregation of platelets and fibrin formation caused by exposure of thrombogenic substances from the rupture of atherosclerotic plaques. The resulting disruption of coronary blood flow leads to critical perfusion deficits in the supplied myocardium resulting in either unstable angina pectoris without evidence of cardiomyocyte necrosis or acute myocardial infarction (MI). The final diagnosis of MI can be established when plasma levels of troponin exceed the lower diagnostic limit signifying cardiomyocyte necrosis (3).

The clinical presentation of acute MI ranges from ventricular arrhythmias and potentially sudden cardiac death over chest pain with minimal or no evidence of left ventricular (LV) dysfunction to cardiogenic shock due to the loss of a significant proportion of myocardial contractile function. In the acute phase of MI, the presence of ST-segment elevations (STEMI) or absence of these (non-STEMI) are the main criteria for decision making and triage indicating either an urgent need of reperfusion (STEMI) or initial medical stabilization (NSTEMI) with subsequent angiographic evaluation of coronary anatomy (CAG) (4,5).

**Normal left ventricular systolic function**

The LV myocardial architecture is organized into distinctive layers with the subendocardial fibers being longitudinally oriented; the midwall fibers circumferentially oriented and finally the subepicardial fibers also longitudinally oriented. The subendocardial fibers are helically oriented with an approximately +60° angle (counterclockwise) and the subepicardial fibers oriented with an average angle of -50° angle (clockwise).

The longitudinal fiber shortening together with circumferential midwall contraction, results in displacement of the base of the...
Left ventricular function after acute myocardial infarction

Loss of myocardial contractile tissue after acute MI leads to regionally impaired myocardial function in the infarcted area and depending on the extent of necrosis also globally depressed LVEF. Accordingly extensive wall motion abnormalities as well as reduced LVEF remain one of the most powerful short- and long term predictors of adverse outcome after acute MI (7, 8). Besides affecting overall LV function to a varying extent in the acute phase, regional myocardial dysfunction can also initiate a detrimental remodeling process in the longer term which involves not only fibrotic repair of the necrotic area, but also cardiomyocyte elongation in non-infarcted areas, chamber dilatation and reduction in LV ejection fraction (LVEF) (9, 10). Increasing wall stress as the LV dilates further a pathological process that, if left unchecked, ultimately leads to distorted LV geometry with a more spherical shape, clinical symptoms of heart failure (HF) due to reduced forward flow and pulmonary congestion and ultimately increased risk of death (11).

Central in this maladaptive cascade is the activation of the renin-angiotensin-system and autonomic nervous system both of which have been targeted in landmark trials that have dramatically improved the clinical course in patients with post MI systolic dysfunction (12-14). However, limited success has been achieved in trials with anti-remodeling pharmacotherapy targeting patients without significant LV systolic dysfunction and LVEF>40% (15). Thus, a method for identifying high risk individuals among patients with acute MI and LVEF > 40% could potentially be applied to identify patients with greater benefit from clinical monitoring, alterations in available therapies and as a selection criterion for future randomized clinical trials.

The ischemia resulting from critical coronary narrowing or total occlusion first affects the subendocardial myocardial fibers due to the transmural distribution of perfusion. The subendocardial myocardial fibers are thus at the center of the wave-front evolution of infarct and will be the first area to exhibit abnormal contractile function when ischemia occurs (16). Thus, abnormalities in longitudinal function can be detected before reductions in LVEF are apparent and importantly, the extent and magnitude of longitudinal fiber dysfunction reflects infarct size as assessed by cardiac magnetic resonance imaging (17, 18).

Apart from the direct effect of ischemic injury several co-morbid conditions including diabetes and hypertension, which may affect patients with MI, have also been associated with longitudinal myocardial fiber dysfunction (19, 20). Thus, potentially a global measure of longitudinal myocardial function may convey information on both acute ischemic injury as well as co-morbid conditions with adverse impact on myocardial systolic function.

Clinical heart failure after acute myocardial infarction

The occurrence of in-hospital HF in the acute phase of MI carries an ominous prognosis and is often preceded by abrupt loss of functioning myocardium (21). However, in-hospital HF also occurs in patients with apparently only minor myocardial injury and preserved or only moderately reduced LVEF and still carries a significantly increased risk of adverse outcome (22). The disconnect between overt LV systolic dysfunction and in-hospital HF has been attributed to impaired myocardial relaxation with patients developing in-hospital HF being more likely to suffer from co-morbid conditions such as diabetes, hypertension and diffuse coronary artery disease (23). Accordingly, patients with in-hospital HF despite absence of LV systolic dysfunction have been included alongside patients with reduced LVEF in trials targeting anti-remodeling therapy in high risk MI (24). In patients with clinical symptoms of HF despite preserved LVEF (HFpEF) abnormalities in longitudinal myocardial mechanics have been reported suggesting, that the discrepancy between near-normal LVEF and clinical symptoms may be partly explained by these indices (25).

However, limited evidence exists on the relationship between acute MI complicated by in-hospital HF and myocardial longitudinal function particularly in the group of patients without significantly decreased LVEF.

Neurohormonal activation in acute myocardial infarction

In patients with chronic systolic HF elevated secretion of neurohormonal peptides from the myocardium is a well recognized predictor of adverse outcome (26). Pro brain natriuretic peptide (proBNP) is secreted from cardiomyocytes in response to increased wall stress and ischemia. From the 108-amino acid precursor proBNP the biologically inactive N-terminal portion (NT-proBNP) is cleaved enzymatically from the bioactive BNP. NT-proBNP is more stable in plasma with a half-life of 120 minutes compared to BNP with a half-life of 20 minutes (27). The biological effect of BNP in vivo is multifaceted with diuretic effects dominating in the short term and anti-remodeling effects in the long term (28, 29). Accordingly, while levels of NT-proBNP are significantly elevated in HF the levels of bioactive BNP may be suppressed.

Several studies have demonstrated that elevated levels of NT-proBNP also contain independent prognostic information on adverse outcome across the spectrum of acute coronary syndrome (30-32). Elevated levels of NT-proBNP in acute MI are correlated with older age, burden of co-morbid conditions, extensive CAD and extent of LV necrosis. Furthermore, rapid induction of proBNP gene expression occurs in border zone surrounding the infarct region and proBNP mRNA in the subendocardial region has been shown to be up-regulated (33, 34).

The subendocardial longitudinal fibers have a smaller curvature compared to the midwall circumferential fibers. The law of LaPlace governing a three-dimensional shell demonstrates that a smaller curvature is related to larger wall stress given constant wall thickness. Thus in theory and as demonstrated in animal experiments, longitudinal subendocardial fiber function should be related to the release of natriuretic peptides (35). Limited literature exists however on the relationship between indices of longitudinal myocardial function and neurohormonal activation in acute MI, particularly in patients with relatively preserved LVEF.

Echocardiographic deformation analysis in acute MI

Risk evaluation after acute MI relies on assessment of global systolic function with LVEF and qualitative wall motion scoring as the most important parameters (36). Characterization of cardiac mechanical function using deformation imaging allows for a detailed quantitative assessment of longitudinal and circumferential fiber function. Impaired longitudinal deformation has been shown to contain prognostic value in STEMI populations (37, 38). Longitudinal deformation is impaired in a number of conditions includ-
Hypothesis: GLS as a measure of global longitudinal fiber function helps the planning of future randomized trials. Echocardiographic risk classification schemes could potentially improve our understanding of early hemodynamic deterioration. In patients with acute MI both overall and in patients with normal to moderately reduced LVEF.

**Study II:** Hypothesis: GLS is related to the occurrence of in-hospital HF independently of other markers of systolic and diastolic function, in patients with acute MI both overall and in patients with normal to moderately reduced LVEF.

**Study III:** Hypothesis: Impaired GLS identifies a group with increased long term risk of HF and death among patients with acute MI and normal to moderately reduced LVEF independently of traditional risk factors.

**MATERIALS AND METHODS**

Study I and II were performed in patients included at Righospitalet and study III was the result of a collaborative effort between Righospitalet and Gentofte Hospital. In the following section are summarized versions of the study design and patient population of study I-II together and study III separately. The echocardiographic methods were equal in all of the studies and are described in one common section. The specific statistical analyses differed somewhat between the studies and are described in separate sections for each of the three studies.

**Study design and patient population**

Detailed descriptions are available in the accompanying manuscripts. Below are summaries as detailed above.

**Study I and II**

We conducted a prospective study of patients referred to Righospitalet for invasive coronary angiography (CAG) due to either STEMI or non-STEMI between September 2009 and October 2010. The inclusion criteria were acute MI, age > 18 years and the exclusion criteria were inability to give written informed consent or life expectancy under 1 year due to non-cardiac conditions. Patients underwent echocardiography within 48 hours of admission to Righospitalet. Patients with non-STEMI were examined prior to CAG and patients with STEMI after CAG. Details regarding data collection of clinical history are given in manuscript I and II.

Peripheral samples of plasma were obtained within 24 hours of echocardiography; in patients with non-STEMI blood sampling was performed prior to the invasive procedure and in patients with STEMI after the invasive procedure. Analysis of NT-proBNP was performed with a commercially available assay (Roche Diagnostics, Mannheim, Germany) immediately after blood sampling. Routine clinical blood work was obtained simultaneously with the echocardiography, with the exception of troponin T (TnT) or troponin I (Tnl) where the peak value during hospitalization was used.

For the assessment of clinical heart failure during admission we evaluated patient charts for the occurrence of objective signs of HF and graded each patient according to the Killip classification scheme (4). If more than one episode of clinical HF occurred during the course of admission the patient was classified according to the highest Killip class. The results of NT-proBNP analyses as well as GLS were not available to the staff delivering care to the patients. Patients were classified as having in-hospital HF whether HF was present on admission or occurred during hospitalization however, the timing of HF was registered as well. Pa-
tients with severe aortic stenosis or atrial fibrillation (AF) or paced rhythm during the examination were not excluded from having echocardiography and blood sampling done however; they were excluded from subsequent echocardiographic deformation analyses.

**Study III**

This study was performed as a collaborative effort between Rigshospitalet and Gentofte Hospital in order to obtain a pre specified sample size of at least 1000 unselected patients with acute MI, to test the hypothesis, that GLS would predict outcome in patients with acute MI and only moderately reduced LVEF. Patients were included from September 2009 and ended at Gentofte in May 2010 and April 2011 at Rigshospitalet. The inclusion criteria were acute MI, age ≥ 18 years and the exclusion criteria were inability to provide written informed consent or life expectancy under 1 year due to non-cardiac conditions.

Patients underwent echocardiography within 48 hours of admission to Rigshospitalet or Gentofte Hospital. Patients with non-STEMI were examined prior to CAG and patients with STEMI after CAG. The details in relation to assessment of in-hospital HF and analysis of patients with AF, paced rhythm or aortic stenosis given above also pertain to this study. The primary outcome in the study was a composite of all cause mortality and hospitalization for HF requiring intravenous loop diuretics. The secondary endpoints were cardiac death and hospitalization due to HF. Endpoints were ascertained from hospital records and verified by an independent reviewer unknowing of echocardiographic information relating to the index MI. NT-proBNP levels were not included in the study due to the cessation of obtaining NT-proBNP measurements after December 2010 for administrative reasons.

**Echocardiography**

Echocardiography was performed prospectively during the period from September 2009 to May 2010 (Gentofte) and April 2011 (Rigshospitalet). Echocardiography was performed using a commercially available system (Vivid E9, GE Healthcare, Horten, Norway) with the MS5 transducer. Raw data were exported to an offline system equipped with EchoPac version BT11.1.0 (GE Healthcare, Horten, Norway).

Measurements performed in Echopac were exported into Microsoft Excel spreadsheets for each patient and subsequently imported into statistical software. All patients were assigned a code upon inclusion allowing for the blinded analysis by one single reviewer (ME). A pre specified echocardiographic protocol with specific attention towards obtaining 2D images suitable for STE analysis (Frame rates of 60-90) was followed in all patients. In case of patients not being in a condition allowing for echocardiography under normal circumstances in the Echolab, bedside examination was performed in the coronary care unit.

Details of the methodology for performing volumetric measurements including LVEF and left atrial maximum volume index (LAVi), Doppler indices and qualitative wall motion analysis (WMSI) are given in the 3 papers.

**Automated Function Imaging**

Myocardial deformation analysis was performed using a novel semi-automatic algorithm (Automated Function Imaging (AFI)) where each apical projection (apical long axis, 4-chamber and 2-chamber) was sequentially identified and followed by manual positioning in each projection of two annular points and one apical point. Aortic valve closure was measured prior to initiating the AFI algorithm from spectral continuous wave Doppler tracings of the LV outflow tract. The algorithm traced the endocardial border automatically and applied a ROI band across the myocardium. The myocardium was then tracked frame-by-frame in the longitudinal direction throughout the cardiac cycle and the software evaluated the tracking quality for each segment. The ROI was adjusted manually if needed to obtain optimal tracking and in case of unacceptable tracking the segment was excluded. Peak longitudinal systolic strain was calculated for each of the 17 segments and a global value incorporating all segments (GLS) reported along with a bulls eye plot of the strain distribution (Figure 1).

**Figure 1**

Example of strain curves from the apical 4-chamber projection with accompanying bulls-eye plot showing the regional distribution of strain.

Overall GLS value was reported automatically only if at least 5 of 6 segments in each projection were adequately tracked. In the case of GLS not being reported due to only one projection being excluded (2 or more segments in that projection with inadequate tracking) GLS was manually calculated from an average of the 2 remaining projections. If 2 projections were without adequate tracking of 2 or more segments the patient was categorized as having insufficient image quality for AFI measurements.

**Statistical Analysis**

All data are presented as mean ± standard deviation (SD) or median (Quartile 1-3) when appropriate. Categorical variables are presented as absolute values and percentages and compared using χ² test or Fishers exact test when indicated. Continuous variables were compared using Students t-test. All tests were two-sided and statistical significance defined as p<0.05.

Reproducibility analysis was performed in 20 randomly selected patients using the method described by Bland and Altman (39).

**Study I**

NT-proBNP was logarithmically (base=10) transformed to stabilize the variance prior to further regression modeling. Univariate association between log(NT-proBNP) and LVEF and GLS was evaluated with scatter plots and coefficients of correlation calculated. Modeling was performed in two separate instances; first the relationship between log(NT-proBNP) and GLS was explored over the whole range of LV systolic function including all patients...
in the study, and secondly, the relationship between log(NT-proBNP) and GLS was explored in the group of patients with LVEF > 45%.

The independent contribution of clinical, biochemical and echocardiographically obtained indices of systolic and diastolic function including GLS, towards explaining the level of log(NT-proBNP) (dependent variable) was analyzed in a general linear model. Overall model performance was assessed with the R² value. All variables with suspected clinical relevance were entered into the model with the subsequent parsimonious model obtained from backward elimination with p<0.1 as retention criterion. Interaction terms were entered into the model to analyze the potential modulating effect of important clinical and echocardiographic covariates on the relationship between GLS and log(NT-proBNP). Comparison between the strength of association between GLS versus NT-proBNP and LVEF versus NT-proBNP was examined in two separate univariate logistic regression models with upper versus lower quartile of NT-proBNP as the binary outcome variable. As a measure of the strength of association the C-statistic also known as the area under the receiver operating curve was calculated and compared between GLS versus NT-proBNP and LVEF versus NT-proBNP using the U-statistic proposed by DeLong et al (40). SAS version 9.2 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

**Study II**

Killip class 1-4 was dichotomized into Killip class 1 versus Killip class > 1 and modeled as the dependent variable under the term ‘in-hospital HF’ in logistic regression analysis. Stepwise multiple logistic regression analysis was performed with the inclusion of GLS, clinical, biochemical and echocardiographic indices with known or suspected relation to in-hospital HF. The incremental importance of GLS in relation to in-hospital HF was examined by first constructing a model containing clinical and biochemical information including log(NT-proBNP). Into this model were entered first LVEF and LAVI, secondly Doppler indices (MV deceleration time and E/e’) and finally GLS. Incremental model performance was assessed with decrease in -2 log likelihood and Akaike Information Criterion (Log likelihood penalized for the number of covariates in the model). Furthermore, direct comparison between LVEF and GLS with or without log(NT-proBNP) in relation to in-hospital HF was examined with C-statistics.

In patients with LVEF>40% two separate multiple logistic regression models were constructed with LVEF and GLS respectively. In both of the models age, peak TnT, log(NT-proBNP), episodes of AF and LAVI were entered as covariates since they were significant in the overall study population. Overall model performance as assessed with the C-statistic as well as the relative importance of each covariate in both of the models was analyzed.

Finally, in the overall study population we performed an internal validation of the stepwise modeling procedure using a bootstrap method. To this end, new datasets were regenrated by random replacement resulting in 200 new bootstrap samples with random combinations of the original observations. Multiple logistic regression analysis with all the covariates from the original model and stepwise elimination was performed in each bootstrap sample. Each of the models created in this process was then applied to and evaluated on the original dataset and in each instance the C-statistic was calculated. The average difference between the C-statistic of the model created in the original dataset and the C-statistic obtained from each run of the bootstrap regression models on the original dataset was calculated and considered a nearly unbiased estimate of the internal validity. Furthermore, in order to assess the stability of the stepwise modeling procedure and consistency of associations, the variables selected for final inclusion in the parsimonious model in each bootstrap sample were counted and reported (41). SAS version 9.2 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

**Study III**

The relationship between GLS and the primary composite outcome was evaluated using Cox proportional hazards regression modeling and the optimal cut-off value of GLS found by maximizing the partial likelihood. Two separate multivariable Cox models were created to evaluate the independent and relative importance of GLS in relation to the primary composite outcome. First, GLS was adjusted for age, diabetes, history of hypertension, Killip class>1, estimated glomerular filtration rate (eGFR), peak troponin level and infarct classification (STEMI/non-STEMI). Peak troponin measurements differed between Gentofte (troponin I) and Rigshospitalet (troponin T) and therefore both types of troponin were re-categorized as quartiles and merged into one covariate consisting of quartiles of peak troponin I or T. Secondly, GLS was adjusted for LAVI, E/e’ ratio, moderate to severe mitral regurgitation, LV mass index, LVEF and WMSI. The incremental value of adding GLS to the aforementioned covariates was assessed with -2 log likelihood and likelihood test. Assumptions of linearity and proportionality were assessed with the cumulated Martingale and Schoenfeld residuals, respectively. No violations of linearity or proportionality were found.

The secondary endpoints were analyzed with cause specific Cox regression models. Subsequent cumulative incidence curves were drawn allowing for the competing risk situation encountered, when cause specific endpoints are modeled. For the cumulative incidence curve for cardiac death the competing risk was death from all other causes, and for HF hospitalizations the competing risk was death from all causes.

Reclassification analysis with calculation of integrated diagnostic improvement (IDI) and net reclassification improvement (NRI) was performed for GLS when added to LVEF and WMSI (42). For NRI we identified arbitrary risk categories of 0-5%, 5-10% and >10%. Furthermore, IDI and NRI was evaluated when adding GLS above the cut-off value to a model consisting of Killip class > 1, diabetes, LVEF and WMSI. This was based on the assumption that these covariates carry the most impact in daily clinical decision making and risk stratification in patients with acute MI and LVEF>40%.

As noted previously, analysis of NT-proBNP was stopped in January 2011 and consequently a significant proportion of the patients reported in paper III did not have measurements of NT-proBNP for which reason NT-proBNP was omitted in the analyses. In this thesis however, an analysis of the population where both GLS and NT-proBNP were available, is provided to explore the relative prognostic value of both measures. Due to the smaller number of patients available in this analysis, the low number of cardiac events and non-existing predictive value of GLS in relation to non-cardiac death, a model is presented where the endpoint is a composite of cardiac death and HF hospitalizations with death from other causes as a competing risk.

All analyses were performed using R software (R development Core Team 2011, http://www.R-project/ ) including the following packages: “Survival”, “RiskRegression” and “Publish.”
SUMMARY OF RESULTS

Study I

Of 611 patients with acute MI a total of 548 were available for analysis in the study. Patients with LVEF>45% were less likely to have diabetes, known IHD, in hospital HF and multivessel disease. Baseline characteristics according to LVEF are given in manuscript. Median NT-proBNP in the overall study population was 115.5 pmol/L (Q1-Q3: 48.3-246.0) and in patients with LVEF> 45% 88.7 pmol/L (Q1-Q3: 36.1-170.0). Log(NT-proBNP) was significantly related to GLS (r=0.62, p<0.0001) and LVEF (r=-0.44, p<0.0001) in the overall study population, however the association between LVEF and log(NT-proBNP) was weaker. In the group of patients with LVEF>45% GLS remained significantly associated with log(NT-proBNP) (r=0.50, p<0.0001), whereas the association between LVEF and log(NT-proBNP) diminished further (r=-0.21, p<0.0001). Scatter plots of GLS and LVEF vs. log(NT-proBNP) both overall, in patients with LVEF>45% and grouped according to type of infarction are given in figure 2.

In multiple linear regression analysis GLS was found to be independently associated with log(NT-proBNP) after adjustment for age, sex, diabetes, hypertension, eGFR, peak troponin T, Killip class>1, type of infarction (STEMI/non-STEMI), LAD involvement, multivessel disease, LAVi, LVMi, E/e’ and MV deceleration time. The final parsimonious model included age, sex, eGFR, troponin T, MV deceleration time, E/e’ and GLS. A one unit increase in GLS (less negative) was estimated to result in an increase of NT-proBNP of 12.4% (95% confidence interval (95%CI); 10.3-14.5%) with all covariates in the model being constant. In the group of patients with LVEF>45% GLS remained significantly and independently related to log(NT-proBNP) along with age, sex, Killip class > 1 and troponin T.

In logistic regression analysis both GLS and LVEF were significantly associated with upper versus lower quartile NT-proBNP however, both overall and in patients with LVEF>45%, the associated C-statistics of the models with GLS (Overall: 0.86; LVEF>45%: 0.76) were significantly higher compared to the ones with LVEF (Overall: 0.75; LVEF>45%: 0.61).

Inter- and intra observer variability analysis revealed a non significant mean difference ± 2 SD for GLS of -0.7 ±2.5% and -0.5 ±1.3% respectively (Figure 3).
Study II
A total of 548 patients out of 611 patients with acute MI were available for analysis. In the overall study population 89 patients (16.2%) experienced in-hospital HF as assessed by Killip class > 1. Incident HF was encountered in 44 patients and HF on presentation in 45 patients. Patients with in-hospital HF had significantly impaired GLS (-10.1 ±3.3% vs. -14.5 ±3.5%, p<0.0001), lower LVEF (43.2 ±12.2% vs. 52.1 ±9.8%, p<0.0001) and higher WMSI (1.75 ±0.3% vs. 1.41 ±0.27%, p<0.0001). Among patients with in-hospital HF, 52 were in Killip class 2, 27 in Killip class 3 and 10 in Killip class 4. There was no significant difference in GLS between patients with incident HF and patients with HF on presentation (-10.3 ±4.0% vs. -9.8 ±2.8%, p=ns). In multiple logistic regression analysis GLS was independently associated with in-hospital HF after adjustment for age, sex, diabetes, known ischemic heart disease, known chronic HF, episodes of atrial fibrillation (AF), type of infarction (STEMI/non-STEMI), LAD involvement, multivessel disease, peak troponin T, log(NT-proBNP), eGFR, WMSI, LVEF, moderate to severe MR, E/e’, LAVi and MV deceleration time. The remaining parsimonious model included age, episodes of AF, peak troponin T, LAVi and GLS. Bootstrap validation of the stepwise modeling procedure revealed that GLS was the most robust measure associated with in-hospital HF.

In patients with LVEF>40% (n=464, 85%) 53 (11.2%) experienced in-hospital HF and exhibited significantly impaired GLS (-11.9% ±2.9% vs. -15.1% ±3.0%, p<0.0001). GLS was independently related to in-hospital HF also in this group when adjusting for age, peak troponin T, log(NT-proBNP), LAVi and episodes of AF. In a parallel model LVEF was entered instead of GLS and was non-significantly associated with in-hospital HF. Adding GLS to a model consisting of age, troponin T, log(NT-proBNP), LVEF, LAVi and episodes of AF increased the C-statistic significantly (0.82 [95%CI, 0.76-0.89] vs. 0.87 [95%CI, 0.82-.091], p=0.02) (Figure 4).

Study III
During the study period not all patients eligible for inclusion were successfully enrolled. The reason for these patients not being included were multi factorial and mainly due to logistic considerations. Importantly, no patients were excluded based on the severity or mildness of their cardiac status. During the inclusion period a total of 2282 patients (1279 NSTEMI and 1003 STEMI) were referred for CAG at Rigshospitalet (n=1873, 82%) and Gentofte Hospital (n=409, 18%) (Source: WebPATS). We included a total of 1110 patients (364 NSTEMI and 746 STEMI) corresponding to 48.6% of the total number of referred patients. However, there was a significant overweight of STEMI patients in our study compared to the referred patients. Of the 1110 patients with MI who were prospectively examined a total of 849 patients were included in this study (Figure 5).
During follow up (median 30.0 months Q1-Q3, 24.3-32.8) 57 patients (6.7%) reached the composite endpoint (42 patients died (5.0%) and 15 (2.0%) were hospitalized for HF). The secondary endpoint cardiac death occurred in 21 patients (2.5%). A cut-off value of -14% maximized the partial likelihood, and identified 373 patients of which a cumulative 7.5% and 10% experienced the composite endpoint by 12 and 24 months respectively. The remaining 475 patients experienced a cumulative of 2% and 3% endpoints by 12 and 24 months respectively (Figure 6).

In a multivariable Cox model GLS was significantly and independently associated with outcome after adjustment for age, diabetes, hypertension, Killip class > 1, quartiles of peak troponin, eGFR and infarct classification (HR, 1.14; 95%CI 1.04-1.26, p=0.007) and addition of GLS to the aforementioned covariates significantly improved the -2 log likelihood (p=0.006). When adjusting for LAVi, E/e', moderate to severe MR, LVMi and WMSi; GLS remained independently associated with the composite endpoint. In cause specific Cox models GLS was significantly associated with the secondary endpoints cardiac death (HR, 1.37; 95%CI 1.17-1.61, p=0.001) and hospitalizations for HF (HR, 1.52; 95%CI 1.23-1.87, p<0.001) and remained significant after multivariable adjustment. The cumulative incidence curve of HF hospitalization according to GLS> -14% versus GLS< -14% demonstrated significantly elevated risk with impaired GLS (Figure 8). The cumulative incidence curves of GLS> -14% versus GLS< -14% for cardiac death and non-cardiac death revealed that the prognostic power of GLS in relation to mortality was driven exclusively by cardiac death (Figure 8).
Reclassification analysis of adding GLS to LVEF and WMSI yielded a significant IDI (0.89%; \( p=0.012 \)) and NRI (0.20; \( p=0.014 \)) driven both by correct net upwards risk reclassification in patients with events (NRIevent\( =0.16; \ p=0.040 \)) and correct net downward reclassification (NRInon-event\( =-0.05; \ p=0.032 \)). When adding GLS\( >-14\% \) to a model consisting of Killip class\( >1 \), diabetes, LVEF and WMSI, significant increase in IDI occurred (0.82%, \( p=0.009 \)) whereas NRI was only borderline statistically significant (0.11, \( p=0.09 \)).

Of the 849 patients with LVEF\( >40\% \), 702 patients (83%) with 32 events (4.6%) (17 cardiac deaths and 15 HF admissions) also had measurements of NT-proBNP available. As expected, a significant univariate association existed between log(NT-proBNP) and the composite outcome (HR, 7.54; 95%CI 3.78-15.04, \( p<0.0001 \)). In multivariable analysis log(NT-proBNP) remained significant (HR4.41; 95%CI 1.95-9.92, \( p=0.0003 \)) after adjustment for age, Killip class\( >1 \), diabetes, LAVi and E/e’. Addition of GLS (HR, 1.26; 95%CI 1.09-1.46, \( p=0.002 \)) significantly improved the model performance (-2 log likelihood decrease from 368 to 358, \( p=0.001 \)), while attenuating the prognostic value of log(NT-proBNP) (HR, 2.57; 95%CI 1.08-6.09, \( p=0.03 \)).

Both GLS\( >-14\% \) (Figure 8 top) and NT-proBNP \( >200\, \text{pmol/L} \) (upper quartile) (Figure 8 middle) were significantly associated with cardiac death/HF, and NT-proBNP \( >200\, \text{pmol/L} \) also had a small borderline significant effect (\( p=0.08 \)) in relation to non-cardiac death, whereas GLS\( >-14\% \) was entirely neutral. The group of patients with both NT-proBNP level \( >200\, \text{pmol/L} \) and GLS\( >-14\% \) (\( n=128 \)) (Figure 8 bottom) suffered a cumulative of 22 cardiac deaths/HF hospitalizations (17.1%) during follow-up with an 11-fold increased risk (HR, 11.1; 95%CI 5.23-23.4, \( p<0.0001 \)) compared to those without both NT-proBNP\( >200\, \text{pmol/L} \) and GLS\( >-14\% \).

**DISCUSSION**

The major findings in this thesis can be summarized as follows: Quantitative analysis of myocardial longitudinal deformation with a fast semi-automatic speckle tracking algorithm, reveals systolic abnormalities in patients with acute MI that are closely associated with increased neurohormonal activation and in-hospital HF both overall and in patients with LVEF\( >40\% \). Furthermore, impaired longitudinal deformation is significantly and independently associated with adverse outcome in patients with LVEF\( >40\% \) and this association is entirely driven by cardiac death and hospitalizations for HF.

**GLOBAL LONGITUDINAL STRAIN IN RELATION TO NT-PROBNP:**

**STUDY I**

The correlation between GLS and NT-proBNP demonstrated in paper I suggest that there could be a pathophysiological coupling between impaired longitudinal fiber function and neurohormonal activation in acute MI. Subendocardial longitudinal fibers are exposed to higher levels of wall stress due to their smaller degree of curvature (35), are affected earlier in the ischemic process and are the site of increased genetic proBNP expression (34). All of these entities lend explanation towards the observed close correlations between GLS and NT-proBNP across the spectrum of LVEF. Another potential explanation could be that GLS discriminates well between small, medium and large infarcts as assessed by cardiac MRI or SPECT imaging (17, 18, 43). Therefore, the observed correlations across the spectrum of LVEF could reflect that NT-proBNP correlates well with infarct size. Two smaller studies demonstrated the association between infarct size and NT-proBNP using MRI (44, 45) however, there were considerable differences compared to our study, since only STEMI patients without in-hospital HF were included. We were not able to assess whether the association between GLS and NT-proBNP was independent of direct infarct size estimation by MRI. However, GLS was related to NT-proBNP independently peak troponin levels although we did not collect information on peak CKMB which is also closely related to infarct size.

Interestingly a measure of global long axis myocardial systolic velocity (\( s’ \)) did not correlate well with NT-proBNP, which could seem counter intuitive in light of the close correlation between GLS and NT-proBNP. One explanation could be that we only measured \( s’ \) in the lateral and medial annular regions whereas GLS is comprised of all myocardial segments. A previous large population study with 1012 subjects found significant associations between elevated levels of proBNP and \( s’ \) as well as the composite eas-index (\( e’ \) divided by the multiple of \( a’ \) and \( s’ \)) (46). The authors measured both \( e’, a’ \) and \( s’ \) in all 6 annular regions. However, direct comparison with our study is difficult, since the
absolute proBNP values were low due to an entirely different population sample of healthy people. Also no direct correlations between continuous TDI measures and proBNP were reported. The ability of deformation based indices to better reflect overall systolic dysfunction compared to annular velocities was demonstrated in a study by Carluccio et al., where TDI based GLS had better discriminative power than mean s’ from 4 sites in separating patients with HFpEF from normal controls.

The results in paper I demonstrates that the association between GLS and elevated levels of NT-proBNP is significant and generates the hypothesis that the association between elevated levels of NT-proBNP and outcome could be attenuated by inclusion of GLS. However, the study was insufficiently dimensioned to address this issue due to a low number of events and limited follow-up time.

The results of the logistic regression analyses and ROC curves in paper I should be interpreted with caution and in the context of the overall aim of the study, namely that GLS is related to neurohormonal activation both overall and in patients with preserved LVEF. The separation of NT-proBNP into binary outcome categories (upper versus lower quartiles) is arbitrary and should not lead the reader to infer any diagnostic considerations, which are usually associated with the use of ROC curves. Rather, the association between GLS and binary NT-proBNP categories can be used in the context of prior outcome based studies in acute MI, which have consistently demonstrated the powerful prognostic value of elevated NT-proBNP in itself and its significant added value to LVEF (47). The stronger correlation between GLS and log NT-proBNP in patients with LVEF<45 compared LVEF and log NT-proBNP is to be expected when truncating the range of LVEF. Thus, the comparison between r-values of GLS/log NT-proBNP and LVEF/log NT-proBNP in the group of patients with LVEF> 45% should be interpreted with caution. Rather, the sustained strong correlation between GLS and log NT-proBNP in patients with LVEF>45% serves to demonstrate that elevated levels of NT-proBNP can be explained in the context of poor LV longitudinal function.

**Limitations: Study I**

A major limitation in this study was that NT-proBNP measurements were not performed on blood samples taken at one standardized time point in all patients. Since blood sampling was performed within 24 hours of echocardiographic examination one can speculate that fluctuations in NT-proBNP within that time frame could impact the results.

Another significant limitation pertains to the pooling of STEMI and non-STEMI patients in this study. The echocardiography was performed before CAG in non-STEMI patients as opposed to STEMI patients where it was performed after primary PCI. This difference could impact our findings however, due to ethical and practical reasons echocardiography in STEMI patients had to be performed after primary PCI. In the case of non-STEMI, the organization of care with fast track management of patients from referral institutions and early discharge meant that post procedural echocardiography was not feasible.

**GLOBAL LONGITUDINAL STRAIN IN RELATION TO IN-HOSPITAL HF: STUDY II**

Clinical HF complicating acute MI has rightly been termed ‘a deadly intersection’ (48) since it carries a poor prognosis irrespective of the magnitude of impairment in LVEF (22). Previous consecutive studies conducted prior to the widespread implementation of mechanical reperfusion therapy documented incidences of HF complicating acute MI in the range of 45% (22). The incidence of this complication has seen a decline over the past decade towards a range between 15-25% (49, 50) although estimates vary. Although clinical HF complicating acute MI is usually accompanied by a significant loss of viable myocardium, this is not always the case. A recent study demonstrated in a very large population, that 50-60% of patients with HF complicating acute MI have an LVEF>40% (51). Impaired myocardial relaxation as assessed by diastolic function parameters such as elevated E'/e', enlarged LA and shortened MV deceleration time have usually been the major echocardiographic criteria used to explain HF complicating acute MI in the absence of overtly reduced LVEF (23). However, in patients with HFpEF where LVEF is near-normal in the presence of clinical symptoms of HF, global myocardial deformation indices have been shown to exhibit distinct abnormalities providing evidence, that even though LVEF is near-normal, this may not necessarily be the case for longitudinal systolic strain (25, 52).

In paper II we provide evidence to suggest that GLS is the most important covariate in relation to in-hospital HF complicating acute MI in our dataset and this association was consistent both overall and in the large group of patients with LVEF<40%. The significant prognostic information carried by clinical HF even in the presence of a near normal LVEF, is underscored by the fact that trials with high risk MI patients have used this as an inclusion criterion on equal terms with significant LV dysfunction (echocardiographic LVEF<35%) (24). Thus, in demonstrating that impaired GLS is significantly associated with in-hospital HF in patients with LVEF<40% we provide evidence to support the hypothesis, that impaired GLS in patients with LVEF>40% with or without HF may have important prognostic information.

**Limitations: Study II**

A number of significant limitations apply to this study. First and foremost, we have equated HF present at admission with HF occurring during hospitalization and the echocardiographic findings are not obtained at a standardized time point in relation to the HF episode. There is evidence to support, that HF occurring during hospitalization may have greater adverse prognostic impact compared to HF on presentation although this association was less clear for patients with STEMI (21). We did not find significant differences in echocardiographic measures when comparing patients with HF on presentation versus incident HF. However, the overall number of patients with HF in our study was small and therefore we could potentially overlook a real difference.

Since the echocardiographic examination was not necessarily performed prior to the HF episodes we cannot draw any reasonable conclusions on the predictive power of GLS in relation to early HF. For this reason the ROC curves should be interpreted with caution and only be used in the context of demonstrating the relative strength of association between the covariates and the occurrence of HF episodes. There is limited literature on the time course of echocardiographic measures of LV systolic function in relation to the evolution and remission of clinical HF. One study demonstrated that LVEF did not change significantly from the time of the acute episode of HF to the time of clinical improvement (53). However more studies on the time course of deformation parameters from the diagnosis of acute HF to clinical remission are needed.

Finally, the assessment of HF was based on observations and hospital records in the semi-intensive setting of the coronary care.
Global Longitudinal Strain in Relation to Outcome: Study III

Paper III is the first large scale echocardiographic study in patients with acute MI and LVEF > 40% to examine the prognostic importance of GLS in relation to outcome. Overall absolute risk of events was low due to the a priori selection of a low risk population. Nevertheless patients with LVEF > 40% constituted nearly 85% of the total MI population included during the study period. This underscores the temporal change in the composition of contemporary MI populations when compared to older studies such as the Bucindolol Evaluation in Acute Myocardial Infarction Trial (BEAT) (22) and Trandolapril Cardiac Evaluation (TRACE) (54) consecutive registries where LVEF < 40% was seen in approximately 67% and 60% respectively. One can speculate that this trend reflects the widespread adoption of mechanical reperfusion therapy, timely intervention planned and initiated with pre-hospital assistance as well as advances in anti thrombotic therapy. However, proper large scale epidemiological studies demonstrating the temporal trend in discharge LVEF over the past decade remain to be conducted.

The results in paper III and the supplemental data presented in this thesis extend on the findings of paper I and II in that GLS besides the close relationship with acute markers of hemodynamic deterioration, provides important long term prognostic information in relation to death and heart failure. The prognostic power of GLS was driven by new episodes of HF and cardiac death, whereas no relation was seen with recurrent MI or non-cardiac death. The relationship between GLS and HF hospitalization is readily interpreted in the context of paper II, in that LV longitudinal dysfunction even with preserved LVEF seems to be linked closely with the propensity to develop acute hemodynamic deterioration. This is also in accordance with the previously mentioned studies in HfPEF (25, 52) although caution must be exerted in comparing post-MI patients having wall motion abnormalities with HfPEF patients.

The connection between cardiac death and impaired GLS is intriguing although the absolute number of events is low as reflected by the wide confidence limits on the estimated HRs. There is however some evidence to suggest that the risk of malignant ventricular arrhythmias either manifested by sudden cardiac death or proper IC discharge is increased with abnormal LV deformation patterns. Such patterns have been described in patients with long QT syndrome (55), arrhythmogenic RV cardiomyopathy (56) and in chronic HF patients followed up after ICD implantation (57).

In all of these studies the authors quantified the time dispersion of the occurrence of segmental LV peak negative strain which provided novel insight into potential mechanisms for malignant ventricular arrhythmias. In the case of patients with chronic HF with or without ischemic etiology (57) it is conceivable that the observed increase in mechanical dispersion to some extent must be accompanied by the presence of a significant proportion segments exhibiting post systolic shortening. Thus, abnormal contraction patterns with some segments exhibiting post systolic shortening with or without complete or partial systolic stretching would be accompanied by impaired GLS. Another study examined the impact of peri-infarct zone strain in chronic ischemic HF patients undergoing ICD implantation for primary prevention (58) and found that the magnitude of strain impairment in the segments neighboring the scar region defined as strain > -4.5% predicted outcome. Again, impaired regional strain surrounding the author-defined scar region would also be accompanied by impaired GLS. We did not report detailed analyses on regional strain values or global strain patterns. However, it is possible that these measures could more accurately reflect the propensity for sudden cardiac death than GLS in a population with acute MI and LVEF > 40%.

Several other studies have demonstrated prognostic importance of GLS in patients with acute MI. In a study of 659 patients, Antoni et al. demonstrated the prognostic power of GLS in STEMI patients (37). Impressively, the authors managed to demonstrate that both GLS, infarct zone strain and infarct zone strain rate were independent predictors of outcome in the same multivariable model despite the presumed high co linearity between these parameters. The authors also managed to calculate GLS in all the available patients without having to exclude studies due to poor image quality. In another study Munk et al. also demonstrated that GLS predicted outcome in patients with STEMI albeit with a somewhat lower rate of success in obtaining GLS (74% of patients) compared to the Antoni study (38) which can possibly be explained by technical differences between the three smaller studies of which the study was based. The feasibility in obtaining GLS reported in our study (95% of patients) places it somewhere between the 100% success rate of Antoni et al. and the feasibility of Munk et al. Another important difference is the composition of the endpoint, where re infarction (Munk et al. and Antoni et al.) and revascularization (Antoni et al.) were included in the composite endpoints. We did not find any significant association between new infarcts and GLS or other markers of systolic dysfunction, whereas diabetes was highly significant. Furthermore, as discussed in detail by Munk et al. the inclusion of elective revascularization as an endpoint is problematic since angiographic follow up will often be scheduled at the baseline procedure in the presence of complex coronary disease.

Although NT-proBNP was not included in paper III data is provided in this thesis to support the argument, that GLS is independently prognostic of NT-proBNP in patients with acute MI and LVEF > 40%. Furthermore, GLS attenuated the value of NT-proBNP by reducing the HR associated with a factor 10 increase in NT-proBNP by almost 50%. This suggests that the strong prognostic association observed by adding NT-proBNP to LVEF in prior studies may be weakened considerably by obtaining GLS. Furthermore, combining GLS and NT-proBNP in the group of patients with acute MI and LVEF > 40% allowed for the selection of a smaller proportion of patients with significantly worse outcome.

Limitations: Study III

There are several limitations to paper III. We included both STEMI and non-STEMI patients in the study and echocardiographic examinations were performed after CAG in STEMI patients whereas in non-STEMI patients it was performed prior to CAG. The reasons for this are detailed under the limitations pertaining to study I. While no evidence exists to support that LV dysfunction has differential prognostic importance in STEMI compared to non-STEMI, it is possible with the overweight of STEMI patients in this study impacted on our results and therefore limits the external validity in respect to non-STEMI patients. However, Infarct type was not associated with outcome in either univariable or multivariable analysis and did not modulate the effect of GLS in inter-
action analysis. We used an echocardiographic platform from one vendor only and it is possible that feasibility and results would differ if another platform had been utilized. However, a recent study demonstrated that GLS was robust across different platforms and types of proprietary software as opposed to strain in the circumferential and radial directions (59).

The statistical models utilized in paper III suffer from some degree of over fitting due to the low number of events which means that the results should be interpreted with caution in regard to future prediction of individual patient risk. We only managed to include approximately 50% of the patients potentially meeting the inclusion criteria during the study period. The fact that the study was not consecutive could introduce bias due to the potential differences between our study population and the patients we did not manage to include.

CONCLUSIONS

Study I
Plasma levels of NT-proBNP in the acute phase of MI are more accurately reflected by longitudinal myocardial deformation assessed by GLS compared with LVEF. In patients with preserved LVEF and elevated levels of NT-proBNP in the acute phase of MI, particular attention should be paid to LV longitudinal dysfunction.

Study II
The results of this study demonstrate that GLS is significantly impaired in patients with MI complicated by in-hospital HF. Measurements of GLS was superior to LVEF and NT-proBNP in providing information about myocardial dysfunction, suggesting that GLS is a powerful marker of hemodynamic deterioration in patients with MI. In patients with preserved LVEF, GLS provided more information than NT-proBNP in relation to in-hospital HF.

Study III
Semi-automated calculation of GLS is significantly related to all-cause mortality or HF admissions in patients with MI and LVEF > 40% above and beyond traditional identifiers of high risk such as diabetes and clinical heart failure.

Abbreviations

(Forkortelser)
CAD = Coronary artery disease
HF = Heart failure
MI = Myocardial Infarction
LV = Left ventricle
STEMI = ST segment elevation myocardial infarction
CAG = Coronary angiography
LVEF = Left ventricular ejection fraction
NT-proBNP = N-terminal pro brain natriuretic peptide
STE = Speckle tracking echocardiography
GLS = Global longitudinal strain
WMSI = Wall motion scoring index
LAVI = Left atrial volume index

SUMMARY

Background
Systolic dysfunction, clinical heart failure and elevated levels of neurohormonal peptides are major predictors of adverse outcome after acute myocardial infarction (MI). In the present thesis we evaluated global longitudinal strain (GLS) in patients with acute MI in relation to neurohormonal activation, in-hospital heart failure and prognosis with specific attention to the group of patients with preserved LVEF that currently do not meet the criteria for anti remodeling therapies.

Results
GLS was found to be significantly associated with neurohormonal activation as assessed by NT-proBNP levels and that this association was present also in patients with preserved LVEF. Patients with clinical HF during hospitalization for acute MI had significantly poorer GLS compared to controls and this relationship was robust when adjusting for known factors associated with elevated LV filling pressure such as left atrial volume and E/e’ ratio. Furthermore, measurement of GLS attenuated the value of NT-proBNP in relation to in-hospital HF in patients with preserved LVEF. Finally, GLS was related to outcome in the largest ever echocardiographic deformation study of patients with acute MI and relatively preserved LVEF. We found that GLS predicted mortality and heart failure admissions and that the effect on mortality was driven by a significantly increased risk of cardiac death in patients with impaired GLS.

Conclusions
In conclusion, the results of this thesis demonstrate that GLS as a measure of LV systolic function is significantly related to elevated neurohormonal activation, early hemodynamic deterioration and predict adverse outcome in a low risk population without indications for anti remodeling therapies. Early measurement of GLS in this population could be used as a risk stratification tool for added monitoring and clinical trials.

LITERATURE


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