Detection of subtle myocardial alterations by echocardiographic techniques for improved prognostic information in patients with heart disease

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LIST OF PAPERS

1. **Right ventricular mechanical dispersion is related to malignant arrhythmias: a study of patients with arrhythmogenic right ventricular cardiomyopathy and subclinical right ventricular dysfunction.**

   Sarvari SI, Haugaa KH, Anfinsen OG, Leren TP, Smiseth OA, Kongsgaard E, Amlie JP, Edvardsen T.

   Eur Heart J 2011; 32:1089-96.

2. **Early Postoperative Left Ventricular Function by Echocardiographic Strain is a Predictor of 1-Year Mortality in Heart Transplant Recipients.**

   Sarvari SI, Gjesdal O, Gude E, Arora S, Andreassen AK, Gullestad L, Geiran O, Edvardsen T.

   J Am Soc Echocardiogr 2012; 25:1007-14.

3. **Layer-Specific Quantification of Myocardial Deformation by Strain Echocardiography May Reveal Significant Coronary Artery Disease in Patients with Non-ST Elevation Acute Coronary Syndrome.**

   Sarvari SI, Haugaa KH, Zahid W, Bendz B, Aakhus S, Aaberge L, Edvardsen T.

   J Am Coll Cardiol Img 2013; 6:535-44.

SELECTED ABBREVIATIONS

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

ACS, acute coronary syndromes;

ARVC, arrhythmogenic right ventricular cardiomyopathy;

AUC, area under the curve;

CAD, coronary artery disease;

CD, contraction duration;

EF, ejection fraction;

FFR, fractional flow reserve;

GCS, global circumferential strain;

GLS, global longitudinal strain;

HTx, heart transplantation;

IQR, interquartile range;

LV, left ventricle;

MRI, magnetic resonance imaging;

NSTE, non–ST elevation;

PVR, pulmonary vascular resistance;

ROC, receiver-operating characteristic;

RV, right ventricle;

RVOT, right ventricular outflow tract;

RVFAC, right ventricular fractional area change;

SAECG, signal-averaged electrocardiography;

TLS, territorial longitudinal strain

WMSI, wall motion score index

INTRODUCTION

Background

Global left ventricular (LV) systolic function, most commonly assessed by echocardiographic ejection fraction (EF), is an important predictor of outcome.[1] Although it is commonly used to evaluate ventricular function, the measurement of EF presents a number of challenges related to image quality, assumptions of ventricular geometry, expertise and is limited to assessing changes in ventricular cavity size during the cardiac cycle. The traditional volume measurement-based echocardiographic parameters are indirect means of assessing myocardial function, and are insensitive to early changes in cardiomyopathy.[2] Furthermore, the assessment of global LV function by EF is based on 2 apical views only, and might underestimate deterioration in regional LV function. Echocardiographic assessment of EF is easily available and feasible, but does not provide information on segmental and layer-specific ventricular function. Increased compensatory contractions in healthy myocardial regions may result in only minor alterations in EF, and may conceal large areas with impaired function. Echocardiographic techniques allow the assessment of strain, a measure of myocardial deformation, an intrinsic mechanical property, that measures myocardial systolic function more directly compared to conventional cavity-based echocardiographic parameters.[3] It is a dimensionless measure of the deformation that occurs upon application of stress. It represents the fractional or percentage change between the unstressed and stressed dimension. Furthermore, recent software allows separate evaluation of endocardial, mid-myocardial and epicardial myocardial deformation.

In this thesis, we evaluated the ability of strain echocardiographic techniques to assess subtle myocardial alterations in order to improve prognostic information in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC), in heart transplant (HTx) recipients and in patients with non-ST elevation (NSTE) acute coronary syndrome (ACS).

Ventricular function and mechanical dispersion in ARVC

ARVC is a chronic, progressive, heritable cardiomyopathy and is one of the leading causes of sudden unexpected cardiac death in previously healthy young individuals.[4] It was initially recognized as a disease of the right ventricle (RV), but involvement of the left ventricle (LV) is now commonly recognized.[5-7] Four clinical stages have been documented: an early concealed phase, overt electrical disorder, isolated right heart failure, and biventricular pump failure.[8]

Recent molecular genetic reports have revealed ARVC as mainly an autosomal dominant inherited desmosomal disease,[9] leading to progressive loss of cardiac myocytes, followed by fibro-fatty replacement. Penetrance is age and gender dependent and the progressive clinical picture is highly variable.[10] Importantly, life-threatening arrhythmia may be the first clinical sign and may occur with only discrete or even absent myocardial structural changes.[11, 12] Risk stratification of so far asymptomatic mutation carriers is therefore challenging.

Echocardiographic studies of the RV in ARVC patients have shown that RV dilatation and reduced regional or global RV function are traits of the disease. Quantitative assessment of RV function is difficult due to complicated anatomy and load dependency. Reviewed guidelines for diagnosing ARVC from 2010 have improved quantitative assessment of RV dysfunction, including measures of RVOT and RV area.[13]

Electrical conduction delay with consequent electrical dispersion has been suggested as a mechanism of ventricular arrhythmia in ARVC patients.[14, 15] Mechanical dispersion (heterogeneous contraction) can be assessed by strain echocardiography and may reflect electrical dispersion. Mechanical dispersion has recently been demonstrated to relate to malignant ventricular arrhythmias in long QT syndrome,[16] after myocardial infarction,[17] and in dilated cardiomyopathy.[18]

The first clinical symptom in ARVC patients may present as life-threatening arrhythmia. Unfortunately, there is no single test to diagnose or exclude ARVC and to predict arrhythmias. Therefore, there is a need for methods identifying patients with increased risk for ventricular arrhythmias.

In the first paper, we focused on the prediction of arrhythmias in patients with ARVC and assessment of LV and RV function in asymptomatic mutation carriers and ARVC patients. The presence of malignant arrhythmias and reduced LV and RV function are important prognostic factors in these patients.

Ventricular function in HTx recipients

End-stage heart failure is a significant problem in the Western world. Cardiac transplantation remains the gold standard therapy for patients with end-stage heart failure, with 1-year survival approaching 90%.[19] Graft dysfunction is a major cause of morbidity and mortality in HTx recipients.[20] Advances in the treatment of antibody mediated rejection and acute cellular rejection have increased early transplant survival.[19] However, not all cases of cardiac allograft dysfunction can be explained by the currently known histopathologic mechanisms of allograft rejection.[21]

The search for non-invasive techniques to assess cardiac allograft function remains a priority objective for HTx professionals. Global LV systolic function, most commonly assessed by echocardiographic EF has, however, several limitations as described earlier. In addition, EF tends to be stable over time and do not always correlate with biopsy-proven rejection or time after transplantation.[22]

Although LV global longitudinal strain (LVGLS) has been used in a growing number of clinical conditions, the association between reduced LVGLS and risk for mortality in HTx recipients is uncertain.

Ventricular function in patients with CAD

The clinical presentation of coronary artery disease (CAD) varies from silent ischemia, stable angina pectoris to ACS and death. ACS comprises NSTE-ACS and ST-elevation myocardial infarction (STEMI). The presence of ST-elevation typically represents coronary occlusion requiring acute reperfusion therapy. In contrast, patients with suspected NSTE-ACS have more heterogeneous findings on coronary angiography. Coronary occlusion and/or significant stenosis may or may not be present, however, revascularization therapy is performed in about two thirds of these patients.[23]

The LV wall of the heart comprises an endocardial, a mid-myocardial and an epicardial layer. Of these 3 layers, the endocardium undergoes the greatest deformation during systole, including thickening and shortening, and is most susceptible to ischemic injury.[24-26] Recent software allows separate evaluation of endocardial, mid-myocardial and epicardial myocardial deformation. Careful evaluation of these layers might increase the diagnostic accuracy of CAD in patients with NSTE-ACS.

In this thesis, patients from the above mentioned categories were included with the overall aim of improving assessment of ventricular function by echocardiographic techniques. Detection of subtle myocardial alterations with these techniques may facilitate more accurate prediction of prognosis and may guide treatment that might improve prognosis in these patients.

AIMS OF THE THESIS

General aim

To assess ventricular function by strain echocardiography in patients with ARVC, HTx recipients and patients with significant CAD, in order to facilitate early detection of subtle myocardial alterations in these patients.

Specific aims

I. To evaluate if pronounced RV and LV mechanical dispersion by myocardial strain is associated with susceptibility to ventricular arrhythmia in patients with ARVC and therefore may be an additional tool for arrhythmia risk stratification (Paper 1).

II. To investigate to what extent LV function assessed by strain echocardiography was reduced along with RV function in patients with ARVC diagnosis and in asymptomatic mutation carriers (Paper 1).

III. To evaluate to what extent reduced LV function assessed by GLS, 1-3 weeks after HTx is associated with increased 1-year mortality, compared to other more established prognostic factors, such as recipient and donor age, renal function and right heart catheterization parameters (Paper 2).

IV. To evaluate if endo-, mid- and epicardial deformation assessed by layer-specific strain echocardiography could identify significant CAD in patients with suspected NSTE-ACS (Paper 3).

MATERIAL

Study population (Paper 1)

In total, 69 patients were included. Of these, 42 were index patients referred to our centre for ventricular tachycardia (VT) or ventricular fibrillation (VF) evaluation and in whom the diagnosis of ARVC was originally made based on the International Task Force criteria from 1994. After the revision of the International Task Force criteria in 2010,[13] the diagnosis of all ARVC patients was reviewed. First degree relatives of mutation positive ARVC index patients underwent cascade genetic screening. Of all screened individuals, 27 tested positive for the ARVC mutation found in the index patient. Importantly, none of the included mutation positive family members had symptoms of the disease in terms of palpitations, syncopes, arrhythmias or heart failure and were defined as asymptomatic mutation carriers.

ARVC related mutations were confirmed in 54 (78%) of all patients, [47 (68%) *PKP2*, 6 (9%) *DSP* and 1 (1%) *RYR2*]. In ARVC patients with arrhythmias, 27 (64%) had ARVC related mutations [23 (55%) *PKP2*, 3 (7%) *DSP* and 1 (2%) *RYR2*]. No mutations were found in 15 (36%) ARVC patients. In asymptomatic mutation carriers 24 (89%) had *PKP2* and 3 (11%) had *DSP* mutations.

The control group consisted of 30 healthy individuals from the hospital staff. Ten healthy mutation negative family members were included after family cascade genetic screening. They were added to explore if RV mechanical dispersion was attributed to the familial ARVC related genetic mutation or to other unidentified familial factors. All individuals in the control group had normal electrocardiogram (ECG), physical examination, and echocardiographic study and were free from disease with potential impact on the cardiovascular system.

Study population (Paper 2)

In total, 176 consecutive adult primary orthotopic HTx recipients, transplanted at the national HTx centre (Oslo University Hospital, Rikshospitalet) between August 2001 and August 2007, were retrospectively evaluated for eligibility in this study. Of these patients, 167 had an analyzable echocardiographic study, performed 13 ± 6 days post HTx. Assessment of LVGLS was not feasible due to poor image quality in 1 (6%) non-survivor and 8 (5%) survivors.

Patients were identified, and the data were collected from our transplant database approved by The Institutional Review Board, the hospital dialysis records and the Norwegian Cancer Registry. Data contains information after HTx, including demographics, clinical history, treatment, examinations and records of hemodynamic data and renal replacement therapy including kidney transplantation. The database is updated continuously as we cross match data from the National Norwegian Population Registry, general hospital registry, Norwegian Cancer Registry and the immunology and pathology registry.

During the first year, 15/167 (9%) patients died 86 ± 72 days after HTx. The primary cause of death was grade 2R or higher acute cellular rejection[27] in 2 patients. Importantly, a combination of acute cellular rejection and antibody mediated rejection was present in 7 cases at the time of death. The primary causes of death in the other 6 patients were multiple organ dysfunction syndrome in combination with sepsis in 3 and in combination with acute respiratory distress syndrome in 1, peroperative myocardial infarction and post-operative ventricular arrhythmia in 1, and heparin induced bleeding in 1 HTx recipient. Among the survivors (n=152), 48 (32%) experienced 1 or more episodes of biopsy proven grade 2R or higher acute cellular rejection and 8 (5%) experienced antibody mediated rejection within the first year.

Study population (Paper 3)

This study was conducted in a single tertiary coronary care center. Seventy-seven patients fulfilled inclusion criteria with symptoms and signs of NSTE-ACS and were referred to our hospital for coronary angiography. These patients were prospectively included in the study.

Exclusion criteria were: age <18 years, history of previous myocardial infarction, percutaneous coronary intervention and open chest surgery, left bundle branch block, severe valvular dysfunction, atrial fibrillation with heart rate >100 bpm, sustained severe arrhythmia, or any condition which interfered with the patients' ability to comply.

ECGs were evaluated by experienced cardiologists at admission. ECGs were described as ischemic if ST depression or T wave changes were present. Echocardiography was performed 1-2 hours prior to coronary angiography and within 48 after the last episode of chest pain.

By coronary angiography, 49 (64%) had significant CAD. Patients with significant CAD were more frequently male compared to patients without. No differences in age, co-morbidity or medication were observed between those with and without significant CAD at admission. Of the patients with significant CAD, 21 (28%) had significant coronary stenosis, and 28 (36%) had coronary artery occlusion in 1 or more coronary arteries.

METHODS

Two-dimensional echocardiography

Patients and control subjects underwent an echocardiographic study by Vivid 5 and 7 (GE, Vingmed, Horten, Norway) in study 1 and 2, and by ARTIDA (Toshiba Medical Systems Corporation, Tokyo, Japan) in study 3. Cineloops from 3 standard apical views (4-chamber, 2-chamber and apical long-axis) were recorded using grey-scale harmonic imaging in all 3 studies. In study 3, also standard parasternal short-axis view of the LV at the level of the papillary muscle was recorded. Data were digitally stored for off-line analysis using software (EchoPac, GE, Vingmed) in study 1 and 2, and (Toshiba Medical Systems Corporation, Tokyo, Japan) in study 3. The echocardiographic data were analyzed blinded to all clinical information.

EF was assessed by the modified biplane Simpson`s method from apical 4- and 2-chamber grey-scale recordings in all 3 studies. End-diastole was defined as the frame closest to the R-wave, and end-systole was defined as the minimal cavity area just before mitral valve opening. According to the recommendations of the American Society of Echocardiography, the inner contour of the LV cavity was manually traced, leaving the papillary muscles and trabeculations within the cavity.[28] In all 3 papers, EF was compared to different strain parameters as a major part of the studies.

From 2D echocardiography the following additional parameters were assessed in the first study: right ventricular outflow tract (RVOT) diameter in the parasternal short axis view, RV end-diastolic area, RV end-systolic area and RV fractional area change (RVFAC) from apical 4 chamber view. [13]

In the third study, wall motion was visually assessed in a 16-segment model, as normal=1, hypokinetic=2, akinetic=3, dyskinetic=4, aneurysm=5, according to the American Society of Echocardiography.[29] Image quality was evaluated and segments were discarded if

the quality were found insufficient for analysis. Wall motion score index (WMSI) was calculated for each patient as the average of analyzed segmental values.

Two-dimensional speckle-tracking echocardiography

Strain is a clinical index of regional myocardial deformation[30] and have been introduced and validated using magnetic resonance imaging (MRI) tagging[31] and sonomicrometry.[32] Tissue Doppler technology, the predecessor of speckle-tracking technology, requires achievement of parallel orientation between the direction of motion and the ultrasound beam. Its use has remained limited due to angle dependency, substantial intraobserver and interobserver variability and noise interference. Strain is defined as tissue elongation relative to length. A positive strain value refers to elongation, whereas a negative strain value describes shortening. Therefore, negative systolic strain values describe a normal contracting myocardial segment (Figure 1).

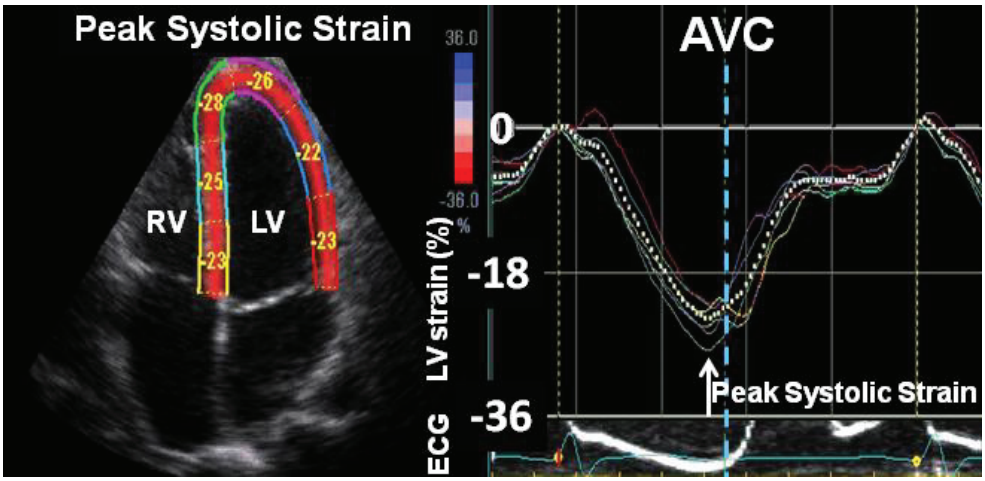


Figure 1. *Left panel demonstrate apical 4-chamber view with the left ventricular (LV) myocardium as region of interest in a healthy individual. Corresponding longitudinal strain curves are shown in the right panel. End-systole is defined by aortic valve closure (AVC),*

and is marked by a vertical stippled blue line. Peak systolic strain is defined as maximum shortening during systole (arrow).

RV = right ventricle

Two-dimensional speckle-tracking echocardiography (2D-STE) has been developed to eliminate the problem of angle-dependency in Doppler-derived deformation analyses.[33] It is a semi-automated quantitative technique for assessment of cardiac function based on gray-scale images. Strain (relative tissue deformation) is evaluated on a frame-by-frame basis by tracking of acoustic markers (speckles) throughout the cardiac cycle and is calculated for each LV segment as the average relative deformation in longitudinal (Paper 1-3), circumferential (Paper 3), or radial directions (Figure 2).

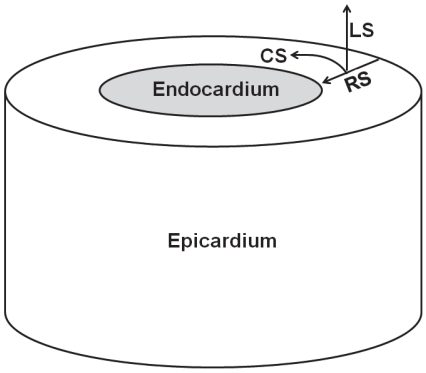


Figure 2. *Schematic illustration of the 3 strain directions in the heart's coordinate system. CS = circumferential strain, LS = longitudinal strain and RS = radial strain.*

The endocardial borders were traced in the end-systolic frame of the 2D images from the 3 apical views for LV longitudinal strain (Paper 1-3), from the 4-chamber view for RV longitudinal strain (Figure 3) (Paper 1) and from short axis view for LV circumferential strain (Paper 3).

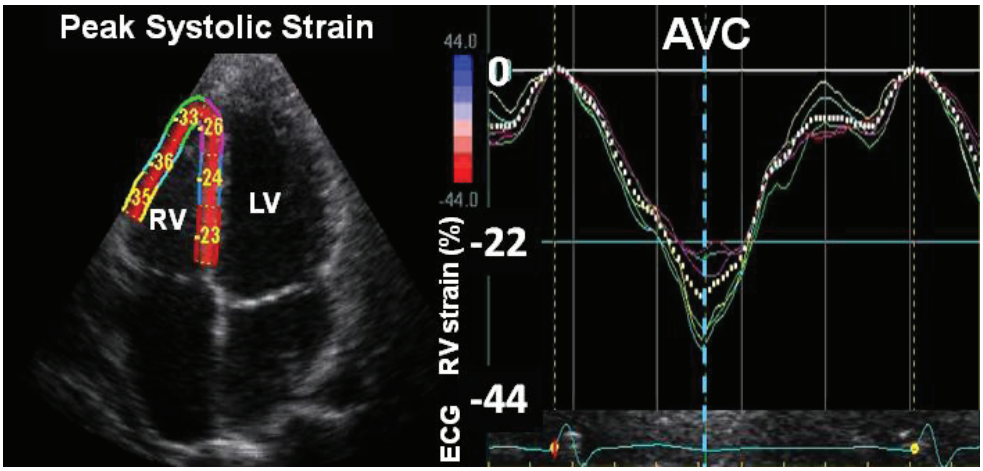


Figure 3. *Left panel demonstrate apical 4-chamber view with the RV myocardium as region of interest in a healthy individual. Corresponding longitudinal strain curves are shown in the right panel.*

AVC = aortic valve closure; LV = left ventricle

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 3 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.[29] Consequently, peak systolic longitudinal strain, defined as maximum systolic longitudinal shortening, was assessed in 16 longitudinal LV segments and averaged to GLS (Paper 1-3). Peak systolic longitudinal strain from 3 RV free wall segments was averaged as a measure of RV function (RV strain) (Paper 1). Finally, global circumferential strain (GCS) was averages from peak systolic circumferential strain in 6 circumferential LV segments (Paper 3). End of

systole was defined by the timing of the aortic valve closure in apical long axis view (Paper 1-3).

Two-dimensional strain and mechanical dispersion

The endocardial borders were traced as described earlier and speckles were tracked in the LV and the RV myocardium throughout the cardiac cycle. Contraction duration (CD) (Figure 4) was measured as the time from onset R on ECG to maximum LV and RV myocardial shortening by strain.

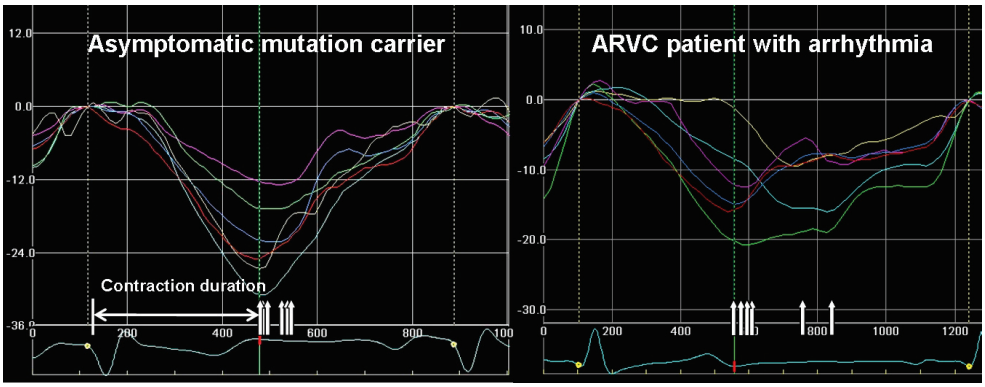


Figure 4. *Mechanical dispersion in an asymptomatic mutation carrier (left panel) and a patient with arrhythmogenic right ventricular cardiomyopathy (ARVC) with recurrent arrhythmias (right panel). Horizontal white arrow indicates contraction duration defined as the time from onset R on ECG to maximum myocardial shortening. Vertical arrows indicate the timing of maximum myocardial shortening in each segment. Right panel shows more pronounced mechanical dispersion.*[34]

Standard deviation of CD was calculated as a parameter of mechanical dispersion, in a 16 LV segment and a 6 RV segment model. We used a 6 RV segment model (3 RV free wall segments plus 3 septal segments) when assessing mechanical dispersion which includes the

usually less affected interventricular septum to elucidate dispersion of contraction between affected and non-affected segments (Figure 5).

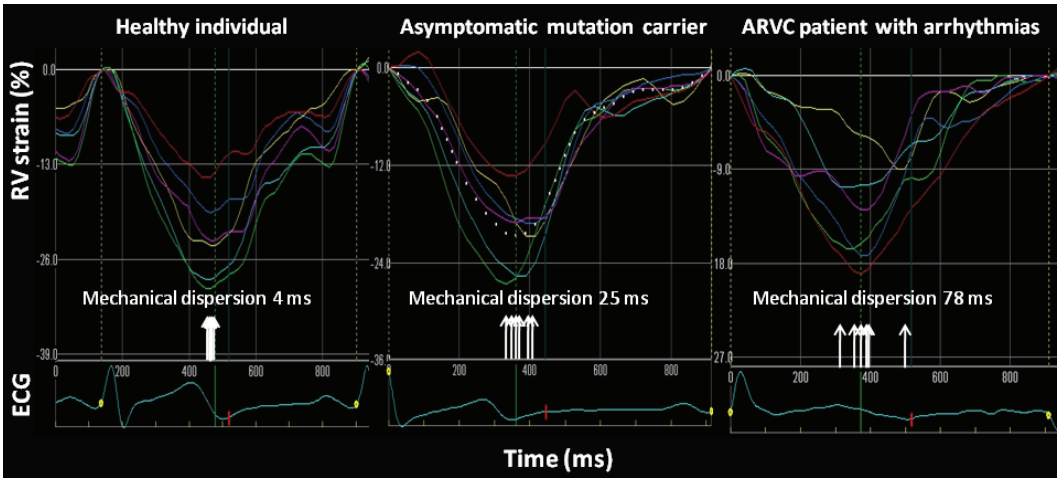


Figure 5. *Right ventricular strain curves in a healthy control, an asymptomatic carrier of arrhythmogenic right ventricular cardiomyopathy (ARVC) specific mutation, and a patient with manifest ARVC. Mechanical dispersion is gradually increasing as the disease is progressing.*

Two-dimensional layer-specific strain

The endocardial borders were traced in the end-systolic frame from the 3 apical views for analyses of longitudinal and from parasternal short-axis view for circumferential endocardial, mid-myocardial and epicardial strains. Peak negative systolic longitudinal and circumferential strains from 3 layers were assessed using off-line software (Toshiba Medical Systems Corporation, Tokyo, Japan) in 16 longitudinal (Figure 6) and 6 circumferential LV segments. All segmental values were averaged to GLS and GCS for each myocardial layer.

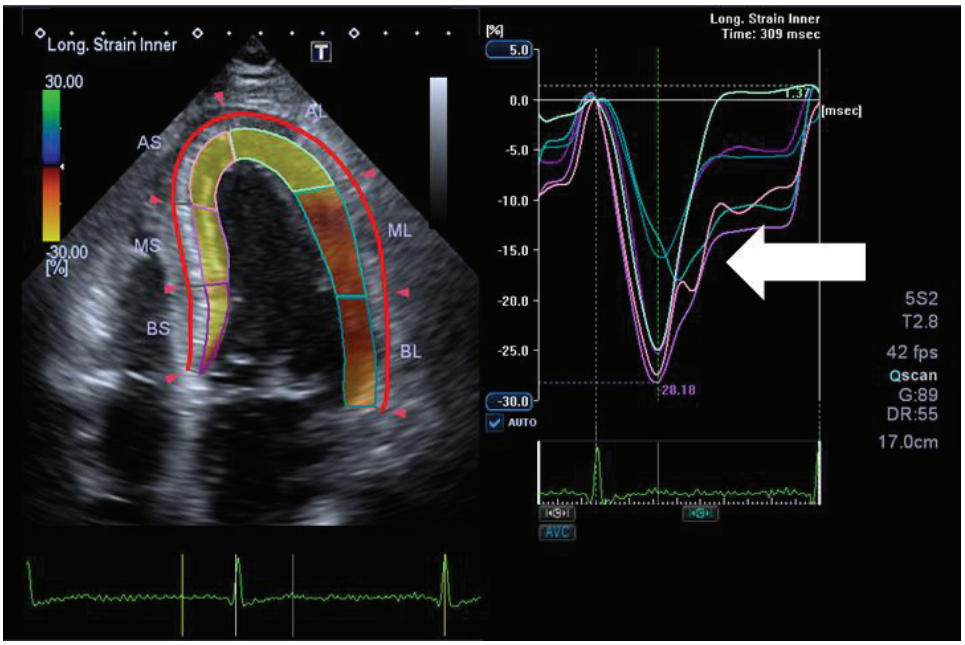


Figure 6. *Six endocardial longitudinal strain segments in apical 4-chamber view in a patient with* non-ST elevation (NSTE) acute coronary syndrome (ACS) *with occluded Left Circumflex (LCX) artery. Automatic strain analysis shows reduced color coded endocardial strain values in the segments supplied by the LCX artery on the left. Color-coding from yellow to green indicates strain from +30% to -30%. Yellow indicates preserved strain. Brown indicates areas with reduced strain. Epicardial shortening was assessed along the red line. Strain curves for the 6 endocardial segments are displayed on the right. The curves representing the segments supplied by the LCX artery show reduced peak systolic longitudinal strain values of -15% (white arrow).*[35]

Endo-, mid-, and epicardial territorial longitudinal strains (TLS) were calculated based on the perfusion territories of the 3 major coronary arteries in a 16-segment LV model,[29] by averaging all segmental peak systolic longitudinal strain values within each territory in each layer.

Magnetic Resonance Imaging

Due to its high reproducibility and high spatial resolution, MRI is considered as the gold standard for cardiac deformation and scar imaging. In addition, MRI can detect fatty replacement of myocardium and scar tissue in patients with ARVC.

In Paper 1, MRI was performed using 1.5 Tesla units (Magnetom Vision Plus or Magnetom Sonata, Siemens, Erlangen, Germany) and a phased array body coil. Axial and sagital T1 TSE images, multiple axial and one sagital cineloop covering the RV and LV were recorded. RV and LV chamber dimensions, wall thickness and myocardial function were assessed. A negative MRI study was defined as normal RV and LV dimensions, normal global and regional wall-motion, no fatty infiltration and/or scar tissue, and no aneurysm formation.

Signal-Averaged ECG

Signal-averaged electrocardiography (SAECG) is a method, in which multiple ECG signals are averaged to remove interference and reveal small variations in the QRS complex, usually called "late potentials". These may represent a predisposition for ventricular arrhythmias.[36]

In Paper 1, SAECG was performed using a MAC® 5000-analysing system (GE Medical Systems, Milwaukee, WI, USA). Time domain analysis was obtained in the bandpass filter 40 to 250 Hz. The SAECG was considered positive for late potentials when at least 2 of the following 3 parameters were abnormal: total filtered QRS duration (fQRSd) >114 ms, the terminal (last 40ms) QRS root mean square voltage (RMS) <20μV and the low amplitude (<40μV) late potential duration (HFLA) >38 ms.

Right heart catheterization

In severe heart failure, pulmonary artery pressures may increase. To assess hemodynamics in the follow up of HTx recipients after a successful heart transplantation, right heart catheterization was performed and pulmonary pressures and pulmonary vascular resistance (PVR) were measured since these parameters are important prognostic factors.

In Paper 2, right heart catheterization was performed using a Swan-Ganz pulmonary artery thermodilution catheter (Baxter Health Care Corp, Santa Ana, CA) 12 ± 8 days post-HTx. Mean pulmonary artery pressures and mean pulmonary capillary wedge pressures were obtained. Cardiac output was measured by thermodilution. The transpulmonary gradient was obtained by subtracting mean pulmonary capillary wedge pressure from mean pulmonary artery pressure. PVR was obtained in Wood units by dividing transpulmonary gradient with cardiac output.

Coronary angiography

In Paper 3, all patients underwent coronary angiography. The assessment of CAD was performed by visual estimate in each single stenosis with multiple projections, avoiding overlap of side branches and foreshortening of relevant coronary stenoses. Coronary occlusion was defined as TIMI flow 0 or 1, while significant coronary artery stenosis was considered as ≥50% reduction of vessel diameter in at least one major coronary artery. Fractional flow reserve (FFR) measurement was performed in case the operator was in doubt about the hemodynamic significance of a stenosis. The lesion was considered functionally significant when FFR ≤0.8. When judged appropriate given angiographic, ECG and clinical data, intervention was performed.

Reproducibility and feasibility

In Paper 1, intraobserver and interobserver intraclass correlation were 0.96 and 0.95, for RV strain measurements, 0.94 and 0.94 for LVGLS measurements, 0.85 and 0.84 for RV time measurements and 0.93 and 0.88 for LV time measurements, respectively.

In Paper 2, intraobserver and interobserver intraclass correlation for LVGLS were 0.91 and 0.88.

In Paper 3, assessment of longitudinal strain could be performed in 1303 (94%) endocardial, 1149 (83%) mid-myocardial and 1142 (82%) epicardial LV segments. The corresponding circumferential analyses could be done in 361 (78%) endocardial, 361 (78%) mid-myocardial and 343 (74%) epicardial segments. Intraobserver and interobserver intraclass correlation were 0.96 and 0.96, for endocardial GLS, 0.87 and 0.92, for mid-myocardial GLS, 0.94 and 0.93 for epicardial GLS, 0.81 and 0.84, for endocardial GCS, 0.84 and 0.81, for mid-myocardial GCS and 0.82 and 0.76 for epicardial GCS measurements, respectively.

Statistical methods

Analyses were carried out using a standard statistical software program (SPSS version 16, SPSS Inc, Chicago, IL). Data were presented as mean ± standard deviation, numbers and percentages, and median and interquartile range (IQR)(Paper 1-3). For normally distributed variables, chi-square test (categorical variables) and Student`s t-test (continuous variables) were used to determine differences between two groups (Paper 1-3). For comparisons between more than two groups, analysis of variance (ANOVA) was performed with the Bonferroni *post hoc* correction for multiple comparisons (Paper 1 and 3). For non-normally distributed variables, the Mann-Whitney U-test (2 groups)(Paper 1-3) and the Kruskal-Wallis test (multiple groups)(Paper1) were used. Logistic regression analysis was performed to determine the independent prognostic value of RV dispersion for predicting arrhythmias in

ARVC patients and asymptomatic mutations carriers (Paper 1) and to determine the diagnostic value of endocardial TLS for predicting significant CAD in patients with suspected NSTE-ACS (Paper 3). The selection of variables to be included in a multivariate logistic regression analysis in Paper 1 was based on statistical significance of $p<0.05$ in the univariate logistic regression analysis, in addition to the LVEF which was forced in. In Paper 3, multivariate logistic regression analysis was performed for factors that could potentially influence myocardial function and strain measurements and to determine the independent diagnostic value of endocardial TLS for predicting significant CAD. The area under the receiver-operating characteristic (ROC) curve (AUC) was calculated for LVEF, LVGLS, RV strain, RVFAC and RV mechanical dispersion in Paper 1, for recipient age, donor age, LVEF, LVGLS, PVR and C-reactive protein in Paper 2 and for endo-, mid-, epicardial TLS, GLS and GCS, WMSI, EF and Troponin T in Paper 3. Comparisons between AUCs were performed by using a non-parametric U-test (Analyze-it®) (Paper 1-3). The value closest to the upper left corner of the ROC curves determined optimal sensitivity and specificity for the ability of the parameters to discriminate between those with and without arrhythmic events in Paper 1, to predict 1-year mortality in HTx recipients in Paper 2 and to predict the presence of significant CAD in NSTE-ACS patients in Paper 3. Correlation between the RV and LV strain was assessed by linear regression analysis in Paper 1. Correlation of strain parameters with Troponin T was tested by the Spearman correlation coefficient in Paper 3.

In Paper 2, univariate Cox regression analysis was performed to determine predictors for 1-year mortality among baseline clinical features, right heart catheterization parameters, biochemical markers and measures of LV function. The variables recipient age, pulmonary vascular resistance, LVGLS, LVEF and CRP with a univariate statistical significance of < 0.05 were selected for inclusion in a multivariate Cox regression analysis to determine the independent prognostic value of LVGLS for predicting 1-year mortality. Survival was

expressed using Kaplan-Meier analysis long-rank tested for significance. We calculated the integrated discrimination index (IDI) and the net reclassification improvement (NRI) between models following the methodology of Pencina et al [37, 38] in Paper 2.

Reproducibility was expressed as intraclass correlation coefficient and was performed in 10 patients in each study.

P-values were two-tailed and values <0.05 were considered significant in all 3 papers.

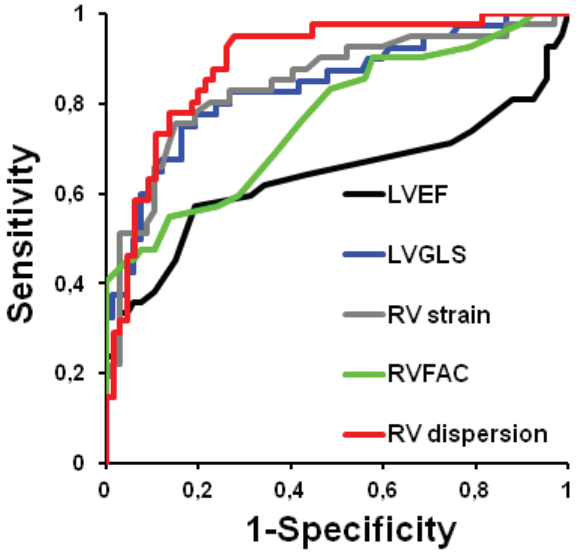
SUMMARY OF RESULTS

Paper 1

We included 69 patients, 42 (61%) had symptomatic ARVC and 27 (39%) were mutation positive asymptomatic family members, diagnosed by cascade genetic screening. Forty healthy individuals served as controls. Ventricular arrhythmias (VT/VF) were documented in all 42 ARVC patients.

Mechanical dispersion was more pronounced in ARVC patients with arrhythmias compared to asymptomatic mutation carriers and healthy individuals in RV (52 [41, 63] ms vs 35 [23, 47] ms vs 13 [9, 19] ms, $p<0.001$). A ROC analysis demonstrated that RV mechanical dispersion was the most sensitive and specific parameter to identify arrhythmic events among the study participants (Figure 7). Furthermore, mechanical dispersion was more pronounced in asymptomatic mutation carriers compared to healthy individuals ($p<0.001$). RV mechanical dispersion predicted ventricular arrhythmias in a multivariate logistic regression analysis ($p<0.03$) with odds ratio 1.66 (95% Confidence Interval 1.06-2.58). RV and LV function by strain (-19 [-16, -21] % and -17 [-16, -19] %) were reduced in symptomatic ARVC patients and correlated significantly ($R=0.81$, $p<0.001$). RV and LV strain were reduced in asymptomatic mutation carriers compared to healthy individuals (-22 [-20, -24] % vs -25 [-23, -27] % and -20 [-18, -21] % vs -22 [-21, -24] %, both $p<0.001$).

RV mechanical dispersion was pronounced in patients with ARVC with ventricular arrythmias. RV mechanical dispersion was present in asymptomatic mutation carriers and may be helpful in risk stratification. RV and LV function correlated in ARVC patients implying that ARVC is a biventricular disease.



	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)
LVEF (%)	0.64	0.51-0.76	63	62	66
LVGLS (%)	0.84	0.76-0.92	-20	83	73
RV strain (%)	0.84	0.76-0.93	-22	83	73
RVFAC (%)	0.77	0.67-0.86	42	69	64
RV dispersion (ms)	0.89	0.83-0.95	29	88	77

AUC, area under the curve; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; RVFAC, right ventricular fractional area change

***Figure** 7. Receiver-operating characteristic curve analyses for the ability of LV and RV parameters to predict arrhythmic events in ARVC patients, asymptomatic mutation carriers and healthy controls (n=109).*[34]

Paper 2

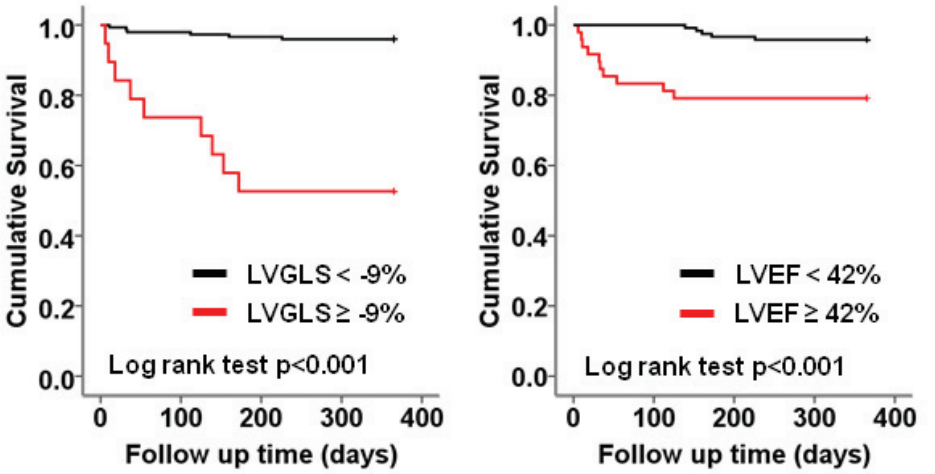
LV function can be accurately assessed by 2D-STE. The association between reduced LVGLS magnitude and risk for mortality in HTx recipients is unclear. We hypothesized that LVGLS could predict 1-year mortality in HTx recipients.

We retrospectively evaluated 176 consecutive adult primary single organ orthotopic HTx recipients. Of these, 167 had acceptable echocardiographic image quality and were included in the study. N-terminal probrain natriuretic peptide, creatinine, C-reactive protein and invasive hemodynamic parameters were measured and echocardiography was performed 1-3 weeks post HTx. LVGLS was averaged from regional strain in 16 LV segments.

During the first year, 15 (9%) patients died 86 ± 72 days after HTx. LV function assessed by LVGLS and LVEF was significantly decreased in non-survivors (-7.9 [-5.7,-10.8] % and 34 [30, 42] %) compared with survivors (-13.7 [-11.8, -15.4] % and 46 [41, 54%], $p<0.001$ respectively). Non-survivors were older and had higher donor age. Mean pulmonary capillary wedge pressure was similar in the two groups while all other hemodynamic parameters were increased in non-survivors ($p<0.05$). LVGLS was the only significant ($p=0.02$) non-invasive independent predictor with hazard ratio 1.42 (95% Confidence Interval 1.07-1.88, $p=0.02$) per 1 % decrease in strain magnitude, while PVR was a significant ($p<0.001$) invasive predictor with hazard ratio 3.98 (95% Confidence Interval 2.01-7.87) of 1-year mortality in a multivariate Cox regression analysis. Kaplan-Meier survival analysis showed that patients with LVGLS ≥ -9% and LVEFs < 42% had significantly worse cumulative survival compared to HTx recipients with LVGLS < -9% and LVEFs ≥ 42% (log-rank test, $p<0.001$) (Figure 8). By ROC analysis, LVGLS ≥ -9% showed the greatest AUC and predicted 1-year mortality with better sensitivity and specificity than LVEF < 42% (Figure 9). Including LVGLS to the prediction model improved classification of risk; relative

integrated discrimination index (IDI) was 3.4 (p<0.01), and net reclassification improvement (NRI) was 0.19 (95% CI 0.05-0.34, p<0.01).

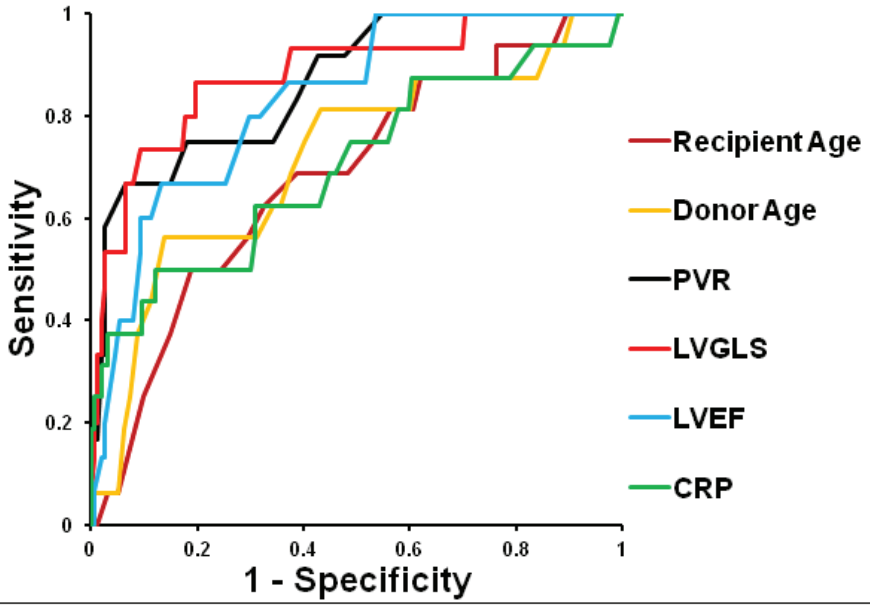
Reduced LV function and increased PVR are related to poor prognosis in HTx recipients. Early assessment of LVGLS might be a non-invasive predictor of 1-year mortality in these patients.



No.at risk						No.at risk					
< -9%	148	145	143	142	142	< 42%	48	40	38	38	38
≥ -9%	19	14	10	10	10	≥ 42%	119	119	115	114	114

Figure 8. *Kaplan-Meier Survival analysis in 167 heart transplant recipients (15 non-survivors and 152 survivors). Patients with LVGLS < -9% and LVEF ≥ 42% show significantly higher cumulative survival at 1-year of follow up.*[39]

LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain



	AUC	95% CI	Optimal cut-off	Senitivity (%)	Specificity (%)
Recipient Age (years)	0.68	0.54-0.82	60	63	68
Donor Age (years)	0.72	0.57-0.86	45	75	60
PVR (WU)	0.87	0.76-0.97	3	67	94
LVGLS (%)	0.88	0.78-0.98	-9	73	91
LVEF (%)	0.83	0.74-0.93	42	70	80
CRP (mg/ml)	0.70	0.54-0.86	36	63	69

Figure 9. *Receiver-operating characteristic curve analyses for the ability of recipient age, donor age, PVR, LVGLS, LVEF, and C-reactive protein to predict 1-year mortality in HTx recipients. The analyses include all study participants (n=167).*[39]

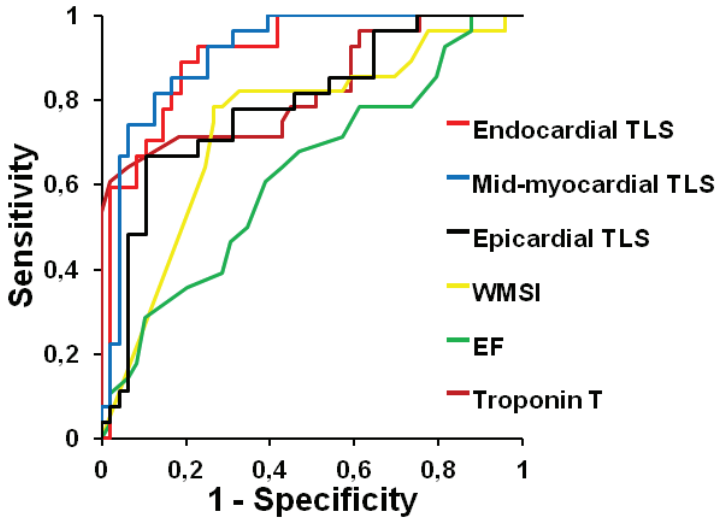
AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; PVR, pulmonary vascular resistance

Paper 3

Seventy-seven patients referred to coronary angiography due to suspected NSTE-ACS were prospectively included. Coronary occlusion was found in 28, significant stenosis in 21 and no stenosis in 28 patients. Echocardiography was performed 1-2 hours before angiography.

Patients with significant CAD had worse function in all 3 myocardial layers assessed by TLS and GCS compared to patients without significant CAD. Endocardial TLS (-14.0 ± 3.3% vs -19.2 ± 2.2%, $p<0.001$) and GCS (-19.3 ± 4.0% vs -24.3 ± 3.4%, $p<0.001$) were most affected. The absolute difference between endo- and epicardial TLS and GCS were lower in patients with significant CAD (Δ2.4 ± 3.6% and Δ6.7 ± 3.8%, $p<0.001$) than in those without significant CAD (Δ5.3 ± 2.1% and Δ10.4 ± 3.0%, $p<0.001$). This reflects a pronounced decrease in endocardial function in patients with significant CAD. Multivariate regression analyses including parameters influencing myocardial function showed that reduced myocardial function by endocardial TLS (per 1% change) was the only significant predictor ($p<0.001$) with odds ratio 2.10 (95% Confidence Interval 1.47-3.09) of the presence of significant CAD. ROC analysis showed that endo- and midmyocardial TLS were superior to identify significant CAD compared to epicardial TLS ($p<0.05$), WMSI ($p<0.01$) and EF ($p<0.001$) (Figure 10).

Assessment of endo- and mid-myocardial territorial longitudinal strain by layer-specific strain echocardiography provided higher accuracy than epicardial strain, WMSI and EF in the identification of patients with NSTE-ACS and significant CAD. Endocardial function was more affected in patients with significant CAD compared to epicardial function.



	AUC	95% CI	Optimal cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Troponin T (ng/L)	0.83	0.72-0.93	8	64	94	86	82
EF (%)	0.63	0.50-0.76	62	61	61	73	47
WMSI	0.74	0.62-0.85	1.07	79	73	63	86
Endocardial TLS (%)	0.91	0.84-0.97	-16.4	89	81	73	93
Mid-myocardial TLS (%)	0.91	0.85-0.98	-14.7	82	88	79	89
Epicardial TLS (%)	0.79	0.68-0.90	-12.6	78	69	58	85

AUC, area under the curve; EF, ejection fraction; WMSI, wall motion score index; TLS, territorial longitudinal strain; PPV, positive predictive value; NPV, negative predictive value

Figure 10. *Receiver-operating characteristic curve analyses for the ability of Troponin T, EF, WMSI and TLS parameters to identify patients with significant coronary artery disease. The analyses include all study participants (n=77).*[35]

DISCUSSION

This thesis introduces new principles in the assessment of myocardial function. The prediction of clinical outcome in different cardiac diseases has long been a primary focus of cardiologists. Different cardiologic diseases have different pathophysiological background, however, many of these share a final common pathway, namely myocardial dysfunction.

Patients with ARVC, HTx recipients and patients with significant CAD showed reduced myocardial function assessed by different strain echocardiographic methods, even though EF was generally within normal range. In all 3 studies, reduced myocardial function by strain was associated with increased myocardial disease and consequently worse prognosis. These findings indicate that subtle myocardial abnormalities induced by different myocardial diseases may be detected by sensitive echocardiographic techniques and improved prognostic information can be achieved.

Mechanisms of reduced myocardial function in ARVC patients, HTx recipients and patients with CAD

The mechanisms of reduced myocardial function in ARVC patients, HTx recipients and patients with CAD have both similarities and differences. Myocardial dysfunction in patients with CAD has primarily regional, while in HTx recipients and patients with ARVC a combination of global and regional character.

ARVC is mainly an autosomal dominant inherited disease. Mutations in the genes encoding desmosomal proteins lead to cytoskeleton dysfunction, cell death and fibrosis.[12] Importantly, reduced myocardial function and malignant arrhythmias origin from different mechanisms at different stages of the disease. In the concealed phase, inflammatory processes due to apoptosis, derangements in cell-to-cell adhesion and altered nuclear signaling are present and may induce arrhythmias even though structural abnormalities may not be detected

by traditional imaging.[12, 40] In later stages, fibro-fatty replacement of myocardial tissue is responsible for reduced myocardial function and for potential re-entry circuits.[41-44]

In HTx recipients, myocardial function is determined by alloimmune response, and a variety of non-immune factors such as ischemia-reperfusion injury, post-surgical sympathetic denervation, reduction of pre-HTx pulmonary hypertension and LV preload, acute renal failure, advanced age, post-transplantation infections, and donor variables including age, ischemic time and LV hypertrophy.[45-49] The mentioned factors influence the myocardium in different ways, however, rejection is the leading cause of allograft dysfunction in these patients.[46] Rejection is characterized histologically by inflammatory cell infiltrates, interstitial edema and myocyte necrosis which ultimately translate into structural and functional abnormalities of the allograft.[50]

In patients with significant CAD there are also several mechanisms leading to reduced myocardial function. Importantly, the main reason for reduced function is the imbalance between myocardial oxygen consumption and supply. The most obvious cause of this imbalance is coronary artery occlusion leading to irreversible myocardial cell death and fibrosis. On the other hand, reversible myocardial dysfunction can be induced by myocardial stunning or hibernation.[51, 52] Myocardial stunning occurs in patients with significant coronary artery stenosis after episodes of ischemia. Recurrent episodes of, or prolonged ischemia and repetitive stunning may lead to chronic, but still reversible myocardial dysfunction known as myocardial hibernation.[51, 53]

As described above, we have examined 3 fundamentally different patient groups. The pathophysiology leading to myocardial dysfunction is essentially different in these groups, however, the reduced ability of the myocardium to contract is shared in these diseases.

Mechanical dispersion and biventricular dysfunction in ARVC

Electrical dispersion (heterogeneous electrical conduction) is a known arrhythmogenic factor.[15] Electrical dispersion leads to mechanical abnormalities. Mechanical abnormalities can be assessed by 2D-STE as mechanical dispersion (heterogeneous contraction).[16-18]

Paper 1 demonstrated that mechanical dispersion is pronounced in both ventricles in patients with ARVC and arrhythmias. Interestingly, reduced myocardial function by strain and increased mechanical dispersion in both ventricles were also present in asymptomatic ARVC mutation carriers. These findings suggest biventricular involvement also in the concealed phase of the disease when structural and functional changes are not apparent if assessed by traditional echocardiography or MRI. Importantly, the occurrence of malignant arrhythmias and reduced myocardial function can precede structural myocardial changes shown by traditional imaging techniques. An accurate assessment of myocardial function is therefore particularly important in early ARVC and in so far asymptomatic ARVC mutation carriers.

Since the disease is inherited in at least 50% of cases,[40] the screening of relatives is essential. Cascade genetic screening helps to identify affected family members and to focus resources. Still, risk stratification of asymptomatic ARVC mutation positive family members is challenging and is emphasized by the fact that the first manifestation of the disease in 20-50% of cases may be cardiac arrest.

The novelty of Paper 1 is the identification of myocardial abnormalities in asymptomatic ARVC mutation carriers and the potential for prediction of arrhythmias. Mechanical dispersion and strain by 2D speckle-tracking echocardiography appears to be a sensitive tool for assessing subtle changes in myocardial function and timing of myocardial contraction in ARVC patients and in asymptomatic ARVC mutation carriers.

Myocardial function in HTx recipients

In Paper 2, we have shown that assessment of LV function by echocardiographic global longitudinal strain is a sensitive marker of poor clinical outcome. Reduced deformation assessed by strain was the only non-invasive independent predictor of 1-year mortality in heart transplant recipients, together with the well established invasive prognostic variable of pulmonary vascular resistance.

But why is LV function reduced in these HTx non-survivors? A transplanted heart is never fully compatible with its new host. Therefore, one could in theory expect a development of low degree chronic rejection status even if it is not necessarily detectable by biopsy. The degree of rejection is clearly dependent on the grade of immune incompatibility and the efficacy of immune suppression. Increasing grade of acute rejection (or combination of the 2 different rejection types) has obviously increasing detrimental effect on LV function.[50] According to our results in Paper 2, markedly reduced myocardial function was present already early after HTx in patients who developed fulminant rejection and graft failure later on, even though rejection was detectable by endomyocardial biopsy in only 1 patient at the time of the echocardiographic study.

On the other hand, all HTx non-survivors without rejections had complications directly or indirectly affecting myocardial function and hemodynamics, and had accordingly reduced LV function. In addition, many of these non-survivors developed multiple organ dysfunction syndrome early after HTx and had increased pulmonary vascular resistance.

Early changes of allograft rejection might be detected by 2D-STE.[54] The results of Paper 2 imply that a single assessment of LVGLS early after HTx might be a useful non-invasive screening tool in the identification of HTx recipients with poor clinical prognosis.

Interestingly, HTx survivors with only modestly reduced or normal LV function by LVEF had markedly reduced function by LVGLS. Reduced LV function assessed by LVGLS

early after HTx, despite normal LVEF, might be explained by non-immune factors, described earlier, besides alloimmune response, and is in accordance with reports demonstrating a higher sensitivity for the detection of incipient heart failure by LVGLS compared to LVEF.[55]

Function in different myocardial layers in patients with CAD

Paper 3 demonstrated impaired LV function in all 3 myocardial layers in patients with NSTE-ACS and significant CAD compared to patients without significant CAD. Interpretation of ECG changes could not distinguish between patients with and without significant CAD. These results confirm earlier studies and suggest that assessment of layer-specific strain may increase the accuracy in the attempts to identify significant CAD before referral to angiography and can be of valuable help as an additional clinical tool in the diagnostic work-up of these patients.

About two thirds of coronary angiographies lead to an intervention in patients with NSTE-ACS.[23] Although, there are several different non-invasive methods for the purpose of identifying significant CAD, [56] many of these are expensive, unavailable and the number of false positive and negative test results are high.

In patients with CAD, the inner oblique myocardium is most susceptible to ischemic injury.[57] Myocardial infarction models as well as reperfusion studies of myocardial infarction have indicated that the endocardial layer is first affected by ischemia[25] causing morphologic[24] and functional alterations predominant in this layer. With increasing severity, ischemia and necrosis propagate in a transmural wavefront extending from the endocardium to the epicardium. [26] Due to the fact that greatest shortening in a healthy myocardium occurs in the endocardial layer and that endocardium is first affected by ischemia, one will expect that significant CAD causes greatest reduction of function in this particular layer. Reduced endocardial function in patients with significant CAD can be explained by either coronary

occlusion and direct myocardial damage or by reversible myocardial dysfunction caused by myocardial stunning or hibernation.[53]

In study 3, endocardial longitudinal and circumferential strains were better than epicardial strains to identify patients with significant CAD. Furthermore, our results indicate that all 3 myocardial layers were affected by both coronary artery occlusion and by significant coronary artery stenosis, however to different extent. The greatest decrease in myocardial function, occurred in the endocardial layer since shortening normally is most prominent in this layer. Therefore, layer-specific strain analyses might increase diagnostic accuracy in these patients.

Methodology: strain by 2-dimensional speckle-tracking echocardiography

Total wall thickness strain, layer-specific strain and mechanical dispersion were assessed by 2D-STE. The advantage of strain echocardiography, is that it assesses myocardial deformation, an intrinsic mechanical property, and therefore is a more direct measure of function than conventional cavity-based echocardiographic parameters such as EF.

In this thesis, detailed strain studies were used to measure subtle alterations in myocardial function in various ways. In ARVC patients and asymptomatic ARVC mutation carriers, we determined timing of maximal myocardial contractions in different myocardial segments and calculated mechanical dispersion, which showed to be related to ventricular arrhythmias. In HTx recipients, reduced myocardial function by strain was detected before these patients developed fulminant allograft rejection. Finally, evaluation of layer-specific deformation showed a pronounced reduction in endocardial function and transmural deformation gradient in patients with significant CAD. In these papers, a combination of previous knowledge of pathophysiology and use of echocardiographic techniques gave valuable prognostic information.

Speckle tracking technique as used in these papers is attractive and robust in use. Reproducibility and feasibility studies including timing and strain measurements were satisfying.

Importantly, in all 3 studies subtle myocardial alterations were detected with strain echocardiographic methods, even if reduced myocardial function was typically absent when assessed by traditional imaging techniques. This thesis shows that strain echocardiographic evaluation may add important prognostic information in patients with subtle myocardial alterations.

LIMITATIONS

Limitations of 2D speckle-tracking echocardiography

The method, 2D-STE, used in all 3 studies is a promising tool for the evaluation of myocardial function, however, it has several limitations.[58]

Feasibility is often mentioned as a limitation of 2D-STE for several reasons. The speckles, used in the process of strain assessments, are created by the 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 nonphysiological 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 or affect neighboring segmental strain values. Speckle tracking is especially challenging in structures with thin wall, such as the RV free wall (Paper 1) and in structures which are difficult to define, such as the 3 myocardial layers (Paper 3). In

case of too many discarded segmental strain values, global strain might be inaccurate. This is especially true in localized myocardial diseases where strain values are unevenly distributed.

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 (Paper 1-3).

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 3 dimensional nature of myocardial deformation. Speckles move in 3 and not 2 dimensions.

A major limitation of the current use of 2D-STE is the differences among vendors. One important difference between the technologies in Toshiba and GE, the 2 vendors used in our studies, is that strain calculations in Toshiba are mainly based on information from the endocardial layer while GE`s are primarily from the mid-myocardial layer.

The discrepancy between vendors is clearly demonstrated by the significant difference between the total wall thickness LVGLS [-22 (-21, -24) %] in healthy controls in Paper 1 by GE scanner and endocardial LVGLS [-20 (-17, -21) %] ($p<0.05$) in patients without significant CAD in Paper 3 by Toshiba scanner. Physiologically, one would expect higher endocardial longitudinal deformation than total wall thickness longitudinal deformation in healthy individuals. Although, the selection of individuals in these 2 groups were somewhat different, the discrepancy in strain might be the result of the difference in scanners. Nevertheless, in an earlier study, the 2 commercially available speckle-tracking softwares in question appeared to be comparable when quantifying LV function in a healthy population.[59]

Importantly, the parameter, global longitudinal strain, which was primarily used in our studies, is a more robust parameter than radial and circumferential strain for the assessment of myocardial function when Toshiba`s and GE`s cardiac ultrasound systems are used for analysis.[59]

Due to variance between strain values between vendors, a joint workgroup of the American Society of Echocardiography (ASE), the European Association of Echocardiography (EAE) and representatives of all Medical Imaging vendors will work for a standardization of strain values from different scanners. The algorithms used by the different software packages are similar in terms of the speckle-tracking analysis, which is based on a block-matching approach of the speckle patterns within the myocardium, however, the strain calculation formulas used are somewhat varying. There are presently ongoing trials with the different scanners using the same formula for strain calculations.

Finally, changes in hemodynamic parameters such as preload, afterload and contractility may influence strain measurements.[60] In paper 1 and 3, patients were hemodynamically stable. However, in paper 2, strain measurements in HTx recipients 1-3 weeks after heart transplantation may have been influenced by hemodynamic instability.

Study specific limitations

The first 2 studies were retrospective in their design, with the limitations inherent in this methodology.

The echocardiographic data were analyzed blinded to all clinical information in all 3 studies. However, in the first study ICD leads were visible on echocardiography, thus demasking the disease status of the patients.

We assessed LVGLS in the first 2 studies but not radial or circumferential strain. This measure was chosen because longitudinal strain has been best validated, measurements are reproducible and are easily obtained. In addition, radial and circumferential strains from the

right ventricle are more difficult to obtain due to the complicated anatomy (Paper 1). In the third study we did not have 3 short axis projections available for the assessment of circumferential strain in a 16 segments model. Therefore, 16 longitudinal segments analyzed by strain were compared with only 6 mid circumferential segments. The latter performed poorer in the diagnosis of significant CAD, probably due to the undetected distal and to some extent mid-coronary stenoses and occlusions which predominantly influence apical and to a certain degree mid-myocardial function.

The follow up in the second paper is limited to 1 year. The echocardiographic image quality is often reduced in the early post-operative phase. However, we were able to assess LVGLS in most of our HTx patients (Paper 2).

All patients with suspected NSTE-ACS in Paper 3 were treated according to guidelines at admission. Obviously, this could have caused dissolvement of thrombi allowing recovery of the affected myocardium. However, one may also speculate that even in case of dissolvement of thrombi, the myocardium might be still stunned or hibernated within the first 48 hours. Furthermore, it is not known if coronary artery occlusions represent transmural or non-transmural infarctions since no viability studies have been performed on our patients. Fractional flow reserve measurements were not performed in all patients, therefore the true hemodynamic relevance of the stenoses is not known (Paper 3).

Deformation of the entire wall thickness of the myocardium has not been assessed in Paper 3 since the software (Toshiba Medical Systems Corporation, Tokyo, Japan) did not allow such analysis. Consequently, we could not evaluate if layer-specific strain analysis has additional value compared to traditional total wall thickness strain analysis in diagnosing CAD.

The gradient of strain across the myocardium is a non linear phenomena and the definition of the layers is arbitrary and is based on simple division into 3 parts. Since the

spatial resolution of ultrasound is limited, there will always be a certain degree of “overlap” (Paper 3).

CONCLUSIONS

General conclusion

Strain echocardiography is a sensitive tool in the early detection of subtle myocardial alterations in patients with ARVC, HTx recipients and patients with significant CAD.

Specific conclusion

I. RV and LV mechanical dispersion by myocardial strain was pronounced in patients with ARVC with ventricular arrhythmias and was present in asymptomatic mutation carriers, therefore mechanical dispersion may be helpful in risk stratification in these patients (Paper 1).

II. ARVC patients with arrhythmias showed significantly reduced RV and LV function assessed by myocardial strain compared to asymptomatic mutation carriers and healthy controls. LV and RV function by strain were correlated in these patients implying that ARVC is a biventricular disease. RV and LV function was reduced in asymptomatic mutation carriers compared to healthy individuals (Paper 1).

III. Reduced LVGLS assessed by strain echocardiography, 1-3 weeks after heart transplantation was the only non-invasive independent predictor of 1-year mortality in heart transplant recipients, together with the well established invasive prognostic variable of pulmonary vascular resistance (Paper 2).

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IV. Assessment of endo- and midmyocardial territorial longitudinal strain by layer-specific strain echocardiography provided higher accuracy than epicardial territorial longitudinal strain, wall motion score index and ejection fraction in the indentification of significant CAD in patients with suspected NSTE-ACS (Paper 3).

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