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Strain of geen strain?

De toepassing van
twee-dimensionale strain echocardiografie in kinderen

PROEFSCHRIFT

Ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus, prof. mr. S.C.J.J. Kortmann,

volgens besluit van het college van decanen

in het openbaar te verdedigen op

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Strain or no strain?

The application of

Two-dimensional Strain Echocardiography

in children

DOCTORAL THESIS

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from Radboud University Nijmegen

on the authority of Rector Magnificus prof. dr. S.C.J.J. Kortmann,

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to be defended in public on

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# Table of Contents

Chapter 1 General introduction and outline of this thesis ........................................ 13

1.1 GENERAL INTRODUCTION ........................................................................... 14
1.2 STRAIN IMAGING ......................................................................................... 15
1.3 OUTLINE OF THIS THESIS .......................................................................... 25
1.4 REFERENCES ................................................................................................. 27

Chapter 2 Reference values for two-dimensional strain echocardiography in a healthy pediatric and young adult cohort ............................................................ 33

2.1 ABSTRACT ................................................................................................... 34
2.2 BACKGROUND ............................................................................................ 34
2.3 MATERIAL and METHODS ......................................................................... 36
2.4 RESULTS ...................................................................................................... 42
2.5 DISCUSSION ............................................................................................... 59
2.6 STUDY LIMITATIONS ................................................................................. 62
2.7 CONCLUSION .............................................................................................. 63
2.8 APPENDIX .................................................................................................. 64
2.9 REFERENCES .............................................................................................. 65

Chapter 3 Synchronicity of systolic deformation in healthy pediatric and young adult subjects assessed with two-dimensional strain echocardiography ............ 71

3.1 ABSTRACT .................................................................................................. 72
3.2 BACKGROUND ............................................................................................ 73
3.3 MATERIAL and METHODS ......................................................................... 75
3.4 RESULTS ...................................................................................................... 80
3.5 DISCUSSION ............................................................................................... 93
3.6 STUDY LIMITATIONS ................................................................................. 97
3.7 CONCLUSION .............................................................................................. 98
3.8 APPENDIX .................................................................................................. 99
Chapter 4 Abnormal two-dimensional strain echocardiography findings in children with congenital valvular aortic stenosis .................................................. 109

4.1 ABSTRACT .................................................................................. 110
4.2 BACKGROUND ........................................................................... 111
4.3 MATERIAL AND METHODS ......................................................... 113
4.4 RESULTS .................................................................................... 117
4.5 DISCUSSION .............................................................................. 124
4.6 STUDY LIMITATIONS ................................................................. 130
4.7 CONCLUSION ............................................................................ 130
4.8 REFERENCES ............................................................................ 131

Chapter 5 Persistent reduction in left ventricular strain using two-dimensional speckle tracking echocardiography after balloon valvuloplasty in children with congenital valvular aortic stenosis .................................................. 137

5.1 ABSTRACT .................................................................................. 138
5.2 BACKGROUND ........................................................................... 139
5.3 MATERIAL and METHODS ......................................................... 140
5.4 RESULTS .................................................................................... 145
5.5 DISCUSSION .............................................................................. 163
5.6 STUDY LIMITATIONS ................................................................. 167
5.7 CONCLUSION ............................................................................ 168
5.8 REFERENCES ............................................................................ 169

Chapter 6 Early detection of myocardial dysfunction in children with mitochondrial disease .......................................................... 175

6.1 ABSTRACT .................................................................................. 176
6.2 BACKGROUND ........................................................................... 177
6.3 MATERIAL and METHODS ......................................................... 178
Chapter 9 Summary, general discussion and future perspectives ................. 253
  9.1 SUMMARY & GENERAL DISCUSSION ......................................................... 254
  9.2 FUTURE PERSPECTIVES ........................................................................... 259
  9.3 TO CONCLUDE ............................................................................................ 262

Chapter 10 Samenvatting, discussie en toekomstperspectieven ................. 265
  10.1 SAMENVATTING & DISCUSSIE ............................................................... 266
  10.2 TOEKOMSTPERSPECTIEVEN ................................................................. 272
  10.3 CONCLUSIE ............................................................................................... 275

Chapter 11 Dankwoord .................................................................................... 279

Chapter 12 Curriculum Vitae .......................................................................... 285

Chapter 13 Affiliation of co-authors ............................................................... 288

Chapter 14 List of publications ....................................................................... 292
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DSTE</td>
<td>Two-dimensional speckle tracking echocardiography</td>
</tr>
<tr>
<td>4C</td>
<td>Four chamber view</td>
</tr>
<tr>
<td>AVR</td>
<td>Aortic valve replacement</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CHD</td>
<td>Congenital heart disease</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>LV</td>
<td>Left ventricle/ left ventricular</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>MIC</td>
<td>Mitochondrial cardiomyopathy</td>
</tr>
<tr>
<td>MV</td>
<td>Mitral valve</td>
</tr>
<tr>
<td>PM</td>
<td>Papillary muscle</td>
</tr>
<tr>
<td>PWS</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>S-CM</td>
<td>Peak systolic circumferential strain at the level of the mitral valve (basal)</td>
</tr>
<tr>
<td>S-CP</td>
<td>Peak systolic circumferential strain at the level of the papillary muscle (midcavity)</td>
</tr>
<tr>
<td>S-L</td>
<td>Peak systolic longitudinal strain</td>
</tr>
<tr>
<td>Sr</td>
<td>Strain-rate</td>
</tr>
<tr>
<td>S-RM</td>
<td>Peak systolic radial strain at the level of the mitral valve (basal)</td>
</tr>
<tr>
<td>S-RP</td>
<td>Peak systolic radial strain at the level of the papillary muscle (midcavity)</td>
</tr>
<tr>
<td>T2P</td>
<td>Time to peak systolic strain</td>
</tr>
<tr>
<td>TDI</td>
<td>Tissue Doppler Imaging</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VAS</td>
<td>Valvular aortic stenosis</td>
</tr>
</tbody>
</table>
Het stenen tijdperk hield niet op omdat de stenen op waren, maar omdat er iets beters voorhanden kwam

Dr. H.H.F. Wijffels
Nederlandse econoom
Chapter 1

General introduction and outline of this thesis
1.1 GENERAL INTRODUCTION

1.1.1 THE VALUE OF ECHOCARDIOGRAPHY

One of the technical revolutions that changed the face and practice of pediatric cardiology was the development of noninvasive imaging techniques. Until fifty years ago, clinical skills were at the core of the profession. It was the stethoscope, the ECG and the chest X-ray that guided the clinician to reach to a diagnosis and form a treatment plan. Imaging techniques to enable the assessment of detailed cardiac anatomy and the quantification of myocardial function were not available.

In the 1970s two-dimensional (2D) echocardiography was developed, which is an attractive imaging modality for several reasons. Images are displayed in real time allowing prompt diagnosis, it is easily available, bed-side, noninvasive, relatively inexpensive to perform and it does not involve ionizing radiation. During the last two decades, echocardiography has become the primary imaging tool in the assessment of both pediatric and adult heart disease. As technology has evolved, the focus of new developments in echocardiographic imaging has shifted from dynamic characterization of cardiac anatomy to the assessment of myocardial performance. It has become clear that the clinical outcome of children with different forms of congenital and acquired heart disease is greatly dependent on myocardial systolic and diastolic functioning. Global cardiac performance is determined by a complex interaction between intrinsic myocardial contractility, preload, afterload, and heart rate. The ultimate goal is to describe intrinsic myocardial contractility. However, due to the fact that loading conditions influences most of the conventional echocardiographic parameters, heart rate and cardiac geometry, the assessment of true myocardial contractility has turned out to become one of the important challenges in the field.
1.1.2 CONVENTIONAL ECHOCARDIOGRAPHIC INDICES USED TO INDICATE SYSTOLIC FUNCTION

Traditionally, dimensional changes (i.e., fractional shortening) or volumetric changes (i.e., ejection fraction) during the ejection phase of the cardiac cycle have been used to assess systolic ventricular function. These techniques are especially load-sensitive and provide no information about regional myocardial contraction. Importantly, they are not always applicable to pediatric hearts with complex congenital malformations. The reason for this is that geometrical assumptions are made in the calculation of volumetric changes, which overlook the complex anatomical and related functional characteristics of congenital heart disease. New developments in echocardiography, as well as other forms of cardiac imaging, have tried to address these shortcomings in recent years.

1.2 STRAIN IMAGING

1.2.1 STRAIN

Strain and strain-rate imaging techniques have emerged as quantitative modalities to accurately estimate regional as well as global myocardial function. Strain is a dimensionless parameter representing deformation (‘contraction or stretching’) of an object relative to its original shape. In cardiac muscle physiology, strain is directly related to fiber shortening. Strain-rate represents the rate of change of deformation.
“Lagrangian” strain and strain-rate are defined by the following formulas:

![Lagrangian formulas](image)

\[
\text{Strain} = \varepsilon = \frac{L - L_0}{L_0} \quad (\%)
\]

\[
\text{Strain rate} = \text{SR} = \frac{\Delta \varepsilon}{\Delta t} \quad (s^{-1})
\]

**Figure 1 - Lagrangian formulas, which are used to calculate strain and strain-rate**

Where \(L\) is the instantaneous length, \(L_0\) is the initial length, and \(\Delta t\) is the time interval between initial and deformed conditions (Figure 1). Strain, symbolically denoted as epsilon (\(\varepsilon\)), is expressed as the percentage (or fractional) change from the original dimension, whilst strain-rate is expressed as \([s^{-1}]\). From these formulas, it is evident that positive strain is lengthening, thickening or ‘stretching’. Negative strain is shortening, thinning or compression in relation to the original length. Strain-rate is negative during compression and positive during stretching.

Strain is often mistaken for motion, a parameter that is obtained using Tissue Doppler Imaging (TDI).\(^6\)\(^,\)\(^7\) However, a moving subject is not necessary undergoing deformation as long as every part of the object moves with the same velocity. The object may then be said to have pure “translational” velocity, but the shape remains unchanged. Over time, the object will change position (displacement). On the other hand, if different parts of the object have different velocities, the object has to change shape and deformation occurs (Figure 2). Strain rate is measured using TDI-based strain-rate imaging.\(^8\)
Figure 2 - Relation between displacement-velocity based (blue) indices and deformation-based (green) parameters

The superiority of strain-based (deformation-based) parameters for assessing cardiac function compared to velocity-displacement-based parameters is explained by the strain-algorithm.

Unlike velocity-based parameters, strain calculations are not affected by movement due to global cardiac motion within the chest (cardiac translation) nor by the effect (pulling or tethering) of surrounding segments on the region of interest. Completely passive segments can show motion relative to the transducer due to tethering, but without active deformation, making velocity and displacement unreliable for the characterization of myocardial function of such regions. Strain parameters on the other hand, are referred to as motion-deformation between two points in the myocardial wall, which is unrelated to the motion towards the transducer. This characteristic of strain discriminates passive movement from true contraction in a myocardial region under investigation.

A second important advantage of strain is that it is geometry-independent and hereby may be valuable for the quantification of ventricular function in cases with
variable abnormal ventricular morphology (e.g., in case of complex congenital heart disease or pathological right ventricular function).

Third, deformation techniques are able to quantify regional myocardial function in addition to global ventricular function. Strain imaging makes it possible to quantify regional myocardial performance in different segments of the (ventricular) wall and to identify regional dysfunction.\textsuperscript{5,9,10,11} In congenital heart disease it is still uncertain how regional myocardial dysfunction affects global (dys-)function. The role of regional functional differences during the progression of myocardial dysfunction needs to be explored. Strain imaging could be helpful in answering (part of) these questions.

Importantly, strain imaging techniques appear to be able to detect sub-clinical myocardial dysfunction at an earlier stage when compared to conventional imaging modalities and applications.\textsuperscript{13,14,15,16,17}

1.2.2 HOW CAN STRAIN BE MEASURED?

\textit{Tagged magnetic resonance imaging (tagged MRI)} of the heart is currently used as the gold standard to evaluate myocardial strain and strain-rate. It is a sensitive, accurate and load-independent technique. However, several characteristics of this technique preclude its use in daily clinical practice. It is characterized by a time and labor-consuming nature, its frame rate cannot be as high as in echocardiography, which is of importance in children, it is not always easily accessible and obviously not bed-side. Furthermore, it is an expensive method, and in case of (young) children often indicates a need for sedation and anesthesia.

A second modality to measure strain is \textit{Tissue Doppler Imaging}.\textsuperscript{18} Doppler echocardiography relies on quantification of the shift in frequency of ultrasound signals reflected by moving objects. Using this principle, conventional Doppler
techniques assess the velocity of blood flow by measuring relatively high frequency, low-amplitude signals from small, fast-moving blood cells. In TDI, the same Doppler principles are used to quantify selectively the higher-amplitude, low frequency signals of myocardial tissue motion. The quantification of strain rate and strain derived from TDI has been extensively validated. Tissue Doppler-derived strain has been utilized over the last decade in a great number of clinical situations, mainly in adults, including the assessment of ischemia, evaluation of dyssynchronous myocardial contraction and the assessment of function in relation to valve abnormalities. However, TDI has several limitations. The first and most important limitation is angle-dependency. The tissue velocities as measured with TDI are, like all Doppler-derived measurements, dependent on the angle of insonation. TDI is only able to reliably estimate strain when the angle between the ultrasound beam and the tissue motion is small. Strain in the perpendicular plane cannot be determined at all using this technique. Although a correction for the angle of insonation can be applied if the tissue under investigation is not moving perfectly in line with the ultrasound beam, the measurement obtained will be subject to inaccuracy. Secondly, TDI is inherently one-dimensional. As a consequence, strain can only be assessed in one direction of contraction.\textsuperscript{18,19}

A third, relatively new technique, is known as two-dimensional strain echocardiography or ‘two-dimensional speckle tracking echocardiography’ (2DSTE). This technique uses sequential, two-dimensional B-Mode (grayscale) images to quantify strain and strain-rate.\textsuperscript{10,11} It was developed in an attempt to (partly) overcome the previously mentioned shortcomings of TDI (and to a lesser extend tagged MRI).

1.2.3 TWO-DIMENSIONAL STRAIN ECHOCARDIOGRAPHY

Two-dimensional strain echocardiography analyzes motion by tracking speckles (acoustic “markers” related to the backscattering of ultrasound by myocardial
tissue) in the 2D ultrasonic image. These markers are a kind of spatial “noise” that is homogeneously distributed throughout the image of the myocardium. In 2D speckle tracking, first the echo amplitude features of the speckle pattern are used to follow the movement of small, partially overlapping, “windows” accurately over consecutive frames. To achieve this, a local search algorithm, aimed to detect the position of the image window with the most similar speckle pattern from one frame to another, is used. This procedure is repeated for each window overlaying the image. The appearance of the speckle pattern within a window may be considered to be relatively stable between two subsequent image frames, whereas a change in its position is assumed to be the result of tissue motion and deformation. When the frame rate is known, the assessment of this change in position of a window, i.e., its displacement, allows determination of the deformation and its rate of change. Finally, two-dimensional strain and strain-rate values are then obtained from the differential displacement between pairs of windows within the myocardium. In-vitro and in-vivo validations of 2DSTE have been undertaken previously (primarily in adults).\textsuperscript{20,21,22} It may be remarked that a level of sophistication has been reached where 2DSTE can be considered ready for more widespread investigations of its clinical utility.

1.2.4 ADVANTAGES OF 2DSTE OVER TDI

One important advantage of 2DSTE is its independence on the angle of insonation, in contrast to TDI where it is necessary for the main motion vector to be (almost) parallel to the ultrasonic beam.

The speckle pattern can be tracked in multiple directions, which allows quantification of myocardial deformation in all three main directions of strain.

These directions of deformation in the myocardium consist of: longitudinal (from base to apex), radial or transmural (perpendicular to the epicardium and to the
longitudinal axis) and circumferential strain (perpendicular to both radial and longitudinal axis) (Figure 3 to 5). \(^{22,23}\)

**Figure 3** - Myocardial strain in all three principal directions. L: longitudinal; C: circumferential; R: radial

**Figure 4** - Schematic cross sectional images of myocardial strain in all three principal directions
Figure 5 - On the left side: Longitudinal plane of the heart from apical 4-chamber view; the image below shows the different segments of the septal and lateral wall of the left ventricle that are indicated (BS: basal septum; MS: mid septum; AS: apical septum; BL: basal lateral wall; ML: mid lateral wall; AL: apical lateral wall). On the right side: Short-axis view (transversal plane) of the heart; the image below shows the different segments of the left ventricular wall that are indicated (antsep: anterior septal wall; ant: anterior wall; lat: lateral wall; post: posterior wall; inf: inferior wall; sep: septal wall).

Importantly, 2DSTE has been shown to be more accurate than strain obtained from TDI. When TDI-, 2DSTE-, and tagged MRI-derived strain measurements were
compared, strain data obtained with TDI were less comparable to those obtained with tagged MRI than 2DSTE-derived data.\textsuperscript{24,25,26,27,28,29}

Furthermore, TDI has been reported to have higher interobserver and intraobserver variabilities compared with strain data assessed by 2DSTE.\textsuperscript{30,31,32,33,34}

Given the high reproducibility, relatively geometry- and angle-independency, and automated tracking system (which facilitates easy acquisition of accurate measurements), 2DSTE has the potential to become a widespread strain imaging technique with many clinical implications.

**1.2.5 (CURRENT) LIMITATIONS OF 2DSTE**

- As with TDI, the ejection phase indices of deformation are known to be influenced by afterload and preload. This means that these indices do not directly reflect pure myocardial intrinsic contractility. However, although influenced by loading conditions, strain and especially strain-rate indices are likely less load-dependent than tissue velocities.\textsuperscript{35,36,37}

- The relatively low inherent quality of the 2D echocardiographic images (spatial resolution and speckle noise phenomenon) remains a limiting factor, which is true for all ultrasound-based imaging modalities. In young, healthy subjects, approximately 7% of all LV segments cannot be analyzed due to poor image quality.

- Tracking algorithms use spatial and temporal smoothing and a priori knowledge of “normal” LV function, which may affect the strain estimates and thus erroneously indicate regional dysfunction or affect neighboring segmental strain values. This is illustrated by the fact that in multi vendor comparison studies, different strain values were obtained. The different implementation of smoothing is considered to be one of the reasons for differences between vendors.\textsuperscript{34}
• Measurements in the radial direction were shown to correlate less well with the reference tool of ultrasonomicrometry. This is due to the relatively limited thickness of the LV wall. A second contributing factor is a greater proportion of movement being perpendicular to the ultrasound beam which is associated with greater variance.\textsuperscript{34,38,39} Consequently, different manufacturers have developed different implementations for deriving the radial strain, resulting in different radial strain values in the same individuals.\textsuperscript{34}

• The method is dependent on frame rate. Too low frame rate will result in too great changes from frame to frame, resulting in poor tracking. This may also limit the use in high heart rates, as the motion and thus frame-to-frame change increases relative to the frame rate. Furthermore, too low frame rates may lead to undersampling, erroneously reducing peak values of strain indices. This is most important for measuring peak values during diastole and isovolumic phases, not so much for systolic strain-rate. Systolic strain is associated with the least frame rate sensitivity of all.\textsuperscript{11,40,41} On the other hand, higher frame rates are obtained by reduced lateral spatial resolution (i.e., reducing the acoustic line density), and thus resulting in poorer tracking (at least in the transverse direction). In addition, too high frame rates may result in erroneous cumulative strain estimates. For each strain estimation, an error is present. When very high frame rates are applied, many strain estimates per cardiac cycle are determined, which may result in a large error when cumulating all these estimates for calculating the strain curve. At present, the optimal frame rate for speckle tracking seems to be 60-90 FPS.\textsuperscript{11,40}

• Finally, another limitation of any two-dimensional technique is that of ‘through plane motion’. Rotation and motion of the heart in the chest cavity cause out-of-plane motion. However, these movements cause the disappearance of the speckles over a multiple frames, rather than within two consecutive frames.
Figure 6 - Radial strain at the level of the papillary muscle in a healthy individual. The peak values of strain and strain-rate are similar for all six myocardial regions and the time to achieve this peak is uniform and synchronous. Radial strain is positive in systole as the LV thickens and so increases in the radial dimension.

1.3 OUTLINE OF THIS THESIS

This thesis concentrates on the application of 2DSTE in children.

The ultimate goal of two-dimensional strain echocardiography is to provide accurate, objective and quantitative indices of myocardial performance that are also applicable to ventricles with different morphology (e.g., right ventricle, congenital heart disease). Previous research using 2DSTE to evaluate ventricular myocardial
function has focused on adults (with or without cardiac disease). The establishment of reference values in a healthy pediatric cohort is a mandatory prerequisite for its use in evaluating (pathologic) changes in ventricular function in children. Data in this area are scarce and include only peak systolic strain in one or two directions, without exact information on the timing of peak systolic strain. 

SECTION ONE

In the first section of this thesis we present reference values for two-dimensional strain echocardiography.

Chapter 2 describes left ventricular myocardial strain in all three principal directions (longitudinal, circumferential and radial) assessed by means of 2DSTE in a large, healthy pediatric and young adult cohort.

Chapter 3 describes the timing of systolic deformation throughout the left ventricular wall and its extent of synchronicity between ventricular wall segments in healthy children and young adults.

SECTION TWO

In this part of the thesis we applied the 2DSTE modality to evaluate LV systolic function in children with various congenital and acquired heart diseases.

Chapter 4 evaluates two-dimensional strain echocardiographic findings in asymptomatic children diagnosed with various degrees of isolated congenital valvular aortic stenosis. None of the patients had a history of prior intervention to relieve pressure overload of the left ventricle.

In Chapter 5 we investigate the recovery of left ventricular myocardial systolic performance using (conventional and) two-dimensional strain echocardiography prior to and after balloon valvuloplasty in children with severe congenital valvular aortic stenosis.
In Chapter 6 we describe left ventricular myocardial performance assessed by means of physical examination, electrocardiography, conventional echocardiography and 2DSTE in a group of children with established mitochondrial disease.

In Chapter 7 we evaluate cardiac anatomy and left ventricular myocardial systolic function in children previously diagnosed with Prader-Willi syndrome. Cardiac evaluation consisted of physical examination, electrocardiography, conventional echocardiography and 2DSTE.

In Chapter 8 we describe signs of early-onset anthracycline induced cardiotoxicity assessed with two-dimensional strain echocardiography and biochemical assays.

SECTION THREE

The most important findings are summarized in Chapter 9 and Chapter 10. Conclusions are presented together with suggestions for future research.

1.4 REFERENCES


Not everything that counts can be counted.

and not everything that can be counted counts.

Albert Einstein
Duits-Amerikaans natuurkundige
(1879 - 1955)
Chapter 2
Reference values for two-dimensional strain echocardiography in a healthy pediatric and young adult cohort

Karen A. Marcus, Annelies M. C. Mavinkurve-Groothuis, Marlieke E. Barends, Arie P. J. van Dijk, Ton Feuth, Chris L. de Korte, Livia Kapusta

The Journal of the American Society of Echocardiography
2011;24(6):625-36
2.1 ABSTRACT

Background: Two-dimensional strain echocardiography or speckle tracking (2DSTE) appears to hold significant promise as a tool to improve the assessment of ventricular myocardial function.

Aim: We aimed to estimate left ventricular myocardial systolic function with 2DSTE in a large cohort consisting of healthy children and young adults to establish reference strain values.

Methods: Transthoracic echocardiograms were acquired in 195 healthy subjects (139 children and 56 young adults) and were retrospectively analyzed. Longitudinal, circumferential and radial peak systolic strain values were determined by means of speckle tracking. Nonlinear regression analysis was performed to assess the effect of aging on these 2DSTE parameters.

Results: There was a strong, statistically significant second-order polynomial relation \((P<0.001)\) between global peak systolic strain parameters and age. Global peak systolic strain values were lowest in the youngest and oldest age groups.

Conclusion: This is the first report to establish age-dependent reference values per cardiac segment for myocardial strain in all three directions assessed using 2DSTE imaging in a large pediatric and young adult cohort. We emphasize the need for using age-specific reference values for the adequate interpretation of 2DSTE measurements.

2.2 BACKGROUND

Despite impressive developments in echocardiographic technology during the past fifty years, there are still important challenges, which new techniques try to meet.
One important, yet partially unresolved, challenge that remains is the quantification of ventricular myocardial function in children with congenital heart disease (CHD). Traditional methods, such as the one-dimensional M-mode technique (e.g., fractional shortening) and two-dimensional imaging (e.g., ejection fraction), are used to evaluate left ventricular function. However, these methods are not always applicable to pediatric hearts with complex congenital malformations. The cardinal reason for this is that they are based on geometric assumptions and overlook the complex characteristics of CHD. Moreover, those current indices provide no information about regional alterations in ventricular myocardial contraction. These disadvantages complicate the assessment of systolic and diastolic function in children with CHD and underscore the need for better quantitative techniques to assess ventricular function in this subset of patients.

New applications, such as strain imaging, have been developed to address the previously mentioned shortcomings. Myocardial strain is a dimensionless assessment of regional ventricular deformation (i.e., a percentage of deformation), whereas myocardial strain rate, which is a time derivate of strain, indicates the rate of deformation of a defined myocardial segment. Multiple studies have proven the accuracy and reliability of strain imaging techniques in the assessment of (regional) ventricular myocardial function. Moreover, these techniques appear to be able to detect sub-clinical myocardial dysfunction at an earlier stage compared with conventional imaging modalities. Several diagnostic imaging modalities to assess myocardial strain and strain rate have been developed in recent years. In addition to tagged magnetic resonance imaging (tagged MRI) and tissue Doppler imaging (TDI), a third method to determine myocardial strain was introduced some years ago. This relatively novel technique is based on two-dimensional echocardiographic images, hence the name: two-dimensional strain echocardiography (2DSTE). It determines myocardial deformation by means of frame-by-frame tracking and motion analysis of speckles within B-mode images using optical flow algorithms. Validation studies with tagged MRI and sonomicrometry in the adult population
have provided evidence that 2DSTE is a reliable method to determine ventricular myocardial function.\textsuperscript{3,4} Despite its limitations, such as sensitivity to signal noise, it has the advantages over TDI-based strain imaging that the strain estimates are not angle-dependent and that strain is obtained in two dimensions.\textsuperscript{4,8,9,10,11} Intraobserver and interobserver reliability scores have been established previously and are high for almost all parameters.\textsuperscript{12} Because this novel technique could be easily accessible and applicable, and moreover is not based on geometric assumptions, it may serve as an important tool in the evaluation of regional and global ventricular function in pediatric subjects with or without CHD.

The establishment of normal values in a healthy pediatric cohort is a mandatory prerequisite for its use in evaluating (pathologic) changes in ventricular function. Data in this area are scarce and include only peak systolic strain in one or two directions.\textsuperscript{13,14} For these reasons, we aimed to evaluate left ventricular myocardial strain in all three directions (longitudinal, circumferential and radial) assessed by means of 2DSTE in a large, healthy pediatric and young adult cohort to establish 2DSTE reference values and to determine the influence of age and growth on these values.

2.3 MATERIAL and METHODS

2.3.1 STUDY POPULATION

Subjects who were routinely referred for echocardiographic evaluation of an asymptomatic, innocent heart murmur between May 1, 2005 and November 1, 2009, were retrospectively analyzed for their eligibility for inclusion in the study. All consecutive healthy subjects, aged from birth to 40 years, were identified from our echocardiographic database from the outpatient clinic at both the Children’s Heart Centre and the Adult Heart Centre, Nijmegen, the Netherlands. Subjects with structural (congenital) heart disease or abnormal cardiac rhythms were excluded.
Other exclusion criteria consisted of hypertension, chronic illness, recent acute illness or poor echocardiographic image quality. Demographic characteristics, including age and gender were collected at the time the echocardiographic study was performed. Informed consent was obtained from each participant. This study was approved by the local medical ethics committee.

2.3.2 2DSTE DATA ACQUISITION

A complete physical examination was performed, including weight, height and blood pressure measurements. Subsequently, all subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography.\textsuperscript{15} Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) phased-array transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). The choice of an S3 or a S5 transducer depended on the age and weight of the subject. Two-dimensional multi-frame B-mode (grayscale) images were obtained in the apical four-chamber (4C), and parasternal midcavity short-axis view (at the level of the papillary muscle: SaxPM) and parasternal basal short-axis view (at the level of the mitral valve: SaxMV).

A sector scan angle of 30 to 60 degrees was chosen and frame rates of 60 to 90 Hz were used, since these rates are considered to be optimal for 2D speckle tracking.\textsuperscript{4,11,16} Data were stored at the same frame rate as the acquisition frame rate. Preferably images from five cardiac cycles triggered by the R wave of the QRS complex were digitally saved in cineloop format. Offline speckle tracking analysis was performed using software for echocardiographic quantification (EchoPAC 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). The timing of aortic valve closure (AVC) and mitral valve opening (MVO) with respect to peak systolic strain were manually obtained, using single gated pulsed-wave (PW-)Doppler or continuous-
wave (CW-) Doppler images of the left ventricular outflow tract. For these measurements, special care was taken to keep the heart rate in the same range as during the 2D grayscale imaging used for 2D strain calculations. Endomyocardial borders of the left ventricle were manually traced within the end-systolic frame. The second, epicardial tracing was automatically generated by the computer algorithm and, when necessary, manually adjusted to cover the whole myocardial wall. The tracking algorithm then followed the myocardial speckles during the cardiac cycle. Tracking was accepted only if both visual inspection as well as the EchoPAC software indicated adequate tracking. This means that tracking of any given segment was accepted only when it was indicated with a green box. The software automatically divided the cross-sectional image into six segments, which were named and identified according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. The left ventricular segments to be analyzed were the apical, middle and basal segments of the septal (ApSept, MidSept, BasSept, respectively) and the lateral wall (ApLat, MidLat, BasLat, respectively) of the 4C view, as well as the anteroseptal (AntSept), anterior (Ant), lateral (Lat), posterior (Post), inferior (Inf) and septal (Sept) segments of the basal and midcavity short-axis views. Strain curves of the three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for further analysis. The Q-Q interval was determined from the electrocardiographic signal to obtain cardiac cycle length. It is known that the duration of the systolic phase of the cardiac cycle in rest does not change with small changes in heart rate, in contrast to the diastolic phase. Therefore, the diastolic phase of the three cardiac cycles was automatically extended and adjusted by the software package to the longest of the three cardiac cycles. This intervention prevents a shift of the peak systolic strain while averaging the three consecutive cardiac cycles. Cardiac cycles with lengths more than ten percent different from the mean length of the three cardiac cycles were excluded from averaging and thus for further analysis. Myocardial longitudinal, radial and circumferential strain values were obtained. To determine global strain, the strain values of the six segments
were averaged for the four-chamber as well as the short-axis views. Strain values are dimensionless and are expressed as percentages. Negative strain values reflect shortening/thinning, while positive strain values reflect lengthening/thickening. All offline measurements with EchoPAC were performed by a single observer (K.A.M.). Interobserver and intraobserver variability was determined by measurement of left ventricular myocardial strain in 40 randomly selected subjects (5 in each age group). To assess intraobserver variability, the same observer (K.A.M.) measured the left ventricular segments again with an interval greater than 2 months to avoid recall bias. To assess interobserver variability, strain measurements were performed by a second observer (M.B.) who was blinded to the results of the first observer (K.A.M.). Figure 7 and 8 show an illustration of 2D strain imaging.

![Illustration of 2D strain imaging](image)

Figure 7 - Short-axis imaging at the level of the papillary muscle with segmentation used for two-dimensional strain echocardiography. Ant: anterior wall; Antsep: anterior septal wall; Inf: inferior wall; Lat: lateral wall; LV: left ventricle; Post: posterior wall; RV: right ventricle; Sep: septal wall.
2.3.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Quantification of cardiac chamber size, ventricular mass and systolic and diastolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography.\textsuperscript{15} Left ventricular systolic function was indicated using fractional shortening (FS), ejection fraction (EF), left ventricular myocardial performance index, or Tei index, end-systolic wall stress (ESWS) and rate-corrected velocity of circumferential fiber...
shortening (VCFc). Ejection fraction (EF) was calculated using the modified Simpson’s rule. The PWD-derived myocardial performance index was calculated by adding the iso-volumetric contraction time (ICT) and the iso-volumetric relaxation time (IVRT), and dividing the sum by the ejection time (ET). \(^{19}\) Left ventricular end-systolic wall stress (ESWS) was calculated using the modified formula of Rowland and Gutgesell. \(^{20}\) VCFc was calculated using the formula obtained from Colan and colleagues. \(^{21}\) Left ventricular mass (LVM) was calculated with the formula for estimation of LVM according to Devereux and Reichek and was subsequently indexed to body surface area (BSA). \(^{22}\)

2.3.4 STATISTICAL ANALYSIS

Patients were divided in eight different age groups for further analysis (see Table 1). All demographic, conventional echocardiographic and 2D strain values are expressed as mean ± standard deviation (SD). The relations between age and global strain parameters were reported using scatterplots, one-way analysis of variance (ANOVA), and second-order polynomial regression analysis. Multiple linear regression analysis was performed to determine the additional effect of anthropometrics (including heart rate, weight and height) and conventional echocardiographic parameters on the myocardial global strain variables.

Intraobserver and interobserver agreement was calculated using the Bland-Altman approach, including the calculation of mean bias (average difference between measurements), the statistical significance of the mean bias on paired t tests (the null hypothesis was zero bias), and the lower and upper limits of agreement (95% limits of agreement of mean bias). In addition, the coefficient of variation was determined (i.e., the standard deviation of the difference of paired samples divided by the average of the paired samples). \(P\)-values less than 0.05 were considered to indicate significance. Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).
2.4 RESULTS

2.4.1 DEMOGRAPHIC AND ANTHROPOMETRIC PARAMETERS

A total of 226 subjects were evaluated for inclusion in the study. Of those subjects, 31 (13.7%) were subsequently excluded in light of incomplete echocardiographic data or suboptimal imaging quality. In total 195 healthy subjects (139 children and 56 young adults) were enrolled in the study. Subject characteristics and anthropometric parameters are described in Table 1.
Table 1 - Demographic and anthropometric characteristics (mean ± standard deviation) of study subjects (n=195) categorized by age group

BSA: body surface area; BMI: body mass index; DiaBP: diastolic blood pressure; HR: heart rate; SysBP: systolic blood pressure.
Table 2 - Conventional echocardiographic parameters (mean ± standard deviation) of study subject (n=195) categorized by age group

EF biplane: ejection fraction measured by modified Simpson’s method; ESWS: end-systolic wall stress; FS: fractional shortening; LVET: left ventricular ejection time; LVETc: LVET corrected for heart rate; LV mass/BSA: left ventricular mass corrected for body surface area; VCFc: heart rate corrected velocity of circumferential fiber shortening.
2.4.2 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional echocardiographic parameters of the study subjects are presented in Table 2. All standard echocardiographic findings were within previously described reference values.

2.4.3 MYOCARDIAL STRAIN PARAMETERS

Myocardial strain parameters are presented in Figures 9 – 11 and Tables 3 - 5. Tracking was feasible in 91% of all segments of the four chamber view, in 96% of all segments in the short-axis view at the level of the papillary muscle and in 91% of all segments in the short-axis view at the level of the mitral valve.

Figure 9 - Global peak systolic longitudinal strain (Global SL) (%) versus age (years) among study subjects (scatter plot). Lines indicate regression line (mean) in the middle and 95% individual prediction interval.
Figure 10 - Midventricular peak systolic circumferential strain (Global SCP) (%) versus age (years) among study subjects (scatter plot). Lines indicate regression line (mean) in the middle and 95% individual prediction interval.
Figure 11 - Midventricular peak systolic radial strain (Global SRP) (%) versus age (years) among study subjects (scatter plot). Lines indicate regression line (mean) in the middle and 95% individual prediction interval.
Table 3 - Longitudinal peak systolic strain (mean ± standard deviation) of study subjects (n=195) categorized by age group

Strain values are presented in percentages; SL: longitudinal peak systolic strain; P5: 5th percentile; P95: 95th percentile; * $P < .05$ when compared with age groups denoted by superscript numerals determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.
Table 4 – Radial peak systolic strain (mean ± standard deviation) of study subjects (n=195) categorized by age group
<table>
<thead>
<tr>
<th>Age group</th>
<th>0 years (1)</th>
<th>1-4 years (2)</th>
<th>5-9 years (3)</th>
<th>10-14 years (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant.Sept. PM</td>
<td>-25.2 ± 5.7</td>
<td>-26.8 ± 3.6</td>
<td>-26.3 ± 2.3</td>
<td>-26.1 ± 3.0</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>-18.2 ± 1.6</td>
<td>-21.3 ± 4.1</td>
<td>-24.4 ± 2.2</td>
<td>-24.1 ± 2.0</td>
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<tr>
<td>Lat. PM</td>
<td>-13.8 ± 2.3</td>
<td>-18.1 ± 2.0</td>
<td>-20.5 ± 1.5</td>
<td>-21.0 ± 1.7</td>
</tr>
<tr>
<td>Post. PM</td>
<td>-10.7 ± 4.0</td>
<td>-16.0 ± 1.9</td>
<td>-19.4 ± 1.1</td>
<td>-20.0 ± 1.4</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>-17.0 ± 6.5</td>
<td>-20.4 ± 2.0</td>
<td>-22.7 ± 2.0</td>
<td>-22.8 ± 1.7</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>-26.5 ± 6.0</td>
<td>-25.3 ± 3.6</td>
<td>-27.2 ± 2.6</td>
<td>-26.9 ± 2.8</td>
</tr>
<tr>
<td><strong>Global S-CP</strong></td>
<td><strong>-18.6 ± 3.3</strong></td>
<td><strong>-21.3 ± 2.0</strong></td>
<td><strong>-23.4 ± 1.7</strong></td>
<td><strong>-23.5 ± 1.8</strong></td>
</tr>
<tr>
<td>P5 Global S-CP</td>
<td>-12.0</td>
<td>-17.3</td>
<td>-20.0</td>
<td>-19.9</td>
</tr>
<tr>
<td>P95 Global S-CP</td>
<td>-25.2</td>
<td>-25.3</td>
<td>-26.8</td>
<td>-27.1</td>
</tr>
<tr>
<td>Ant.Sept. MV</td>
<td>-25.1 ± 3.8</td>
<td>-27.1 ± 3.9</td>
<td>-25.3 ± 3.8</td>
<td>-24.3 ± 3.3</td>
</tr>
<tr>
<td>Ant. MV</td>
<td>-16.5 ± 4.2</td>
<td>-20.3 ± 4.4</td>
<td>-21.1 ± 2.1</td>
<td>-21.8 ± 2.0</td>
</tr>
<tr>
<td>Lat. MV</td>
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<td>-15.4 ± 3.6</td>
<td>-17.4 ± 2.9</td>
<td>-19.2 ± 1.5</td>
</tr>
<tr>
<td>Post. MV</td>
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<td>-12.4 ± 2.7</td>
<td>-15.3 ± 2.9</td>
<td>-18.2 ± 1.2</td>
</tr>
<tr>
<td>Inf. MV</td>
<td>-16.0 ± 7.6</td>
<td>-17.8 ± 3.4</td>
<td>-20.1 ± 2.1</td>
<td>-20.7 ± 1.8</td>
</tr>
<tr>
<td>Sept. MV</td>
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<td>-24.9 ± 4.7</td>
<td>-26.4 ± 3.4</td>
<td>-24.7 ± 2.3</td>
</tr>
<tr>
<td><strong>Global S-CM</strong></td>
<td><strong>-17.5 ± 2.5</strong></td>
<td><strong>-19.7 ± 2.0</strong></td>
<td><strong>-20.9 ± 2.0</strong></td>
<td><strong>-21.5 ± 1.7</strong></td>
</tr>
<tr>
<td>P5 Global S-CM</td>
<td>-12.5</td>
<td>-15.7</td>
<td>-16.9</td>
<td>-18.1</td>
</tr>
<tr>
<td>P95 Global S-CM</td>
<td>-22.5</td>
<td>-23.7</td>
<td>-24.9</td>
<td>-24.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group</th>
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<th>20-24 years (6)</th>
<th>25-29 years (7)</th>
<th>30-40 years (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant.Sept. PM</td>
<td>-27.5 ± 2.8</td>
<td>-25.2 ± 2.1</td>
<td>-24.7 ± 2.5</td>
<td>-24.1 ± 2.0</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>-22.9 ± 2.0</td>
<td>-22.5 ± 1.7</td>
<td>-20.9 ± 2.6</td>
<td>-20.4 ± 2.3</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>-21.1 ± 2.2</td>
<td>-19.7 ± 1.5</td>
<td>-18.6 ± 2.1</td>
<td>-17.8 ± 2.0</td>
</tr>
<tr>
<td>Post. PM</td>
<td>-19.2 ± 2.5</td>
<td>-18.0 ± 1.6</td>
<td>-18.0 ± 2.1</td>
<td>-17.3 ± 2.4</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>-22.4 ± 2.1</td>
<td>-20.9 ± 1.9</td>
<td>-19.6 ± 1.5</td>
<td>-20.7 ± 2.8</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>-28.6 ± 2.5</td>
<td>-24.8 ± 2.8</td>
<td>-24.6 ± 3.2</td>
<td>-23.5 ± 2.7</td>
</tr>
<tr>
<td><strong>Global S-CP</strong></td>
<td><strong>-23.6 ± 2.0</strong></td>
<td><strong>-21.8 ± 1.5</strong></td>
<td><strong>-21.1 ± 1.9</strong></td>
<td><strong>-20.6 ± 2.2</strong></td>
</tr>
<tr>
<td>P5 Global S-CP</td>
<td>-19.6</td>
<td>-18.8</td>
<td>-17.3</td>
<td>-16.2</td>
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<tr>
<td>P95 Global S-CP</td>
<td>-27.6</td>
<td>-24.8</td>
<td>-24.9</td>
<td>-25.0</td>
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<tr>
<td>Ant.Sept. MV</td>
<td>-24.0 ± 2.5</td>
<td>-24.8 ± 2.6</td>
<td>-25.1 ± 4.1</td>
<td>23.2 ± 2.3</td>
</tr>
<tr>
<td>Ant. MV</td>
<td>-22.4 ± 1.9</td>
<td>-20.8 ± 2.0</td>
<td>-20.6 ± 1.5</td>
<td>-19.7 ± 1.6</td>
</tr>
<tr>
<td>Lat. MV</td>
<td>-19.3 ± 2.4</td>
<td>-18.2 ± 1.4</td>
<td>-18.8 ± 1.2</td>
<td>-18.3 ± 1.1</td>
</tr>
<tr>
<td>Post. MV</td>
<td>-17.7 ± 2.0</td>
<td>-18.2 ± 2.1</td>
<td>-16.9 ± 2.0</td>
<td>-16.2 ± 1.2</td>
</tr>
<tr>
<td>Inf. MV</td>
<td>-21.9 ± 2.7</td>
<td>-20.3 ± 1.8</td>
<td>-20.1 ± 1.6</td>
<td>-20.2 ± 2.2</td>
</tr>
<tr>
<td>Sept. MV</td>
<td>-26.0 ± 2.5</td>
<td>-24.0 ± 2.2</td>
<td>-24.3 ± 3.4</td>
<td>-23.4 ± 3.9</td>
</tr>
<tr>
<td><strong>Global S-CM</strong></td>
<td><strong>-21.9 ± 2.1</strong></td>
<td><strong>-21.1 ± 1.3</strong></td>
<td><strong>-21.0 ± 1.6</strong></td>
<td><strong>-20.2 ± 1.4</strong></td>
</tr>
<tr>
<td>P5 Global S-CM</td>
<td>-17.7</td>
<td>-18.5</td>
<td>-17.8</td>
<td>-17.4</td>
</tr>
<tr>
<td>P95 Global S-CM</td>
<td>-26.1</td>
<td>-23.7</td>
<td>-24.2</td>
<td>-23.0</td>
</tr>
</tbody>
</table>

*Table 5 - Circumferential peak systolic strain (mean ± standard deviation) of study subjects (n=195) categorized by age group*
Caption Table 4:

Strain values are presented in percentages; Global S-RM: global radial peak systolic strain at the level of the mitral valve; Global S-RP: global radial peak systolic strain at the level of the papillary muscle; MV: at the level of the mitral valve; PM: at the level of the papillary muscle; P5: 5th percentile; P95: 95th percentile; * P < .05 when compared with age groups denoted by superscript numerals determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.

Caption Table 5:

Strain values are presented in percentages; Global S-CM: global circumferential peak systolic strain at the level of the mitral valve; Global S-CP: global circumferential peak systolic strain at the level of the papillary muscle; MV: at the level of the mitral valve; PM: at the level of the papillary muscle; P5: 5th percentile; P95: 95th percentile; * P < .05 when compared with age groups denoted by superscript numerals determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.

The most striking observation was a quadratic relationship (P<0.001) between global peak systolic parameters and age. This means that global peak systolic strain values were lowest in the youngest and oldest age groups. In contrast to teens, who showed the highest global peak systolic values. There were statistically significant differences (P<0.05) between most of the age groups for all global peak systolic parameters as assessed by means of the one-way analysis of variance with Bonferroni correction for multiple comparisons (Tables 3 - 5). A second-order polynomial regression analysis revealed that age (together with its square) accounted for 37% of variation in global longitudinal peak systolic strain (R²=0.368; P<0.05). Age (and its square) accounted for 22% and 29% of variation in global circumferential peak systolic strain at the level of the papillary muscle (R²=0.222; P<0.05) and mitral valve (R²=0.293; P<0.05) respectively. In the radial direction, age (together with its square) accounted for 12% and 14% of variation in global radial
peak systolic strain at the level of the papillary muscle \( R^2=0.117; P<0.05 \) and mitral valve \( R^2=0.142; P<0.05 \) respectively.

Multiple linear regression analyses among the anthropometric and standard echocardiographic parameters were performed to determine the additional effect of these parameters on the myocardial global strain variables (Table 6). Of these parameters, only VCFc and LVETc appear to be significantly associated with global strain values after adjustment for age. VCFc was associated with global circumferential strain \( P<0.05 \). A 1-unit (i.e., circ/sec) increase of VCFc corresponded to a 1.5% decrease in global circumferential peak systolic strain at the level of the papillary muscle (95% CI: 0.3% - 2.8%) and 1.7% decrease in global circumferential peak systolic strain at the level of the mitral valve (95% CI 0.5% – 2.9%). LVETc was associated with both global circumferential and global longitudinal peak systolic strain \( P<0.05 \). A 1-millisecond increase of LVETc corresponded to a 0.015% decrease in global longitudinal peak systolic strain (95% CI: 0.006% – 0.023%).

As with global peak systolic circumferential strain, a 1-millisecond increase in LVETc corresponded to a 0.05% decrease in global peak systolic circumferential strain at the level of the papillary muscle (95% CI: 0.034% -0.067%) and a 0.024% decrease at the level of the mitral valve (95% CI: 0.009% – 0.039%). No gender-based differences were present. Figure 12 and 13 illustrate peak systolic strain in longitudinal direction versus some of the key morphometric variables.
Table 6 - Coefficient of determination $R^2$

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>GlobalSL</th>
<th>GlobalSCP</th>
<th>GlobalSCM</th>
<th>GlobalSRP</th>
<th>GlobalSRM</th>
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</thead>
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<td><strong>Predictors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age//Age²</td>
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<td>0.22</td>
<td>0.29</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
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<td>0.34</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Age//Age² // VCFc</td>
<td>0.37</td>
<td>0.24</td>
<td>0.31</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Age//Age² // Tei index</td>
<td>0.37</td>
<td>0.23</td>
<td>0.29</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Age//Age² // FS</td>
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<td>0.22</td>
<td>0.29</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Age//Age² // EF biplane</td>
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<td>0.22</td>
<td>0.29</td>
<td>0.13</td>
<td>0.14</td>
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<td>Age//Age² // ESWS</td>
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<td>0.23</td>
<td>0.29</td>
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<td>0.14</td>
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<td>Age//Age² // LVIDs</td>
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<td>0.29</td>
<td>0.12</td>
<td>0.14</td>
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<tr>
<td>Age//Age² // LVIDd</td>
<td>0.37</td>
<td>0.22</td>
<td>0.30</td>
<td>0.12</td>
<td>0.14</td>
</tr>
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</tr>
<tr>
<td>Age//Age² // weight</td>
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<td>0.22</td>
<td>0.29</td>
<td>0.12</td>
<td>0.15</td>
</tr>
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<td>Age//Age² // height</td>
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<td>0.22</td>
<td>0.29</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
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<td>0.22</td>
<td>0.29</td>
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<tr>
<td>Final Model g</td>
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<td>0.28</td>
<td>0.37</td>
<td>0.12</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Predictors used in Final Model f:
- Age//Age² (P<0.0001)
- Age//Age² (P<0.0001)
- Age//Age² (P<0.0001)
- Age//Age² (P<0.0001)
- Age//Age² (P<0.0001)
Caption Table 6:
Predictors: LVETc: LV ejection time corrected for heart rate; VCFc: heart-rate corrected velocity of circumferential fiber shortening; FS: fractional shortening; EF biplane: ejection fraction measured by modified Simpson’s method; ESWS: end-systolic wall stress; LVIDs: LV internal diameter during systole; LVIDd: LV internal diameter during diastole; LVEDV: LV end diastolic volume; HR: heart rate; BMI: body mass index; BSA: body surface area.

Outcome variables: GlobalSL: Global peak systolic longitudinal strain; Global SCP: Global peak systolic circumferential strain at the level of the papillary muscle (midcavity); Global SCM: Global peak systolic circumferential strain at the level of the mitral valve (basal); Global SRP: Global peak systolic radial strain at the level of the papillary muscle (midcavity); Global SRM: Global peak systolic radial strain at the level of the mitral valve (basal).

Each $R^2$ is based on the linear model that uses all subjects with known values for all predictors used in that particular model.

a: $P<0.05$; b: $P<0.01$; c: $P<0.001$; d: $P<0.0001$; e: $P<0.0001$

a, b, c, d: P-value based on partial F-test comparing model with indicated predictors to model using only age and age$^2$ as predictors.

e: P-value based on global F-test for linear model using age and age$^2$ as predictors.

f: P-values based on partial F-test comparing the Final Model to the model based on all variables in Final Model minus the variable of concern.

g: The Final Model uses as predictors age and age$^2$ and the variables that contribute significantly over age and age$^2$, as shown in the upper part of the table, and remained significant in multivariable analysis.
Figure 12 - Global peak systolic longitudinal strain versus various morphometric variables among study objects
Figure 13 - Global peak systolic longitudinal strain versus various morphometric variables among study objects
Caption Figure 12:

Scatterplots with locally weighted line smoothers (loess regression lines) to indicate curved linear relationships.

GlobalSL: Global longitudinal peak systolic strain (%); LV_mass: left ventricular mass (g);
LV_mass_BSA: left ventricular mass corrected for body surface area (g/m²);
LVEDV: left ventricular end diastolic volume (ml); LVESV: left ventricular end systolic volume (ml).

Caption Figure 13:

Scatterplots with locally weighted line smoothers (loess regression lines) to indicate curved linear relationships.

GlobalSL: Global longitudinal peak systolic strain (%); height (m); BSA: body surface area (m²); HR: heart rate (bpm); EF_biplane: left ventricular ejection fraction (%).
The intraobserver and interobserver variability results are shown in Table 7. There were no important differences in variability scores between the various age groups or various segments.

### Table 7 - Intraobserver and interobserver variability of global peak systolic strain

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bias</th>
<th>LLA</th>
<th>ULA</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTRAOBSERVER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global S-L</td>
<td>0.03</td>
<td>-2.89</td>
<td>2.95</td>
<td>7.15</td>
</tr>
<tr>
<td>Global S-CP</td>
<td>-0.39</td>
<td>-3.51</td>
<td>2.74</td>
<td>7.22</td>
</tr>
<tr>
<td>Global S-CM</td>
<td>-0.02</td>
<td>-3.19</td>
<td>3.14</td>
<td>7.89</td>
</tr>
<tr>
<td>Global S-RP</td>
<td>-0.32</td>
<td>-10.01</td>
<td>9.36</td>
<td>8.91</td>
</tr>
<tr>
<td>Global-S-RM</td>
<td>-0.23</td>
<td>-9.19</td>
<td>8.73</td>
<td>8.52</td>
</tr>
<tr>
<td><strong>INTEROBSERVER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global S-L</td>
<td>-0.04</td>
<td>-3.35</td>
<td>3.27</td>
<td>8.12</td>
</tr>
<tr>
<td>Global S-CP</td>
<td>0.46</td>
<td>-3.61</td>
<td>4.54</td>
<td>9.44</td>
</tr>
<tr>
<td>Global S-CM</td>
<td>-0.09</td>
<td>-4.52</td>
<td>4.34</td>
<td>11.04</td>
</tr>
<tr>
<td>Global S-RP</td>
<td>-0.39</td>
<td>-14.78</td>
<td>14.01</td>
<td>13.22</td>
</tr>
<tr>
<td>Global-S-RM</td>
<td>0.28</td>
<td>-13.21</td>
<td>13.78</td>
<td>12.83</td>
</tr>
</tbody>
</table>

Global S-L: global peak systolic longitudinal strain; Global-S-CP: global peak systolic circumferential strain at the level of the papillary muscle; Global-S-CM: global peak systolic circumferential strain at the level of the mitral valve; Global-S-RP: global peak systolic radial strain at the level of the papillary muscle; Global-S-RM: global peak systolic radial strain at the level of the mitral valve;

Bias: mean difference between paired measurements;
LLA: 95% lower limit of agreement;
ULA: 95% upper limit of agreement;
CV: coefficient of variation.
2.5 DISCUSSION

Reference values for 2D strain echocardiography measurements in the pediatric and young adult population are scarce. Two previously published reports have focused on this subject. Lorch et al. investigated 2D strain echocardiography in a large healthy pediatric population.\(^{14}\) Their study provided reference values for 2D longitudinal strain outcomes, however circumferential and radial strain measurements were not included. Bussadori et al. described reference values for circumferential and longitudinal strain and strain rate measurements in a small pediatric (n=15) and adult (n=30) population.\(^{13}\) Data on circumferential and radial 2D strain measurements in a large healthy pediatric and young adult population, to establish their reference values and investigate the influence of age on them, were still lacking.

In the present study, we collected 2D strain echocardiography data in different left ventricular wall segments and in all three directions, longitudinal, radial and circumferential, in a large healthy cohort consisting of children and young adults. Our findings are in disagreement with the previous study performed by Lorch et al. that showed a relative invariability of longitudinal peak systolic strain with age.\(^{14}\) Regression analysis performed on our data showed a statistically significant relationship between age and global peak systolic strain values in all directions. Subjects at both ends of the age spectrum displayed the lowest peak systolic strain values in contrast to teenagers, whose strain values are significantly higher. In several previous studies, the growth and function of the human heart was analyzed by means of echocardiography and ergometry, which gave evidence for an increase in cardiac contractility during puberty.\(^{23,24}\) Increased cardiac contractility is probably one of the most important factors that enables a substantial increase in physical working capacity during puberty.\(^{25}\) The systolic thickness of ventricular walls increases disproportionally when related to the rise in afterload during pubertal development. With this observation, it seems that cardiac systolic function prepares
a reserve, probably for future increase in myocardial energy expenditure due to an increase in functional working capacity. The same relationship between age on one hand and left ventricular mass (LVM) on the other has been reported in healthy, normotensive humans. A study performed by Cain et al. identified age as the major determinant of LVM in healthy subjects.²⁶ LVM strongly increased when children reached pubertal age and subsequently declined during adult life to a level that is approximately 80% of early adulthood despite increasing blood pressure and exercise activity with increasing age in their study population. The underlying biological trigger that causes the sudden surge in cardiac contractility and LVM has not been identified. A possible explanation could be a physiological response as an adaptation process to an increased oxygen demand due to an increasing body mass and functional working tasks. Other factors, such as genetic and hormonal factors should also be considered. There is evidence that hormones, such as growth hormone and sex hormones whose concentrations surge during puberty, influence cardiac growth and systolic function. The heart possesses specific myocardial receptors for growth hormone (GH) and its anabolic mediator, insulin-like growth factor-1 (IGF-1). Both GH and IGF-1 have been reported able to exert an acute positive inotropic effect.²⁷ The administration of recombinant IGF-1 to healthy human subjects has been reported to increase cardiac performance, as assessed by impedance cardiography or echocardiography.²⁸ IGF-1 increases intracellular calcium content and enhances the calcium sensitivity of myofilaments in cardiomyocytes.²⁹,³⁰ Another contributing factor to increasing contractility during puberty could be cardioregulatory actions of sex hormones. Androgen exposure for example has been shown to directly stimulate the contractility of isolated rat ventricular myocytes and thus may play a role in regulating cardiac performance.³¹,³² Similar to testosterone, estrogen has been shown to improve cardiac performance and hemodynamic function.³³,³⁴,³⁵

A remarkable result of our study was the observation of low global peak systolic strain values in infants. Previous reports have not reached consensus on the cardiac
contractile state in this age group compared with older children. Some have concluded that newborn infants have a higher basal contractile state that cannot be accounted for completely by a lower afterload.\textsuperscript{36,37,38} Pena \textit{et al.}, for instance, found higher peak systolic radial and longitudinal strain assessed by means of TDI in healthy neonates during their first day of life compared with a second measurement at the age of one month.\textsuperscript{39} Others have reported lower TDI velocities in infants compared with older children, which is in accordance with the results of invasive animal studies.\textsuperscript{40} These studies describe a lower myocardial contractile state in the newborn age group.\textsuperscript{41} Besides lower global systolic strain results, we also found a greater range of variation in the youngest age group in comparison with older subjects. This variation could be explained by a previously reported greater sensitivity of myocardial performance to changes in afterload.\textsuperscript{38} Indeed, several important hemodynamic changes occur during early neonatal life. Preload increases due to the closure of the ductus arteriosus, with subsequent increase of pulmonary blood flow. A postnatal increment in afterload is related to the removal of the low-resistance placental circulation and increased systemic arterial blood pressure. When taking these alterations in loading conditions into account, it seems likely that they exert at least some influence on myocardial deformation. Therefore, it is important to note that our study population did not include infants below the age of 1 month. Besides hemodynamic and other biological factors, it is possible that (part) of the large variations in myocardial strain in neonates reflect a technical shortcoming of 2DSTE analysis. Thin left ventricular walls of infants with small absolute deformation distances could be more susceptible to error.

After adjustment for age, of the anthropometric and conventional echocardiographic parameters tested in this study only VCFc and LVETc appeared to be significantly associated with global strain values. Unlike the study by Lorch \textit{et al.}, we did not find a strong relation between cardiac size (e.g., left ventricular end diastolic volume) and peak systolic strain parameters.\textsuperscript{14} Although our findings did not indicate one specific anthropometric or hemodynamic variable that explains the
observed differences in myocardial deformation between the various age groups, it is likely that each individual morphometric variable (and their alterations during growth and development) contributes to the overall changes in peak systolic strain which we have observed.

Our results indicate a significant gradient of deformation from base to apex for longitudinal strain in the left ventricular septum and lateral free walls. In addition, circumferential and radial strain increased from base to midventricle as well. These findings are in agreement with those of Bussadori et al.\textsuperscript{13} However, considering that in this latter study a different 2D strain method was used that analyzed only the left side of the septum for longitudinal strain and only the subendocardial layer for circumferential strain, it is interesting to observe that in our study population this base to apex gradient was significant even using speckle tracking that uses kernels distributed throughout the whole thickness of the myocardial wall and septum. In previous studies, different imaging techniques have given variable results regarding the uniformity of left ventricular strain from base to apex. Tagged MRI and 2DSTE studies reported higher strain in the apex, in contrast to tissue velocity imaging reports which did not show significant variation from base to apex.\textsuperscript{42,43,44,45} The latter could be a reflection of the implications of the angle of insonation on apical measurements when using TDI. In addition to technical factors contributing to the base-to-apex gradient of deformation, we consider that it is possible that there exists a physiological substrate in which the base-to-apex gradient is the result of the torsional mechanism of the left ventricular system and the direction of contraction of the descending fibers in the internal loop of helical ventricular myocardial band.

\textbf{2.6 STUDY LIMITATIONS}

This study is limited by its retrospective nature. Our study population did not include infants below the age of one month. Furthermore, the dependence of
2DSTE on frame-by-frame tracking of the myocardial pattern makes it dependent on image factors including reverberation artifacts and attenuation. Indeed, technical proficiency remains important in image processing. Also, we did not investigate radial and circumferential strain at the apical level of the left ventricle. Comparison with an independent external technique, such as tagged MRI, was not performed in the current study, primarily because the validation of speckle-tracking software has already been compared with MRI previously.\textsuperscript{46,47} Overall, the variability of radial peak systolic strain measurement was higher when compared to circumferential and longitudinal measurement, which is in accordance with another study.\textsuperscript{48} However, the reproducibility of peak systolic strain measurements in all three directions of deformation (expressed by intraobserver and interobserver variability scores) was somewhat higher compared with this previous study. In addition, we used custom-made software, which is not commercially available. The custom-made software was specially developed to improve the reliability of timing of peak systolic strain measurements while averaging strain curves, as well as to include peak systolic strain measurements, which occur (shortly) before aortic valve closure. Although the custom-made software is not commercially available, this method can be implemented in generally available software such as Matlab (The MathWorks Inc., Natick, MA; see appendix for instructions).

\textbf{2.7 CONCLUSION}

With this study, we present age-specific reference values for 2D strain echocardiography parameters, which are essential for its use in evaluating (pathologic) changes in ventricular myocardial function. This study shows that age is an important determinant of peak systolic strain values in healthy subjects and therefore emphasizes the need for age-specific reference values for adequate interpretation of 2DSTE measurements.
2.8 APPENDIX

Three consecutive cardiac cycles were analyzed. Results from all views and segments were separately digitally stored into text files on the local Hard Drive of the GE workstation (with disabled drift compensation).

The data files then were exported from the system and stored on the network for further analysis in a custom-made software package “CardiacCurveAnalysisTool” (CCAT) using Matlab version 7.4.0.287 (r2007a).

CUSTOM-MADE SOFTWARE - STEP BY STEP

- automatic load of the three consecutive cardiac cycles results
- up sampling (cubic spline) to 2000 samples per cardiac cycle (in order to be able to select precise time stamps for the QQ-definition)
- interactive QQ-onset defining (on the three consecutive cardiac cycles)
- curve length check (If length difference is >10% then delete longest cycle)
- deletion of data before and after the selected QQ timestamps
- padding zeros (NaN’s) at the end of the diastolic phase for the shortest cycles (to generate equal data lengths)
- drift compensation (using Matlab detrend linear function)
- curve averaging (using Matlab mean function)
- maximum detection
- estimation of averaged peak values per segment

* The custom-made software used in this study was specially developed to improve the timing of systolic deformation (rate) while averaging multiple cardiac cycles. This method does not affect the (average) maximum systolic strain (rate) measurements when compared to commercially available EchoPAC software to determine the average peak systolic strain (rate) values (per cardiac segment).
2.9 REFERENCES


15. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Steward WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18(12):1440-63.


The only reason for time is so that everything doesn’t happen at once

Albert Einstein
Duits-Amerikaans natuurrundige
(1879 - 1955)
Chapter 3
Synchronicity of systolic deformation in healthy pediatric and young adult subjects assessed with two-dimensional strain echocardiography

Karen A. Marcus, Jan Janoušek, Marlieke E. Barends, Gert Weijers, Chris L. de Korte, Livia Kapusta

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3.1 ABSTRACT

Background: Two-dimensional speckle tracking echocardiography (2DSTE) offers valuable information in the echocardiographic assessment of ventricular myocardial function. It enables the quantification and timing of systolic ventricular myocardial deformation. In addition, 2DSTE can be used to identify mechanical dyssynchrony, which is an important parameter in predicting the response to cardiac resynchronization therapy for heart failure. Detailed knowledge of normal timing of systolic deformation and its degree of synchronicity in children is lacking.

Aim: We aimed to establish the normal timing of left ventricular myocardial systolic deformation using 2DSTE in a large cohort of healthy children and young adults.

Methods: Transthoracic echocardiograms were acquired in 195 healthy subjects (139 children and 56 young adult < 40 years of age) and were retrospectively analyzed. Time to peak systolic longitudinal, circumferential and radial strain was determined by means of speckle tracking.

Results: Strong, statistically significant relations between age as well as various anthropometric variables (e.g., heart rate) and timing of systolic deformation \( (P<0.0001) \) were present. The degree of synchronous deformation decreased with age.

Conclusion: This is the first report that establishes reference values per cardiac segment for time to peak systolic myocardial strain values in all three directions assessed with 2DSTE in a large pediatric and young adult cohort. We emphasize the need for using age-specific reference values as well as heart rate correction for the adequate interpretation of 2DSTE measurements.
3.2 BACKGROUND

Coordinated contraction of the left ventricular myocardium is a mandatory prerequisite for an efficient contractile function. In heart failure patients, this contractile function is depressed due to various cellular and extracellular biochemical abnormalities that often lead to asynchronous ventricular contraction. Electromechanical dyssynchrony, in turn, causes a sequence of events that may result in pathological ventricular remodeling and contributes independently to further impairment in global systolic function.\(^1\) Cardiac resynchronization therapy (CRT) is an emerging option for treating dyssynchrony-associated heart failure in patients with pediatric or congenital heart disease. CRT has proven to be beneficial for both the acute manipulation of cardiac output after surgery of congenital heart defects, and for the management of chronic systemic ventricular failure.\(^2,3,4,5,6,7,8,9,10\)

An important issue in CRT that remains is the selection of patients. Current selection criteria (for adults) consist of: (1) symptomatic heart failure, indicated by left ventricular ejection fraction \(\leq 35\%\) and New York Heart Association (NYHA) class II-IV (2) QRS duration \(\geq 120\) (150) msec.\(^{11,12,13,14}\) Results from the MIRACLE trial showed that approximately 30% of the adult patients did not experience any significant clinical response to CRT when current selection criteria were used. In pediatric and congenital heart disease, the non-response rate is 20%.\(^2,9,13\) In fact, the rate of echocardiographic response in adults is even lower than clinical improvement.\(^15\) Previous studies have indicated that electrocardiographic indices, such as QRS duration, are poor predictors of hemodynamic improvement achieved by CRT.\(^{16,17,18,19}\) These parameters have been shown to underestimate intraventricular dyssynchrony in a significant number of patients, whereas mechanical asynchrony is absent in nearly 30% of patients with prolonged QRS duration.\(^{20,21}\) Besides their inaccuracy in predicting hemodynamic and clinical response to CRT, the current adult criteria cannot be easily translated to the pediatric population.\(^22\) However, given the variety of anatomic and functional
substrates subjected to CRT in the pediatric age group, evaluation of mechanical dyssynchrony has become a substantial part of the decision process despite the fact that reference values are lacking.

At present, several (additional) visualization methods of assessing dyssynchrony have been proposed, varying from conventional echocardiographic techniques to more recently developed applications such as two-dimensional strain echocardiography or speckle tracking (2DSTE). This latter echocardiographic technique determines the extent and timing of myocardial deformation by means of frame-by-frame tracking and motion analysis of speckles within the B-mode images using correlation or optical flow search algorithms. Validation studies with tagged MRI and sonomicrometry in the adult population have provided the evidence that 2DSTE is a reliable method to determine ventricular myocardial function. The fact that the estimation of the timing and extent of deformation using 2DSTE is less affected by passive translational motion of the heart or tethering effects of neighboring myocardial segments than velocity-motion based techniques, makes strain an attractive modality to assess regional myocardial dyssynchrony. Previous studies have indicated that speckle tracking provides promising echocardiographic parameters to predict beneficial effects of CRT in adult patients with heart failure.

In healthy adult individuals, the timing of myocardial deformation as well as the extent of asynchronous contraction between various ventricular segments appear to be related to age. Therefore, the application of 2DSTE indices for the assessment of ventricular dyssynchrony is dependent on establishing reference ranges across a wide range of age groups in a large group of healthy individuals. However, data regarding the normal timing of deformation and the extent of synchrony of contraction between ventricular segments in healthy children is lacking. Mapping of normal contraction times in the healthy pediatric heart may give valuable insight into normal contraction patterns and may serve as a reference
for interpreting data of patients considered for CRT and patients with ischemia-induced myocardial damage. Therefore, the primary aim of this study was to establish reference values for the normal timing of left ventricular deformation in pediatric and young adult age groups as assessed with 2DSTE. In addition, we examined the relation between timing of myocardial deformation and multiple anthropometric as well as echocardiographic variables.

3.3 MATERIAL and METHODS

3.3.1 STUDY POPULATION

Subjects that were routinely referred for echocardiographic evaluation of an asymptomatic, innocent heart murmur and screening purposes between May 1, 2005 and November 1, 2009, were retrospectively analyzed for their eligibility for inclusion in the study. All consecutive healthy subjects, aged from birth to 40 years, were identified from our echocardiographic database from the outpatient clinic at both the Children’s Heart Centre and the Adult Heart Centre, Nijmegen, the Netherlands. This study was approved by the local Medical Ethics Committee. Demographic characteristics, including age and gender were collected at the time the echocardiographic study was performed. A complete physical examination was performed, including weight, height and blood pressure measurements. For each subject a 12-lead electrocardiogram was recorded and subsequently evaluated using age-appropriate reference values. Subjects were included only if clinical examination, transthoracic echocardiogram, and electrocardiogram (ECG) showed no evidence of preexisting cardiac disease or other significant coexisting illness. On the 12-lead ECG, an abnormal cardiac rhythm, prolonged QRS duration, bundle branch block, pathological Q waves, ventricular hypertrophy, or changes consistent with myocardial ischemia resulted in exclusion as did evidence of (congenital) structural heart disease, significant valvular abnormality, impaired systolic or diastolic left ventricular function, or left ventricular hypertrophy on the
transthoracic echocardiogram. Other exclusion criteria consisted of hypertension, chronic illness, recent acute illness as well as poor echocardiographic image quality.

3.3.2 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

All subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography. Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) phased-array transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). The choice of an S3 or a S5 transducer depended on the age and weight of the subject. Quantification of cardiac chamber size, ventricular mass and systolic and diastolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography. Left ventricular systolic function was determined using fractional shortening (FS), ejection fraction (EF), left ventricular myocardial performance (Tei-) index, end-systolic wall stress (ESWS), and rate-corrected velocity of circumferential fiber shortening (VCFc). Ejection fraction (EF) was calculated using the modified Simpson’s rule. The pulsed-wave Doppler-derived myocardial performance index was calculated by adding the iso-volumetric contraction time (ICT) and the iso-volumetric relaxation time (IVRT), and dividing the sum by the ejection time (ET). Left ventricular end-systolic wall stress (ESWS) was calculated using the modified formula of Rowland and Gutgesell. VCFc was calculated using the formula obtained from Colan and colleagues. Left ventricular mass (LVM) was calculated using the formula for estimation of LVM according to Devereux and Reichek and was subsequently indexed to body surface area (BSA).
3.3.3 2DSTE DATA ACQUISITION

Two-dimensional multi-frame B-mode (grayscale) images were obtained in the apical four-chamber (4C), and parasternal midcavity short-axis view (at the level of the papillary muscle: SaxPM) and parasternal basal short-axis view (at the level of the mitral valve: SaxMV). A sector scan angle of 30 to 60 degrees was chosen and frame rates of 60 to 90 Hz were used, since these rates are considered to be optimal for 2D speckle tracking.\textsuperscript{24,40,41} Data were stored at the same frame rate as the acquisition frame rate. Preferably images from five cardiac cycles triggered by the R wave of the QRS complex were digitally saved in cineloop format. Offline speckle tracking analysis was performed using software for echocardiographic quantification (EchoPAC 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). The timing of aortic valve closure (AVC) and mitral valve opening (MVO) with respect to peak systolic strain were manually obtained, using single gated pulsed-wave Doppler or continuous-wave Doppler images of the left ventricular outflow tract. For these measurements, special care was taken to keep the heart rate in the same range as during the 2D grayscale imaging used for 2D strain calculations. Endomyocardial borders of the left ventricle were manually traced within the end-systolic frame. The second, epicardial tracing was automatically generated by the EchoPAC software and, when necessary, manually adjusted to cover the whole myocardial wall. The tracking algorithm then followed the myocardial speckles during the cardiac cycle. Tracking was accepted only if both visual inspection as well as the EchoPAC software indicated adequate tracking. This means that tracking of any given segment was accepted only when it was indicated with a green box. The software automatically divided the cross-sectional image into six segments, which were named and identified according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.\textsuperscript{42} The left ventricular segments to be analyzed were the apical, middle and basal segments of the septal (ApSept, MidSept, BasSept, respectively) and the lateral wall (ApLat, MidLat, BasLat, respectively) of the 4C view, as well as the
anteroseptal (AntSept), anterior (Ant), lateral (Lat), posterior (Post), inferior (Inf) and septal (Sept) segments of the basal and midcavity short-axis views. Speckle tracking was performed for all (three) consecutive cycles separately. Strain curves of the three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for further analysis (see Appendix). The Q-Q interval was determined from the electrocardiographic signal to obtain cardiac cycle length. It is known that the duration of the systolic phase of the cardiac cycle in rest does not change with small changes in heart rate, in contrast to the diastolic phase.\(^{43}\) Therefore, the diastolic phase of the three cardiac cycles was automatically extended and adjusted by the software package to the longest of the three cardiac cycles. This intervention prevents a shift of the peak systolic strain while averaging the individually measured three consecutive cardiac cycles. Cardiac cycles with lengths more than ten percent different from the mean length of the three cardiac cycles were excluded from averaging and thus for further analysis. Timing of myocardial longitudinal, radial and circumferential peak systolic strain was obtained. For a parameter \(x\) we determined \(t(x_i)\) as the time interval measured from the beginning of the QRS complex of the surface electrocardiogram to the peak value of the parameter \(x\) in the myocardial segment \(i\) within the analyzed heart cycle period:

\[
t(x_i) = \Delta t [ \text{peak} \ (x_i) - QRS_{ECG} ].
\]

To determine global time to peak systolic strain, the time to peak systolic strain values of the six segments were averaged for the 4C as well as the short-axis views. To measure the extent of synchronicity of regional deformation, a method previously described by Yu et al. was used, which calculates the standard deviation of the time to peak systolic strain measurements.\(^{21}\) An increased index would indicate increased regional heterogeneity with respect to time to peak systolic strain. In addition, the difference in time to peak systolic longitudinal strain between the lateral wall and septum was calculated. Correction for the influence of
variation in heart rate on time to peak systolic strain values was achieved by dividing time to peak systolic strain measurements by the square root of the R-R interval in these subjects (i.e., Bazett’s formula). All offline measurements with EchoPAC were performed by a single observer (K.A.M.). Interobserver and intraobserver variability scores have been described previously by our research group. Time to peak systolic strain is expressed as milliseconds. Figure 14 shows an illustration of strain curves.

**Figure 14 - Radial and circumferential strain curves of the septal wall at the level of the papillary muscle**

![Strain Curves Illustration](image)

ECG: Electrocardiogram; ES: end-systole; SAX-PM: short-axis view at the level of the papillary muscle.
3.3.4 STATISTICAL ANALYSIS

All demographic, conventional echocardiographic and 2DSTE values are expressed as mean ± standard deviation (SD). The relation between different anthropometric and echocardiographic parameters on one hand, and time to (global) strain parameters on the other was reported using scatter plots, one-way analysis of variance (ANOVA) with Bonferroni correction for multiple comparison and linear regression analyses. \( P \)-values less than 0.05 were considered to indicate significance. Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).

3.4 RESULTS

3.4.1 DEMOGRAPHIC AND ANTHROPOMETRIC PARAMETERS

A total of 226 subjects were evaluated for inclusion in the study. Of those subjects, 31 (13.7%) were subsequently excluded in light of incomplete echocardiographic data or suboptimal imaging quality. In total 195 healthy subjects (139 children and 56 young adults) were enrolled in the study. Subject characteristics and anthropometric parameters are described in Table 8. None of the subjects used medication for cardiovascular illness.

3.4.2 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional echocardiographic parameters of the study subjects are presented in Table 9. All standard echocardiographic findings were within previously described reference values for age.
Table 8 - Demographic and anthropometric characteristics (mean ± standard deviation) of study subjects (n=195) categorized by age group

<table>
<thead>
<tr>
<th>Age group No</th>
<th>0 years</th>
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<th>5-9 years</th>
<th>10-14 years</th>
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<tr>
<td>Number</td>
<td>24</td>
<td>34</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Male</td>
<td>13 (54%)</td>
<td>19 (56%)</td>
<td>25 (69%)</td>
<td>16 (55%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.3 ± 0.3</td>
<td>2.9 ± 1.0</td>
<td>7.2 ± 1.2</td>
<td>12.8 ± 1.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.62 ± 0.11</td>
<td>0.95 ± 0.10</td>
<td>1.26 ± 0.09</td>
<td>1.59 ± 0.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6.3 ± 2.6</td>
<td>14.6 ± 3.4</td>
<td>24.7 ± 4.4</td>
<td>46.3 ± 12.0</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.32 ± 0.10</td>
<td>0.62 ± 0.10</td>
<td>0.93 ± 0.12</td>
<td>1.43 ± 0.23</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.9 ± 2.2</td>
<td>15.9 ± 1.4</td>
<td>15.4 ± 1.3</td>
<td>17.9 ± 2.1</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>118 ± 12</td>
<td>101 ± 14</td>
<td>84 ± 13</td>
<td>77 ± 14</td>
</tr>
<tr>
<td>SysBP (mmHg)</td>
<td>82 ± 8</td>
<td>98 ± 10</td>
<td>104 ± 8</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>DiaBP (mmHg)</td>
<td>56 ± 6</td>
<td>62 ± 10</td>
<td>70 ± 8</td>
<td>72 ± 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group No</th>
<th>15-19 years</th>
<th>20-24 years</th>
<th>25-29 years</th>
<th>30-40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>21</td>
<td>25</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Male</td>
<td>9 (43%)</td>
<td>16 (64%)</td>
<td>8 (62%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>17.0 ± 1.3</td>
<td>21.7 ± 1.2</td>
<td>27.3 ± 1.3</td>
<td>35.6 ± 2.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.09</td>
<td>1.78 ± 0.10</td>
<td>1.82 ± 0.08</td>
<td>1.77 ± 0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.4 ± 12.0</td>
<td>70.3 ± 11.9</td>
<td>76.5 ± 11.9</td>
<td>77.8 ± 11.0</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.81 ± 0.20</td>
<td>1.86 ± 0.20</td>
<td>1.96 ± 0.19</td>
<td>1.95 ± 0.18</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.2 ± 2.3</td>
<td>22.1 ± 2.0</td>
<td>23.1 ± 2.2</td>
<td>24.9 ± 2.9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>65 ± 9</td>
<td>63 ± 11</td>
<td>60 ± 14</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>SysBP (mmHg)</td>
<td>116 ± 11</td>
<td>118 ± 12</td>
<td>121 ± 11</td>
<td>123 ± 12</td>
</tr>
<tr>
<td>DiaBP (mmHg)</td>
<td>75 ± 9</td>
<td>75 ± 8</td>
<td>77 ± 8</td>
<td>78 ± 9</td>
</tr>
</tbody>
</table>

BSA: body surface area; BMI: body mass index; DiaBP: diastolic blood pressure; HR: heart rate; SysBP: systolic blood pressure.
### Table 9 - Conventional echocardiographic parameters (mean ± standard deviation) of study subjects (n=195) categorized by age group

<table>
<thead>
<tr>
<th></th>
<th>0 years (n = 24)</th>
<th>1-4 years (n = 34)</th>
<th>5-9 years (n = 36)</th>
<th>10-14 years (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group No</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>LVET (sec)</strong></td>
<td>0.19 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td><strong>LVETc (sec)</strong></td>
<td>0.28 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td><strong>QRS duration (sec)</strong></td>
<td>0.055 ± 0.02</td>
<td>0.057 ± 0.02</td>
<td>0.061 ± 0.04</td>
<td>0.062 ± 0.03</td>
</tr>
<tr>
<td><strong>QTc (sec)</strong></td>
<td>0.411 ± 0.02</td>
<td>0.399 ± 0.02</td>
<td>0.404 ± 0.02</td>
<td>0.407 ± 0.02</td>
</tr>
<tr>
<td><strong>VCFc (circ/sec)</strong></td>
<td>1.32 ± 0.22</td>
<td>1.28 ± 0.20</td>
<td>1.25 ± 0.19</td>
<td>1.19 ± 0.19</td>
</tr>
<tr>
<td><strong>Tei index</strong></td>
<td>0.37 ± 0.01</td>
<td>0.37 ± 0.01</td>
<td>0.38 ± 0.01</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td><strong>FS</strong></td>
<td>0.37 ± 0.05</td>
<td>0.38 ± 0.05</td>
<td>0.37 ± 0.04</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td><strong>EF biplane</strong></td>
<td>0.73 ± 0.07</td>
<td>0.71 ± 0.06</td>
<td>0.67 ± 0.05</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td><strong>ESWS (g/cm²)</strong></td>
<td>30.7 ± 11.0</td>
<td>42.8 ± 13.9</td>
<td>43.6 ± 10.7</td>
<td>45.3 ± 11.6</td>
</tr>
<tr>
<td><strong>LV mass/BSA (g/m²)</strong></td>
<td>36.2 ± 12.1</td>
<td>48.5 ± 11.6</td>
<td>57.2 ± 12.3</td>
<td>59.9 ± 14.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>15-19 years (n = 21)</th>
<th>20-24 years (n = 25)</th>
<th>25-29 years (n = 13)</th>
<th>30-40 years (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group No</strong></td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td><strong>LVET (sec)</strong></td>
<td>0.29 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td><strong>LVETc (sec)</strong></td>
<td>0.30 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td><strong>QRS duration (sec)</strong></td>
<td>0.073 ± 0.02</td>
<td>0.078 ± 0.04</td>
<td>0.082 ± 0.04</td>
<td>0.081 ± 0.05</td>
</tr>
<tr>
<td><strong>QTc (sec)</strong></td>
<td>0.415 ± 0.02</td>
<td>0.418 ± 0.02</td>
<td>0.421 ± 0.02</td>
<td>0.416 ± 0.02</td>
</tr>
<tr>
<td><strong>VCFc (circ/sec)</strong></td>
<td>1.31 ± 0.20</td>
<td>1.21 ± 0.43</td>
<td>1.20 ± 0.43</td>
<td>1.20 ± 0.41</td>
</tr>
<tr>
<td><strong>Tei index</strong></td>
<td>0.40 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.38 ± 0.01</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td><strong>FS</strong></td>
<td>0.39 ± 0.06</td>
<td>0.39 ± 0.07</td>
<td>0.37 ± 0.05</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td><strong>EF biplane</strong></td>
<td>0.67 ± 0.07</td>
<td>0.74 ± 0.04</td>
<td>0.77 ± 0.04</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td><strong>ESWS (g/cm²)</strong></td>
<td>44.4 ± 13.4</td>
<td>40.0 ± 10.4</td>
<td>45.7 ± 16.2</td>
<td>41.8 ± 10.1</td>
</tr>
<tr>
<td><strong>LV mass/BSA (g/m²)</strong></td>
<td>71.8 ± 16.0</td>
<td>78.6 ± 18.0</td>
<td>77.3 ± 19.1</td>
<td>75.4 ± 17.2</td>
</tr>
</tbody>
</table>

EF biplane: ejection fraction measured by modified Simpson’s method; ESWS: end-systolic wall stress; FS: fractional shortening; LVET: left ventricular ejection time; LVETc: LVET corrected for heart rate; LV mass/BSA: left ventricular mass corrected for body surface area; QTc: QT interval corrected for heart rate; VCFc: heart rate corrected velocity of circumferential fiber shortening.
3.4.3 MYOCARDIAL STRAIN PARAMETERS

Tracking was feasible in 91% of all segments of the four-chamber view, in 96% of all segments in the short-axis view at the level of the papillary muscle and in 91% of all segments in the short-axis view at the level of the mitral valve. Left ventricular time to peak systolic strain parameters corrected for heart rate using Bazett’s formula are presented in Tables 10-12. Even after correction for heart rate, statistically significant differences in the timing of peak systolic deformation between various age groups (Tables 10-12) were present, as assessed by one-way analysis of variance with Bonferroni correction for multiple comparisons. The time to reach maximum strain during systole increased until the age of ten years in all three principal directions of contraction. After this age, 2DSTE measurements remained fairly stable. In the longitudinal plane, time to peak systolic strain was shorter at the apex compared with the base of the ventricle (Table 10). This pattern was also shown in the circumferential and radial plane, where time to reach peak systolic strain was shorter midventricular compared with the base of the ventricle. In addition, time to reach peak systolic strain in all three directions of contraction was shorter in the septum compared with the lateral free wall (Tables 10-12).

No significant sex-related differences in time to peak systolic strain were found. Figures 15-18 show the degree of synchronous deformation among left ventricular myocardial segments in longitudinal, circumferential and radial direction according to age. Even after correction for heart rate, the degree of synchrony decreased significantly with age (Table 13 and Figures 15-18). Our data indicate that the degree of synchrony decreases with age and body growth (indices) (Table 14). There were no statistically significant correlations between synchronicity parameters and FS or LVEF. QRS duration, QT interval corrected for heart rate (QTc) and blood pressure did not correlate with the strain parameters under investigation (Table 14).
Table 10 - Time to peak systolic longitudinal strain corrected for heart rate (mean ± standard deviation) of study subjects (n=195) categorized by age group

<table>
<thead>
<tr>
<th>No. age group</th>
<th>0 years</th>
<th>1-4 years</th>
<th>5-9 years</th>
<th>10-14 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>24</td>
<td>34</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Bas. Sept.</td>
<td>362 ± 20</td>
<td>394 ± 22</td>
<td>424 ± 25</td>
<td>425 ± 28</td>
</tr>
<tr>
<td>Mid. Sept.</td>
<td>334 ± 18</td>
<td>360 ± 21</td>
<td>390 ± 24</td>
<td>392 ± 26</td>
</tr>
<tr>
<td>Apic. Sept.</td>
<td>313 ± 16</td>
<td>342 ± 17</td>
<td>368 ± 18</td>
<td>368 ± 20</td>
</tr>
<tr>
<td>Apic. Lat.</td>
<td>329 ± 16</td>
<td>358 ± 17</td>
<td>382 ± 17</td>
<td>380 ± 20</td>
</tr>
<tr>
<td>Mid. Lat.</td>
<td>352 ± 17</td>
<td>384 ± 19</td>
<td>400 ± 21</td>
<td>413 ± 22</td>
</tr>
<tr>
<td>Bas. Lat.</td>
<td>372 ± 19</td>
<td>401 ± 21</td>
<td>435 ± 23</td>
<td>439 ± 24</td>
</tr>
<tr>
<td>Global T2P SL</td>
<td>341 ± 18*</td>
<td>373 ± 19*</td>
<td>400 ± 20*</td>
<td>402 ± 23*</td>
</tr>
<tr>
<td>P5 Global T2P SL</td>
<td>305</td>
<td>335</td>
<td>361</td>
<td>356</td>
</tr>
<tr>
<td>P95 Global T2P SL</td>
<td>377</td>
<td>411</td>
<td>439</td>
<td>448</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. age group</th>
<th>15-19 years</th>
<th>20-24 years</th>
<th>25-29 years</th>
<th>30-40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>21</td>
<td>25</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Bas. Sept.</td>
<td>433 ± 26</td>
<td>422 ± 29</td>
<td>443 ± 25</td>
<td>453 ± 33</td>
</tr>
<tr>
<td>Mid. Sept.</td>
<td>400 ± 25</td>
<td>388 ± 24</td>
<td>397 ± 26</td>
<td>422 ± 28</td>
</tr>
<tr>
<td>Apic. Sept.</td>
<td>370 ± 19</td>
<td>362 ± 21</td>
<td>380 ± 24</td>
<td>362 ± 27</td>
</tr>
<tr>
<td>Apic. Lat.</td>
<td>392 ± 20</td>
<td>374 ± 23</td>
<td>392 ± 26</td>
<td>385 ± 30</td>
</tr>
<tr>
<td>Mid. Lat.</td>
<td>417 ± 24</td>
<td>395 ± 25</td>
<td>422 ± 28</td>
<td>440 ± 30</td>
</tr>
<tr>
<td>Bas. Lat.</td>
<td>445 ± 25</td>
<td>448 ± 29</td>
<td>454 ± 23</td>
<td>468 ± 32</td>
</tr>
<tr>
<td>Global T2P SL</td>
<td>410 ± 23*</td>
<td>398 ± 24*</td>
<td>415 ± 25*</td>
<td>422 ± 31*</td>
</tr>
<tr>
<td>P5 Global T2P SL</td>
<td>364</td>
<td>350</td>
<td>365</td>
<td>360</td>
</tr>
<tr>
<td>P95 Global T2P SL</td>
<td>456</td>
<td>446</td>
<td>465</td>
<td>484</td>
</tr>
</tbody>
</table>

Time values are presented in milliseconds (ms); SL: longitudinal peak systolic strain; T2P: time to peak systolic strain; correction for heart rate was calculated by dividing the time to peak systolic strain by √R-R interval for each individual; * P < .05 when compared with age groups denoted by superscript numerals, determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.
Table 11 - Time to peak systolic radial strain corrected for heart rate (mean ± standard deviation) of study subjects (n=195) categorized by age group

<table>
<thead>
<tr>
<th>No. age group</th>
<th>0 years</th>
<th>1-4 years</th>
<th>5-9 years</th>
<th>10-14 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>24</td>
<td>34</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Ant.Sept. PM</td>
<td>332 ± 16</td>
<td>372 ± 20</td>
<td>381 ± 23</td>
<td>380 ± 24</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>343 ± 15</td>
<td>381 ± 22</td>
<td>404 ± 24</td>
<td>387 ± 23</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>379 ± 20</td>
<td>412 ± 23</td>
<td>431 ± 25</td>
<td>430 ± 27</td>
</tr>
<tr>
<td>Post. PM</td>
<td>381 ± 19</td>
<td>414 ± 22</td>
<td>434 ± 24</td>
<td>437 ± 23</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>353 ± 18</td>
<td>390 ± 20</td>
<td>416 ± 25</td>
<td>413 ± 23</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>325 ± 16</td>
<td>340 ± 21</td>
<td>355 ± 22</td>
<td>361 ± 24</td>
</tr>
<tr>
<td>Global T2P S-RP</td>
<td>352 ± 18</td>
<td>384 ± 22</td>
<td>404 ± 24*</td>
<td>401 ± 25*</td>
</tr>
<tr>
<td>P5 Global T2P S-RP</td>
<td>316</td>
<td>340</td>
<td>356</td>
<td>351</td>
</tr>
<tr>
<td>P95 Global T2P S-RP</td>
<td>388</td>
<td>428</td>
<td>452</td>
<td>451</td>
</tr>
<tr>
<td>Ant.Sept. MV</td>
<td>372 ± 24</td>
<td>401 ± 23</td>
<td>420 ± 21</td>
<td>418 ± 25</td>
</tr>
<tr>
<td>Ant. MV</td>
<td>392 ± 25</td>
<td>409 ± 26</td>
<td>431 ± 22</td>
<td>426 ± 26</td>
</tr>
<tr>
<td>Lat. MV</td>
<td>419 ± 31</td>
<td>449 ± 28</td>
<td>479 ± 25</td>
<td>473 ± 29</td>
</tr>
<tr>
<td>Post. MV</td>
<td>429 ± 30</td>
<td>457 ± 28</td>
<td>488 ± 30</td>
<td>493 ± 30</td>
</tr>
<tr>
<td>Inf. MV</td>
<td>417 ± 22</td>
<td>447 ± 22</td>
<td>455 ± 32</td>
<td>464 ± 30</td>
</tr>
<tr>
<td>Sept. MV</td>
<td>370 ± 21</td>
<td>399 ± 20</td>
<td>410 ± 22</td>
<td>414 ± 25</td>
</tr>
<tr>
<td>Global T2P S-RM</td>
<td>400 ± 22</td>
<td>427 ± 24</td>
<td>447 ± 27</td>
<td>448 ± 28</td>
</tr>
<tr>
<td>P5 Global T2P S-RM</td>
<td>356</td>
<td>379</td>
<td>393</td>
<td>392</td>
</tr>
<tr>
<td>P95 Global T2P S-RM</td>
<td>444</td>
<td>475</td>
<td>501</td>
<td>504</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. age group</th>
<th>15-19 years</th>
<th>20-24 years</th>
<th>25-29 years</th>
<th>30-40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>21</td>
<td>25</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Ant.Sept. PM</td>
<td>370 ± 21</td>
<td>391 ± 25</td>
<td>386 ± 27</td>
<td>395 ± 29</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>389 ± 25</td>
<td>408 ± 26</td>
<td>406 ± 31</td>
<td>416 ± 29</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>425 ± 27</td>
<td>448 ± 29</td>
<td>443 ± 30</td>
<td>458 ± 33</td>
</tr>
<tr>
<td>Post. PM</td>
<td>433 ± 26</td>
<td>458 ± 27</td>
<td>461 ± 29</td>
<td>458 ± 32</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>414 ± 24</td>
<td>424 ± 25</td>
<td>431 ± 29</td>
<td>442 ± 31</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>360 ± 23</td>
<td>372 ± 24</td>
<td>370 ± 27</td>
<td>369 ± 28</td>
</tr>
<tr>
<td>Global T2P S-RP</td>
<td>399 ± 25 *1</td>
<td>417 ± 26 *1</td>
<td>416 ± 29 *1,2</td>
<td>423 ± 30 *1,2</td>
</tr>
<tr>
<td>P5 Global T2P S-RP</td>
<td>349</td>
<td>366</td>
<td>358</td>
<td>363</td>
</tr>
<tr>
<td>P95 Global T2P S-RP</td>
<td>449</td>
<td>470</td>
<td>474</td>
<td>483</td>
</tr>
<tr>
<td>Ant.Sept. MV</td>
<td>412 ± 25</td>
<td>428 ± 26</td>
<td>427 ± 29</td>
<td>418 ± 30</td>
</tr>
<tr>
<td>Ant. MV</td>
<td>423 ± 27</td>
<td>430 ± 30</td>
<td>439 ± 30</td>
<td>484 ± 31</td>
</tr>
<tr>
<td>Lat. MV</td>
<td>460 ± 31</td>
<td>486 ± 33</td>
<td>483 ± 30</td>
<td>492 ± 36</td>
</tr>
<tr>
<td>Post. MV</td>
<td>488 ± 33</td>
<td>494 ± 35</td>
<td>495 ± 35</td>
<td>499 ± 38</td>
</tr>
<tr>
<td>Inf. MV</td>
<td>474 ± 31</td>
<td>482 ± 33</td>
<td>483 ± 33</td>
<td>492 ± 34</td>
</tr>
<tr>
<td>Sept. MV</td>
<td>402 ± 22</td>
<td>410 ± 28</td>
<td>406 ± 30</td>
<td>400 ± 38</td>
</tr>
<tr>
<td>Global T2P S-RM</td>
<td>443 ± 30</td>
<td>456 ± 33 *1</td>
<td>457 ± 33 *2</td>
<td>464 ± 36 *1</td>
</tr>
<tr>
<td>P5 Global T2P S-RM</td>
<td>383</td>
<td>390</td>
<td>391</td>
<td>392</td>
</tr>
<tr>
<td>P95 Global T2P S-RM</td>
<td>503</td>
<td>522</td>
<td>523</td>
<td>536</td>
</tr>
</tbody>
</table>
Table 12 - Time to peak systolic circumferential strain corrected for heart rate (mean ± standard deviation) of study subjects (n=195) categorized by age group

<table>
<thead>
<tr>
<th>No. age group</th>
<th>0 years</th>
<th>1-4 years</th>
<th>5-9 years</th>
<th>10-14 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>N =</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Ant.Sept. PM</td>
<td>317 ± 17</td>
<td>376 ± 20</td>
<td>384 ± 24</td>
<td>379 ± 26</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>332 ± 20</td>
<td>386 ± 22</td>
<td>389 ± 25</td>
<td>387 ± 27</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>368 ± 23</td>
<td>419 ± 26</td>
<td>439 ± 28</td>
<td>426 ± 29</td>
</tr>
<tr>
<td>Post. PM</td>
<td>367 ± 24</td>
<td>440 ± 27</td>
<td>441 ± 28</td>
<td>446 ± 28</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>337 ± 18</td>
<td>407 ± 23</td>
<td>411 ± 25</td>
<td>421 ± 27</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>316 ± 17</td>
<td>367 ± 21</td>
<td>366 ± 24</td>
<td>367 ± 27</td>
</tr>
<tr>
<td>Global T2P S-CP</td>
<td>339 ± 18</td>
<td>399 ± 23 *1</td>
<td>405 ± 25 *1</td>
<td>404 ± 27 *1</td>
</tr>
<tr>
<td>P95 Global T2P S-CP</td>
<td>375</td>
<td>445</td>
<td>455</td>
<td>458</td>
</tr>
<tr>
<td>Ant.Sept. MV</td>
<td>338 ± 16</td>
<td>361 ± 22</td>
<td>377 ± 25</td>
<td>381 ± 29</td>
</tr>
<tr>
<td>Ant. MV</td>
<td>351 ± 20</td>
<td>375 ± 24</td>
<td>391 ± 26</td>
<td>400 ± 30</td>
</tr>
<tr>
<td>Lat. MV</td>
<td>385 ± 25</td>
<td>415 ± 27</td>
<td>436 ± 25</td>
<td>442 ± 35</td>
</tr>
<tr>
<td>Post. MV</td>
<td>402 ± 26</td>
<td>423 ± 27</td>
<td>442 ± 31</td>
<td>449 ± 34</td>
</tr>
<tr>
<td>Inf. MV</td>
<td>364 ± 20</td>
<td>400 ± 26</td>
<td>428 ± 30</td>
<td>443 ± 34</td>
</tr>
<tr>
<td>Sept. MV</td>
<td>332 ± 20</td>
<td>353 ± 23</td>
<td>375 ± 26</td>
<td>372 ± 29</td>
</tr>
<tr>
<td>Global T2P S-CM</td>
<td>362 ± 22</td>
<td>388 ± 25 *8</td>
<td>408 ± 27 *8</td>
<td>415 ± 30 *8</td>
</tr>
<tr>
<td>P5 Global T2P S-CM</td>
<td>318</td>
<td>338</td>
<td>354</td>
<td>355</td>
</tr>
<tr>
<td>P95 Global T2P S-CM</td>
<td>406</td>
<td>438</td>
<td>462</td>
<td>417</td>
</tr>
<tr>
<td>15-19 years</td>
<td>5</td>
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<td>7</td>
<td>8</td>
</tr>
<tr>
<td>N = 21</td>
<td>25</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ant.Sept. PM</td>
<td>366 ± 28</td>
<td>365 ± 30</td>
<td>384 ± 31</td>
<td>384 ± 35</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>373 ± 28</td>
<td>368 ± 33</td>
<td>395 ± 29</td>
<td>395 ± 39</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>420 ± 26</td>
<td>425 ± 34</td>
<td>451 ± 34</td>
<td>455 ± 42</td>
</tr>
<tr>
<td>Post. PM</td>
<td>428 ± 31</td>
<td>439 ± 34</td>
<td>469 ± 36</td>
<td>463 ± 42</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>422 ± 29</td>
<td>409 ± 32</td>
<td>429 ± 35</td>
<td>440 ± 34</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>362 ± 27</td>
<td>362 ± 30</td>
<td>381 ± 28</td>
<td>370 ± 36</td>
</tr>
<tr>
<td>Global T2P S-CP</td>
<td>395 ± 28 *1</td>
<td>395 ± 30 *1</td>
<td>419 ± 31 *1</td>
<td>418 ± 35 *1</td>
</tr>
<tr>
<td>P5 Global T2P S-CP</td>
<td>339</td>
<td>335</td>
<td>357</td>
<td>348</td>
</tr>
<tr>
<td>P95 Global T2P S-CP</td>
<td>451</td>
<td>455</td>
<td>481</td>
<td>488</td>
</tr>
<tr>
<td>20-24 years</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>N = 21</td>
<td>25</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ant.Sept. PM</td>
<td>366 ± 28</td>
<td>365 ± 30</td>
<td>384 ± 31</td>
<td>384 ± 35</td>
</tr>
<tr>
<td>Ant. PM</td>
<td>373 ± 28</td>
<td>368 ± 33</td>
<td>395 ± 29</td>
<td>395 ± 39</td>
</tr>
<tr>
<td>Lat. PM</td>
<td>420 ± 26</td>
<td>425 ± 34</td>
<td>451 ± 34</td>
<td>455 ± 42</td>
</tr>
<tr>
<td>Post. PM</td>
<td>428 ± 31</td>
<td>439 ± 34</td>
<td>469 ± 36</td>
<td>463 ± 42</td>
</tr>
<tr>
<td>Inf. PM</td>
<td>422 ± 29</td>
<td>409 ± 32</td>
<td>429 ± 35</td>
<td>440 ± 34</td>
</tr>
<tr>
<td>Sept. PM</td>
<td>362 ± 27</td>
<td>362 ± 30</td>
<td>381 ± 28</td>
<td>370 ± 36</td>
</tr>
<tr>
<td>Global T2P S-CM</td>
<td>395 ± 28 *1</td>
<td>395 ± 30 *1</td>
<td>419 ± 31 *1</td>
<td>418 ± 35 *1</td>
</tr>
<tr>
<td>P5 Global T2P S-CM</td>
<td>339</td>
<td>335</td>
<td>357</td>
<td>348</td>
</tr>
<tr>
<td>P95 Global T2P S-CM</td>
<td>451</td>
<td>455</td>
<td>481</td>
<td>488</td>
</tr>
</tbody>
</table>

Corrected for heart rate (mean ± standard deviation) of study subjects (n=195) categorized by age group.
Caption Table 11:

Time values are presented in milliseconds (ms); MV: at the level of the mitral valve; PM: at the level of the papillary muscle; S-RM: radial peak systolic strain at the level of the mitral valve; S-RP: radial peak systolic strain at the level of the papillary muscle; T2P: time to peak systolic strain; correction for heart rate was calculated by dividing the time to peak systolic strain by $\sqrt{R-R}$ interval for each individual; * $P < .05$ when compared with age groups denoted by superscript numerals, determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.

Caption Table 12:

Time values are presented in milliseconds (ms); MV: at the level of the mitral valve; PM: at the level of the papillary muscle; S-CM: circumferential peak systolic strain at the level of the mitral valve; S-CP: circumferential peak systolic strain at the level of the papillary muscle; T2P: time to peak systolic strain; correction for heart rate was calculated by dividing the time to peak systolic strain by $\sqrt{R-R}$ interval for each individual; * $P < .05$ when compared with age groups denoted by superscript numerals, determined by means of one-way analysis of variance with Bonferroni correction for multiple comparisons.
Table 13 - Coefficient of determination $R^2$

<table>
<thead>
<tr>
<th>Rate uncorrected outcome variable</th>
<th>Age</th>
<th>Heart Rate</th>
<th>$\sqrt{R^2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diff. Sep-Lat S-L</td>
<td>0.61 *</td>
<td>0.21 *</td>
<td>0.33 *</td>
</tr>
<tr>
<td>SD T2P S-L</td>
<td>0.58 *</td>
<td>0.19 *</td>
<td>0.34 *</td>
</tr>
<tr>
<td>SD T2P S-RP</td>
<td>0.63 *</td>
<td>0.20 *</td>
<td>0.32 *</td>
</tr>
<tr>
<td>SD T2P S-RM</td>
<td>0.61 *</td>
<td>0.22 *</td>
<td>0.33 *</td>
</tr>
<tr>
<td>SD T2P S-CP</td>
<td>0.47 *</td>
<td>0.18 *</td>
<td>0.29 *</td>
</tr>
<tr>
<td>SD T2P S-CM</td>
<td>0.60 *</td>
<td>0.17 *</td>
<td>0.29 *</td>
</tr>
</tbody>
</table>

Each $R^2$ is based on the linear model that uses all subjects with known values for the predictor used in that particular model. T2P: time to peak; Diff.Sep-Lat: difference in T2P between septum and lateral wall; SD T2P: synchrony index (standard deviation); S: peak systolic global strain; L: longitudinal; R: radial; C: circumferential; P: at the level of the papillary muscle; M: at the level of the mitral valve; * $P<0.001$. 

88 | Chapter 3
Table 14 - Coefficient of determination $R^2$

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Outcome variable Corrected for heart rate</th>
<th>Age</th>
<th>BSA</th>
<th>BMI</th>
<th>LV Mass/BSA</th>
<th>LVIDs</th>
<th>LVESV</th>
<th>ESWS</th>
<th>QRS duration</th>
<th>QTc</th>
<th>Syst. BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diff. S-L / VR-R</td>
<td>0.43$^d$</td>
<td>0.28$^d$</td>
<td>0.26$^d$</td>
<td>0.15$^d$</td>
<td>0.18$^d$</td>
<td>0.20$^d$</td>
<td>0.01</td>
<td>0.12$^d$</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>SD T2P S-L / VR-R</td>
<td>0.40$^d$</td>
<td>0.30$^d$</td>
<td>0.21$^d$</td>
<td>0.12$^d$</td>
<td>0.24$^d$</td>
<td>0.27$^d$</td>
<td>0.04$^b$</td>
<td>0.13$^d$</td>
<td>0.04$^b$</td>
<td>0.07$^c$</td>
<td></td>
</tr>
<tr>
<td>SD T2P S-RP / VR-R</td>
<td>0.49$^d$</td>
<td>0.36$^d$</td>
<td>0.26$^d$</td>
<td>0.14$^d$</td>
<td>0.23$^d$</td>
<td>0.23$^d$</td>
<td>0.01</td>
<td>0.15$^d$</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>SD T2P S-RM / VR-R</td>
<td>0.42$^d$</td>
<td>0.22$^d$</td>
<td>0.13$^d$</td>
<td>0.14$^d$</td>
<td>0.20$^d$</td>
<td>0.13$^d$</td>
<td>0.01</td>
<td>0.14$^d$</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>SD T2P S-CP / VR-R</td>
<td>0.33$^d$</td>
<td>0.23$^d$</td>
<td>0.13$^d$</td>
<td>0.08$^d$</td>
<td>0.17$^d$</td>
<td>0.14$^d$</td>
<td>0.01</td>
<td>0.17$^d$</td>
<td>0.03$^a$</td>
<td>0.03$^a$</td>
<td></td>
</tr>
<tr>
<td>SD T2P S-CM / VR-R</td>
<td>0.42$^d$</td>
<td>0.34$^d$</td>
<td>0.29$^d$</td>
<td>0.24$^d$</td>
<td>0.34$^d$</td>
<td>0.32$^d$</td>
<td>0.01</td>
<td>0.15$^d$</td>
<td>0.04$^b$</td>
<td>0.05$^b$</td>
<td></td>
</tr>
</tbody>
</table>

Each $R^2$ is based on the linear model that uses all subjects with known values for the predictor used in that particular model.

a: $P<0.05$; b: $P<0.01$; c: $P<0.001$; d: $P<0.0001$; BSA: body surface area; BMI: body mass index; LV mass/BSA: left ventricular mass corrected for body surface area; LVIDs: left ventricular internal diameter at end systole; LVEDV: left ventricular end diastolic volume; ESWS: end-systolic wall stress; QTc: QT duration corrected for heart rate using Bazett’s method; Syst. BP: systolic blood pressure; T2P: time to peak; Diff. Sep-Lat: difference in T2P between septum and lateral wall; SD T2P: synchrony index (standard deviation); S: peak systolic global strain; L: longitudinal; R: radial; C: circumferential; P: at the level of the papillary muscle; M: at the level of the mitral valve; all outcome variables are corrected for heart rate by dividing the timing measurements by the square root of the R-R interval of that particular subject.
Figure 15 – Difference in time to peak systolic longitudinal strain between lateral wall and septum corrected for heart rate with Bazett’s formula

Age in years; DiffT2PSepLatCorrected: difference in time to peak systolic longitudinal strain between lateral wall and septum in milliseconds corrected for heart rate by Bazett’s formula; lines indicate regression line (mean) in the middle and 95% individual prediction interval.
Figure 16 – Synchronicity index: standard deviation of time to peak systolic longitudinal strain corrected for heart rate with Bazett’s formula according to age

Age in years; deviationT2PS_L Corrected: standard deviation of time to peak systolic longitudinal strain in milliseconds corrected for heart rate by Bazett’s formula; lines indicate regression line (mean) in the middle and 95% individual prediction interval.
Figure 17 – Synchronicity index: standard deviation of time to midventricular peak systolic circumferential strain corrected for heart rate with Bazett's formula according to age

Age in years; deviationT2PSCP Corrected: standard deviation of time to peak systolic circumferential strain at the level of the papillary muscle in milliseconds corrected for heart rate by Bazett's formula; lines indicate regression line (mean) in the middle and 95% individual prediction interval.
3.5 DISCUSSION

Electromechanical dyssynchrony, with early and late contracting ventricular myocardial segments, results in inefficient ventricular performance. Late contracting segments are stretched by the early contracting regions and perform a higher local myocardial workload. This workload is to some part wasted because late contraction appears after semilunar valve closure and at the end of the ventricular ejection phase.\textsuperscript{9,49} The uneven distribution of work load leads to changes in both regional blood flow and metabolism in addition to pathologic remodeling.\textsuperscript{50}
Oosterhout et al. showed development of asymmetric myocardial hypertrophy due to electromechanical dyssynchrony with decrease in regional wall thickness and volume at the early contracting sites and increase in the areas of late contraction.\textsuperscript{51} Together, these series of events lead to reduced overall cardiac efficiency and increases myocardial energy demands, which eventually result in (progression of) heart failure.\textsuperscript{52} Furthermore, dyssynergistic ventricular contraction and relaxation have been associated with a pro-arrhythmogenic effect.\textsuperscript{53}

Several visualization methods of assessing the extent of ventricular dyssynchrony have been proposed to overcome the above-mentioned limitations, varying from conventional echocardiographic techniques to more novel applications such as Tissue Doppler imaging (TDI) and two-dimensional strain echocardiography. TDI initially showed promising results for the assessment of dyssynchrony in small clinical trials. However, TDI is limited by several characteristics of Doppler-based techniques. Firstly, TDI measures only the vector of motion that is parallel to the direction of the ultrasound beam and is thus inherently angle-dependent. Secondly, TDI measures absolute tissue velocity and is unable to discriminate passive motion (related to translation or tethering) from active motion (fiber shortening or lengthening). Recently, large, prospective randomized clinical trials have shown that TDI is inadequate to predict response from CRT in patients with heart failure and is associated with considerable intraobserver and interobserver variability.\textsuperscript{26,54,55}

Strain imaging derived from two-dimensional speckle tracking echocardiography enables quantification of regional myocardial function without tethering effect and Doppler angle-dependency. It measures the extent and timing of regional myocardial deformation with high temporal and spatial resolution. Although 2DSTE is sensitive to signal noise, it may facilitate the detection of ventricular dyssynchrony and the prediction of outcome after CRT.\textsuperscript{26} A prospectively designed study, performed by Suffoletto et al., showed that 2DSTE is able to quantify dyssynchrony and predict immediate and long-term response to CRT with good
results. In addition, they demonstrated the feasibility of 2DSTE to detect the site of latest mechanical activation. Identification of the most delayed segment is of importance, since previous reports have indicated that left ventricular lead placement at the most delayed segment resulted in the greatest immediate improvements from CRT.\textsuperscript{27,56} Previous speckle tracking studies, in pediatric patients, have revealed abnormal timing of systolic deformation in various congenital and acquired cardiac conditions. These studies have indicated that various pathological conditions are associated with a prolonged required amount of time to reach maximum peak systolic strain, in addition to a greater extent of inter- and intraventricular dyssynchronous deformation.\textsuperscript{57,58,59,60,61} If validated by additional prospective studies, 2DSTE may prove particularly helpful in selecting appropriate candidates with congenital heart disease for CRT in whom conventional criteria are not applicable. However, the application of 2DSTE indices in the assessment of ventricular dyssynchrony is dependent on establishing reference values across a wide range of age groups; since both timing of myocardial deformation and the extent of dyssynchrony between various ventricular segments appear to be related to age.\textsuperscript{28,29,30}

While mapping of normal contraction times in the healthy (pediatric) heart may give valuable insight into normal contraction patterns, data regarding the normal timing of deformation and the extent of synchrony of contraction between ventricular segments in healthy children and young adults were lacking. Therefore, in the present study reference values for the normal timing of left ventricular deformation in the pediatric and young adult age groups were assessed using 2DSTE.

Our results demonstrated a significant correlation between time to peak systolic strain and age, as well as heart rate. The influence of heart rate on strain measurements was previously described by others in a TDI study.\textsuperscript{62} Since heart rate decreases with age, we subsequently assessed the relationship between age and time to peak systolic strain after correction for heart rate. As expected, the present
study identified strong relationships between timing of myocardial deformation and age, as well as multiple anthropometric variables and echocardiographic indices. No significant sex-related differences in time to peak systolic strain were found. Surprisingly, we did not find a significant relation between timing of left ventricular deformation and conventional echocardiographic functional indices such as left ventricular ejection fraction, fractional shortening, and end-systolic wall stress, neither after correction of timing of deformation for heart rate. These findings are in contrast to those of Rosen et al. A possible explanation for these contradictory findings could be that our study included only subjects free from risk factors for cardiac disease in contrast to the study by Rosen et al. where the majority of subjects had cardiovascular risk factors (such as smoking, diabetes mellitus, hypertension, and obesity). Conventional echocardiographic functional indices in our study showed little variation, which could offer an explanation for the absence of a relation between these conventional parameters and 2DSTE timing indices. In all age groups, time to peak systolic longitudinal strain was shorter in the septum than in the lateral wall, which is in accordance to previous reports. Zwanenburg et al. showed an opposite spatial pattern of the onset of longitudinal deformation. As a result, duration of deformation was longest in the lateral wall, for which it is known that peak strains are largest.

Our results demonstrate that increased age is associated with a greater extent of variation in timing of deformation among various left ventricular segments, indicating that patterns of regional non-uniformity of myocardial deformation are altered with age. These findings are in agreement with previous studies describing ventricular synchrony in healthy adult cohorts as well as animal studies, with the exception of a study performed by Ng et al. which did not find decreased synchronicity with advanced age. Several possible explanations for a decreasing extent of synchronicity with advancing age have been suggested previously. A study by Kitzman et al. demonstrated age-related alterations in left ventricular diastolic function as an intrinsic biologic effect of aging, irrespective of
other physiologic and pathologic changes that frequently accompany the aging process such as hypertension, coronary artery disease and diabetes. Others described an age-associated increase in the dynamic stiffness of the left ventricle in addition to prolonged contraction duration due to a slower removal of calcium from the contractile proteins, independent of myocardial catecholamine content. In elderly, delayed myocardial contraction and dyssynchrony may result from myocardial fibrosis. Especially in hypertensive patients with silent ischemia or infarction, potentially contributing to further electromechanical uncoupling. Importantly, increased ventricular dyssynchrony and prolonged duration of contraction may impinge on early diastolic relaxation through increased post-systolic shortening and dyscoordinate myocardial strain.

3.6 STUDY LIMITATIONS

A technical limitation is that speckle-tracking echocardiography is dependent on frame rate, as well as image resolution and image quality. Low frame rates result in a too high frame-to-frame change of the speckle pattern, which prevents the precise characterization of regional myocardial deformation. However, since in this study we are primarily interested in time to reach peak strain, low frame rates only result in less resolution of the timing values, but not in incorrect estimation. The optimal frame rate for precise detection of myocardial deformation has been reported to be approximately 60-90 Hz. We used custom-made software which is not commercially available. The custom-made software was specially developed to improve the reliability of timing of peak estimations by averaging strain curves, as well as to include peak systolic strain measurements that occur (shortly) before aortic valve closure. This custom-made software uses Q-Q intervals instead of the R-R interval used by commercially available software. Although the custom-made software is not commercially available, this method can be implemented in generally available software such as Matlab (The MathWorks Inc.,
Natick, MA; see appendix for instructions). However, when not the strain curves are averaged before calculating maximum strain and the time to reach these values, but the timing parameters are calculated from the individual curves and are averaged afterwards, similar values will be obtained. Comparison with an independent external technique, such as tagged MRI, was not performed in the current study, primarily because the validation of speckle-tracking software has already been compared with MRI previously with excellent results.\textsuperscript{70,71} In addition, the frame rate of tagged MRI cannot be as high as in echocardiography, which is of importance in children in whom heart rate is higher compared with adults. Furthermore, in case of (young) children it often indicates a need for sedation and anesthesia. Further prospective studies are needed to compare the usefulness and prognostic value of dyssynchrony quantification through 2DSTE in children.

3.7 CONCLUSION

The present study provides reference values for timing of deformation assessed with 2DSTE in children and young adults. Our findings indicate that aging results in a decrease of synchronicity of left ventricular systolic deformation. The results of the present study should be further used to assess mechanical dyssynchrony in children with various cardiac conditions. Since the currently used selection criteria for CRT appear not very suitable for application in the pediatric age groups, it is possible that 2DSTE indices could provide valuable additional information on myocardial synchrony and systolic function, which could be of assistance in selecting patients who could benefit from CRT.
3.8 APPENDIX

Three consecutive cardiac cycles were analyzed. Results from all views and segments were separately digitally stored into text files on the local Hard Drive of the GE workstation (with disabled drift compensation). The data files then were exported from the system and stored on the network for further analysis in a custom-made software package “CardiacCurveAnalysisTool” (CCAT) using Matlab version 7.4.0.287 (r2007a).

CUSTOM-MADE SOFTWARE, STEP BY STEP:

- automatic load of the three consecutive cardiac cycles results
- up sampling (cubic spline) to 2000 samples per cardiac cycle
- interactive QQ-onset defining (on the three consecutive cardiac cycles)
- curve length check (If length difference is >10% then delete longest cycle)
- deletion of data before and after the selected QQ timestamps
- padding zeros (NaN’s) at the end of the diastolic phase for the shortest cycles (to generate equal data lengths)
- curve averaging (using Matlab mean function)
- maximum detection
- estimation of averaged peak values per segment

3.9 REFERENCES


34. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18(12):1440-63.


Chapter 3 | 103


We all labor against our own cure, since death is the cure of all diseases

Thomas Browne
Engelse auteur
(1605 – 1682)
Chapter 4
Abnormal two-dimensional strain echocardiography findings in children with congenital valvular aortic stenosis

Even in case of asymptomatic, mild stenosis

Karen A. Marcus, Chris L. de Korte, Ton Feuth, Johan M. Thijssen, Livia Kapusta

Ultraschall in der Medizin / The European Journal of Ultrasound
2011 Mar, Epub ahead of print
4.1 ABSTRACT

**Background:** Congenital valvular aortic stenosis (VAS) causes a pressure overload to the left ventricle. In the clinical setting, the severity of stenosis is graded by the pressure drop over the stenotic valve (pressure gradient). This parameter is dependent on the hemodynamic status and does not provide information on myocardial performance.

**Aim:** This study was undertaken to reveal the potentials of two-dimensional strain echocardiography (2DSTE) for the detection of myocardial functional changes due to congenital VAS in children.

**Methods:** A total of 86 patients (aged from birth to 18 years) with various degrees of isolated congenital VAS were enrolled in this study. None of the patients had undergone any form of surgical or balloon intervention. A number of 139 healthy children served as a control group. Two-dimensional cine-loops recordings of apical four-chamber, midcavity short-axis and basal short-axis views were digitally stored for offline analysis. Longitudinal, circumferential and radial peak systolic strain and strain rate values were determined, as well as the time to peak systolic strain (T2P). Two-way analysis of variance was performed to assess the relation between VAS severity and 2DSTE parameters.

**Results:** In all patients conventional echocardiographic findings did not indicate systolic left ventricular dysfunction. All strain parameters of the control group were significantly different from those of VAS patients. There was a statistically significant, inverse relation between global peak systolic strain parameters in all three directions and the degree of VAS ($P<0.05$). Local peak systolic strain (rate) in the interventricular septum was most affected. T2P increased significantly with VAS severity ($P<0.05$). The decline in LV longitudinal systolic performance preceded that in other directions.
Conclusion: 2DSTE detects alterations in myocardial systolic deformation in children diagnosed with congenital VAS, whose conventional echocardiographic findings did not indicate ventricular systolic dysfunction.

4.2 BACKGROUND

Congenital isolated valvular aortic stenosis (VAS) is one of the most common forms of congenital heart disease (CHD) with an incidence of 1.1 - 4.3 per 10,000 live births and an increasing prevalence with age, since certainly not all cases are diagnosed at time of birth. It is a progressive disease with an overall 25-years survival rate of 85% after the diagnosis has been made and a worrisome potential incidence of sudden cardiac death, which underscores the essential need for lifelong, periodic cardiac evaluation and monitoring. However, the survival rate of 85% is described for both symptomatic and asymptomatic patients and, therefore, is not really reflective of the prognosis for just the asymptomatic patient group. In clinical practice, the severity of stenosis is defined using the ultrasound Doppler-derived peak instantaneous aortic flow velocity or the Bernoulli equation- derived pressure drop across the stenotic aortic valve (pressure gradient). With this method, mild stenosis is defined as a peak systolic flow velocity between 2.5 and 3.0 m/s, moderate VAS between 3.0 and 4.0 m/s, while patients with a peak jet velocity of more than 4.0 m/s, which corresponds to a peak pressure gradient of more than 64 mmHg, are considered to have severe VAS.

In contrast to the adult population, there is little consensus and evidence concerning the timing of intervention in children with VAS. Most authors agree that balloon valvuloplasty or surgery is indicated in symptomatic children or in asymptomatic individuals with severe VAS. There is no consensus however, as to whether, how and when to treat asymptomatic patients with moderate VAS. The main reason for intervention in this subset of patients, even in the absence of
electrocardiographic abnormalities or of echocardiographic signs of left ventricular hypertrophy, is to avoid further deterioration and ultimately irreversible myocardial damage.\(^4\) Nevertheless, it remains a challenge to define which patients require intervention. Both balloon and surgical valvulotomy are firmly established and are comparable techniques in terms of immediate gradient relief, procedural mortality, morbidity and long-term survival.\(^5,6\) However, the major concern, mainly in young patients after any sort of valvulotomy, is severe valvular aortic regurgitation (resulting in volume overload), which is less well tolerated than moderate residual VAS (pressure overload). The decision concerning the timing of an intervention will hopefully be greatly improved if an appropriate non-invasive, easily applicable (bedside) diagnostic tool to monitor left ventricular function becomes available. Furthermore, it would enable accurate evaluation of the effectiveness of such treatment, which would make it possible to adjust medical treatment to the patient’s individual needs in a timely manner.

Two-dimensional speckle tracking echocardiography (2DSTE), a relatively new echocardiographic technique to quantify myocardial strain (deformation), represents a promising bedside tool for the careful evaluation of myocardial function. The spectrum of potential clinical applications is very wide. Due to its ability to differentiate between active and passive deformation of myocardial segments, to quantify intraventricular dyssynchrony and to evaluate individual components of myocardial function such as longitudinal myocardial shortening, 2DSTE is able to detect myocardial dysfunction at an early stage.\(^7,8,9,10\) However, the value and applicability of 2DSTE in the evaluation of ventricular myocardial function in children with CHD, such as VAS, have not been elucidated. We hypothesized that regional myocardial function in congenital VAS is altered early, i.e., before the overt manifestation of cardiomyopathy and heart failure. The aim of this study is to introduce 2DSTE as a useful technique for the early detection of such subtle (regional) myocardial dysfunction.
4.3 MATERIAL AND METHODS

4.3.1 STUDY POPULATION

All asymptomatic children with isolated congenital VAS visiting the outpatient clinic at the Children’s Heart Centre Nijmegen between January 1, 2004 and November 30, 2009 were analyzed for their eligibility for inclusion in the study. Inclusion criteria consisted of an isolated congenital VAS (e.g. thickening of the valve or functional bicuspid aortic valve), accompanied by a Doppler-derived peak instantaneous aortic flow velocity of at least 2.5 m/s (which corresponds to a pressure gradient of 25 mmHg). Exclusion criteria were: (1) moderate or severe aortic regurgitation (i.e. > grade 1), (2) cardiac rhythms other than sinus rhythm, (3) balloon and/or surgical valvulotomy in the past, (4) acute or chronic illness at the time of echocardiographic evaluation, (5) diagnosis of a metabolic and/or genetic syndrome. Demographic and anthropometric characteristics were collected at the same time the echocardiographic study was performed. A complete history, as well as physical examination and ECG were performed. This study was approved by the local medical ethics committee.

4.3.2 CONTROL GROUP

Subjects who were routinely referred for echocardiographic evaluation of an asymptomatic, innocent heart murmur between May 1, 2005 and November 1, 2009, were retrospectively analyzed for their eligibility for inclusion in the study to serve as a control group. Subjects with structural (congenital) heart disease, abnormal cardiac rhythms and/or (a past history of) chronic or acute illness were excluded.
4.3.3 2DSTE DATA ACQUISITION

All subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography and a local research protocol previously described by Mavinkurve-Groothuis et al.\textsuperscript{11,12} Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) phased-array transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). The choice of an S3 or a S5 transducer depended on the weight and age of the child. Two-dimensional multiframe B-Mode (grayscale) images were obtained in the parasternal apical four-chamber (4C), midcavity short-axis view (at the level of the papillary muscle: PM) and basal short-axis view (at the level of the mitral valve: MV). A sector scan angle of 30 to 60 degrees was chosen and frame rates of 60 to 90 Hz were used. Preferably images from five cardiac cycles triggered by the R wave of the QRS complex were digitally saved in cineloop format. Offline strain analysis was performed using software for echocardiographic quantification (Echopac 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). The timing of aortic valve closure and mitral valve opening with respect to peak strain and peak systolic strain were manually obtained, using single gated pulsed-wave (PW-)Doppler or continuous-wave (CW-) Doppler images of the left ventricular outflow tract. For these measurements, special care was taken to keep the heart rate in the same range as during the 2D grayscale imaging used for 2D strain calculations. Endo-myocardial borders of the left ventricle were interactively tracked within the end-systolic frame. The second, epicardial, tracing was generated by the EchoPAC software and, when necessary, manually adjusted to cover the whole myocardial wall. The tracking algorithm then followed the speckles (the echographic pattern present in an echogram) in the myocardium through the cardiac cycle. Tracking was accepted only if both visual inspection as well as the EchoPAC software indicated adequate tracking. The software automatically divided the cross-sectional image into six
segments, which were named and identified according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.\textsuperscript{13} The left ventricular segments to be analyzed were the apical, middle and basal segments of the septal and the lateral wall of the 4C view, as well as the anteroseptal, anterior, lateral, posterior, inferior and septal segments of the basal (MV) and midcavity (PM) short-axis views. Strain (rate) curves of the three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for further analysis. The Q-Q interval was determined from the electrocardiogram signal to obtain cardiac cycle length. It is known that the duration of the systolic phase of the cardiac cycle in rest does not change with small changes in heart rate, in contrast to the diastolic phase.\textsuperscript{14} Therefore, the diastolic phase of the three cardiac cycles was automatically extended and adjusted by the software package to the longest of the three cardiac cycles. This intervention prevents a shift of the peak systolic strain while averaging the three consecutive cardiac cycles. Cardiac cycles with lengths more than ten percent different from the mean length of the three cardiac cycles were excluded for further analysis. Myocardial longitudinal, radial and circumferential strain (-rate) values of each of the cardiac segments were obtained, as well as the time to reach peak systolic strain. To determine global strain (-rate), the strain (-rate) values of the six segments were averaged for the 4C- as well as the short-axis views. All 2DSTE analyses were performed by the same investigator (K.A.M.) to avoid interobserver variability. Intraobserver (and interobserver) reliability scores have been established previously by our research group in children and young adults and are high for almost all parameters.\textsuperscript{12} Strain values are dimensionless and are expressed as percentages. Strain-rate is the time derivative of strain and is expressed as $s^{-1}$. Time to peak systolic strain is expressed as milliseconds (ms). Negative strain values reflect shortening/thinning, while positive strain values reflect lengthening or thickening.
4.3.4 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Quantification of cardiac chamber size, ventricular mass and systolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography.\textsuperscript{11} Left ventricular systolic function was characterized using fractional shortening (FS), ejection fraction (EF), left ventricular myocardial performance index, or Tei index and rate-corrected velocity of circumferential fiber shortening (VCFc). Ejection fraction was calculated using the modified Simpson’s rule. The PW-Doppler-derived myocardial performance index was calculated as: Tei = (iso-volumetric contraction time + iso-volumetric relaxation time)/ ejection time.\textsuperscript{15} VCFc was calculated using the formula obtained from Colan and colleagues.\textsuperscript{16} Left ventricular mass (LVM) was calculated using the formula for estimation of LVM according to Devereux and Reichek and was subsequently indexed to body surface area (BSA).\textsuperscript{17} The obtained values of LVM (corrected for BSA) were compared with those previously described in healthy children.\textsuperscript{18,19}

4.3.5 STATISTICAL ANALYSIS

All patients were divided in three groups according to VAS severity for further analysis. Mild VAS was defined as a peak flow velocity between 2.5 and 3.0 m/s, moderate VAS between 3.0 and 4.0 m/s, whereas patients with a peak jet velocity of more than 4.0 m/s were considered to have severe VAS.\textsuperscript{3} All demographic, anthropometric, conventional echocardiographic and 2D strain values are expressed as mean ± standard deviation (SD) or median and range. A cohort consisting of 139 healthy children was used as control group. The relation of VAS severity (none, mild, moderate and severe) with each conventional echocardiographic parameter, as well as with anthropometric characteristics was investigated using one-way analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons or Kruskal-Wallis nonparametric test when outcomes were not normally distributed.
The relation between VAS severity (none, mild, moderate and severe) and each global peak systolic strain (-rate) parameter, as well as each time to global peak systolic strain parameter was reported using two-way analysis of variance with the Bonferroni correction for multiple comparisons. A two-way analysis of variance was chosen to adjust for age, hereby avoiding possible bias caused by age. All 2DSTE parameters were normally distributed. To study the additional effect of demographic, anthropometric and conventional echocardiographic parameters over the effects of age and VAS severity, multiple linear regression analysis was used for each global 2DSTE variable taken as outcome variable. P-values less than 0.05 were considered to indicate significance. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).

4.4 RESULTS

4.4.1 DEMOGRAPHIC AND ANTHROPOMETRIC PARAMETERS

In total 159 children with congenital VAS were recruited from our outpatients clinic for possible inclusion in the study. Seventy-three of those patients were subsequently excluded: in 12 cases VAS was accompanied by another congenital heart defect, 6 patients suffered from moderate to severe aortic regurgitation, 51 patients had a past history of valvuloplasty, 3 patients had been diagnosed with Turner syndrome and one patient suffered from heart failure. Finally, 86 pediatric patients aged 0 to 18 years were eligible for inclusion in the study. A group of 139 healthy children (control group) with approximately the same age range (median 7.5 years; range 0.1 – 17.9 for control group versus median: 9.3 years; range 0.2 – 17.9 for VAS patient group) was included and examined. Patient demographic characteristics and anthropometric parameters are described in Table 15, together with the data of the control group.
Table 15 - Demographic and anthropometric characteristics (mean ± standard deviation or median and range) of study subjects categorized by VAS severity

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Mild VAS</th>
<th>Moderate VAS</th>
<th>Severe VAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>139</td>
<td>45</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>82 (59%)</td>
<td>31 (69%)</td>
<td>23 (74%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>7.5 (0.1 – 17.9)</td>
<td>10.2 (1.7 – 17.9) *</td>
<td>9.0 (0.4 – 17.0)</td>
<td>8.0 (0.2 – 15.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28.4 (3.3 – 83.4)</td>
<td>36.2 (9.1 – 75.3) *</td>
<td>31.0 (7.0 – 86.0)</td>
<td>32.5 (6.4 – 58.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>126 ± 38.5</td>
<td>137 ± 29.6 *</td>
<td>133 ± 30.3</td>
<td>128 ± 37.8</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.99 ± 0.51</td>
<td>1.20 ± 0.44 *</td>
<td>1.13 ± 0.45</td>
<td>1.01 ± 0.43</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.8 ± 2.6</td>
<td>17.9 ± 3.2</td>
<td>18.1 ± 3.6</td>
<td>16.7 ± 1.5</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>90 ± 23</td>
<td>81 ± 20</td>
<td>83 ± 22</td>
<td>90 ± 34</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>71.7 ± 10.5</td>
<td>74.6 ± 8.5</td>
<td>76.0 ± 9.9 *</td>
<td>72.8 ± 7.6</td>
</tr>
<tr>
<td>Syst.BP (mmHg)</td>
<td>98 ± 16</td>
<td>103 ± 13</td>
<td>100 ± 11</td>
<td>100 ± 11</td>
</tr>
</tbody>
</table>

BMI: body mass index; BSA: body surface area; HR: heart rate; MAP: mean arterial blood pressure; Syst.BP: systolic blood pressure. *P < 0.05 statistically significant difference compared with control subjects determined by one-way analysis of variance with Bonferroni correction for multiple comparisons or Kruskal-Wallis nonparametric test.

4.4.2 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

The conventional functional echocardiographic findings did not indicate systolic left ventricular dysfunction and were all within normal limits. There were no statistically significant differences in LVETc, VCFc, Tei index, FS, and LVEF between VAS severity categories (none, mild, moderate and severe) as assessed by one-way analysis of
variance with Bonferroni correction for multiple comparisons. Only left ventricle mass corrected for BSA increased significantly ($P < 0.001$) with VAS severity, but did not reach the threshold for left ventricular hypertrophy, not even in the group of children with severe VAS ($< 90^{th}$ percentile). Median z-scores were normal, in both control and patient groups. Conventional echocardiographic findings are described in Table 16.

Table 16 – Conventional echocardiographic findings (mean ± standard deviation or median and range) of study subjects categorized by VAS severity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=139)</th>
<th>Mild VAS (n=45)</th>
<th>Moderate VAS (n=31)</th>
<th>Severe VAS (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak velocity (m/s)</td>
<td>1.2 (0.8 – 1.4)</td>
<td>2.8 (2.6 – 2.9) *</td>
<td>3.4 (3.1 – 3.9) * ¥</td>
<td>4.5 (4.1 – 6.2) * ¥</td>
</tr>
<tr>
<td>LVETc (s)</td>
<td>0.31 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>0.32 ± 0.02</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>VCFc (circ/s)</td>
<td>1.26 ± 0.20</td>
<td>1.26 ± 0.18</td>
<td>1.27 ± 0.16</td>
<td>1.28 ± 0.25</td>
</tr>
<tr>
<td>Tei index</td>
<td>0.38 ± 0.04</td>
<td>0.38 ± 0.06</td>
<td>0.39 ± 0.05</td>
<td>0.39 ± 0.06</td>
</tr>
<tr>
<td>FS</td>
<td>0.39 ± 0.05</td>
<td>0.39 ± 0.04</td>
<td>0.41 ± 0.05</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>LVEF biplane</td>
<td>0.70 ± 0.06</td>
<td>0.69 ± 0.05</td>
<td>0.73 ± 0.06</td>
<td>0.73 ± 0.10</td>
</tr>
<tr>
<td>LV mass/BSA (g/m$^2$)</td>
<td>53.2 ± 16.3</td>
<td>65.6 ± 18.7 *</td>
<td>74.0 ± 20.2 *</td>
<td>74.3 ± 18.3 *</td>
</tr>
<tr>
<td>z-score LV mass (median – range)</td>
<td>-1.1 (-2.3 – 1.8)</td>
<td>-1.2 (-2.4 – 1.7)</td>
<td>-0.2 (-2.5 – 2.3)</td>
<td>0.3 (-1.5 – 1.9)</td>
</tr>
</tbody>
</table>

FS: fractional shortening; LVEF biplane: left ventricular ejection fraction measured by modified Simpson's method; LVETc: left ventricular ejection time corrected for heart rate; LV mass/BSA: left ventricular mass corrected for body surface area; Peak velocity: peak instantaneous systolic flow velocity over the aortic valve; Tei index: left ventricular myocardial performance index; VCFc: heart-rate corrected velocity of circumferential fiber shortening.

* $P < 0.05$ statistically significant difference when compared to control subjects; ¥ $P < 0.05$ statistically significant difference when compared to previous VAS category; determined by one-way analysis of variance with Bonferroni correction for multiple comparisons or Kruskal-Wallis nonparametric test.
4.4.3 MYOCARDIAL STRAIN PARAMETERS

Tracking was feasible in 98% of all segments of the four-chamber view, in 95% of all segments in the short-axis view at the level of the papillary muscle and in 89% of all segments in the short-axis view at the level of the mitral valve. The most striking observation was that all global systolic 2DSTE parameters in VAS patients were statistically significant different from those determined in the control group. There was a statistically significant, inverse relation ($P<0.001$) between each of the global peak systolic strain (-rate) parameters and VAS severity, determined by means of a two-way analysis of variance with Bonferroni correction for multiple comparisons. After adjustment for age and performing Bonferroni correction, there were statistically significant differences ($P<0.001$) between most of the VAS severity categories for all global peak systolic parameters (Table 17).

In children with VAS, the decline in left ventricular longitudinal peak systolic strain values preceded that in the other two directions. Global longitudinal peak systolic deformation was significantly lower in children with VAS among all degrees of stenosis when compared to healthy subjects, even in case of mild stenosis ($P<0.001$). The decline in longitudinal systolic strain as well as longitudinal systolic strain rate was more pronounced in segments of the interventricular septum, especially its basal parts. In children with severe VAS, global peak systolic longitudinal strain was on average 5.9% ± 0.9 lower in the interventricular septum (6.2% ± 0.9 in the basal part) and 4.5% ± 1.3 lower in the lateral free wall, when compared to healthy age matched children (data not shown; $P<0.001$).

The strain values in circumferential and radial direction decreased when the degree of VAS was more severe. Significantly lower peak systolic strain values in the circumferential plane were detected in children with moderate and severe VAS. The radial peak systolic strain values were only significantly negatively affected in case of severe VAS. Radial peak systolic strain appears to be relatively unaffected in
children with moderate VAS. In contrast, in children with mild VAS global radial peak systolic strain was increased compared with age-matched reference values.

The strain rate values among children with various degrees of VAS showed a pattern comparable to that seen with global peak systolic strain. Left ventricular longitudinal peak systolic strain rate was significantly lower in children with congenital VAS, even in case of mild stenosis, when compared to healthy control subjects ($P < 0.001$). The lowest values of longitudinal strain rate were observed in the group of children with severe VAS. Similar to circumferential strain, circumferential peak systolic strain rate was significantly decreased in children with moderate and severe VAS ($P < 0.001$). In children with all degrees of VAS, radial peak systolic strain rate values at the level of the papillary muscle were comparable to those found in the healthy control subjects. At the level of the mitral valve, radial peak systolic strain rate was significantly higher in the group of children with mild VAS ($P < 0.01$).

Time to global peak systolic strain results in all three directions (longitudinal, circumferential and radial) increased significantly ($P < 0.05$) with increasing VAS severity (none to severe), especially in severe VAS. Differences in timing of deformation between the groups under investigation cannot be attributed to differences in heart rate between the various groups of children (Table 15). As with strain parameter results, the time to peak systolic strain was most affected in longitudinal direction, with abnormal timing of maximal deformation even in the group of children with mild stenosis. Time to peak systolic circumferential and radial strain was prolonged when the degree of stenosis was more severe (Table 15; Figure 19).
Table 17 – Global strain parameters (mean ± standard deviation) of study subjects categorized by severity of valvular aortic stenosis

<table>
<thead>
<tr>
<th>2DSTE Parameters</th>
<th>Controls (n = 139)</th>
<th>Mild VAS (n = 45)</th>
<th>Moderate VAS (n = 31)</th>
<th>Severe VAS (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global S-L (%)</td>
<td>-20.9 ± 1.9</td>
<td>-19.5 ± 1.2 *</td>
<td>-18.4 ± 1.3 ¥</td>
<td>-15.5 ± 1.2 ¥ *</td>
</tr>
<tr>
<td>Global S-CP (%)</td>
<td>-22.1 ± 2.8</td>
<td>-22.9 ± 1.5</td>
<td>-21.1 ± 2.0 ¥</td>
<td>-18.9 ± 2.2 ¥ *</td>
</tr>
<tr>
<td>Global S-CM (%)</td>
<td>-20.3 ± 2.4</td>
<td>-20.9 ± 1.5</td>
<td>-19.0 ± 1.6 ¥</td>
<td>-16.3 ± 1.3 ¥ *</td>
</tr>
<tr>
<td>Global S-RP (%)</td>
<td>55.1 ± 6.8</td>
<td>57.4 ± 2.3</td>
<td>56.2 ± 2.7</td>
<td>53.0 ± 2.2 *</td>
</tr>
<tr>
<td>Global S-RM (%)</td>
<td>52.4 ± 5.4</td>
<td>54.8 ± 2.8 *</td>
<td>53.3 ± 2.9</td>
<td>50.0 ± 2.1 *</td>
</tr>
<tr>
<td>T2P Global S-L (ms)</td>
<td>345 ± 39</td>
<td>368 ± 26 *</td>
<td>383 ± 25 *</td>
<td>382 ± 26 *</td>
</tr>
<tr>
<td>T2P Global S-CP (ms)</td>
<td>331 ± 35</td>
<td>342 ± 33</td>
<td>349 ± 31 *</td>
<td>376 ± 29 *</td>
</tr>
<tr>
<td>T2P Global S-CM (ms)</td>
<td>347 ± 36</td>
<td>350 ± 32</td>
<td>366 ± 29 *</td>
<td>390 ± 28 *</td>
</tr>
<tr>
<td>T2P Global S-RP (ms)</td>
<td>335 ± 31</td>
<td>324 ± 27</td>
<td>339 ± 24 ¥</td>
<td>365 ± 23 ¥ *</td>
</tr>
<tr>
<td>T2P Global S-RM (ms)</td>
<td>351 ± 29</td>
<td>342 ± 27</td>
<td>354 ± 23 ¥</td>
<td>391 ± 22 ¥ *</td>
</tr>
<tr>
<td>Global Sr-L (s⁻¹)</td>
<td>-1.37 ± 0.1</td>
<td>-1.28 ± 0.2 *</td>
<td>-1.12 ± 0.1 ¥</td>
<td>-0.93 ± 0.1 ¥ *</td>
</tr>
<tr>
<td>Global Sr-CP (s⁻¹)</td>
<td>-1.90 ± 0.1</td>
<td>-1.91 ± 0.1</td>
<td>-1.67 ± 0.2 ¥</td>
<td>-1.59 ± 0.2 *</td>
</tr>
<tr>
<td>Global Sr-CM (s⁻¹)</td>
<td>-1.47 ± 0.2</td>
<td>-1.49 ± 0.2</td>
<td>-1.26 ± 0.2 ¥</td>
<td>-1.09 ± 0.3 *</td>
</tr>
<tr>
<td>Global Sr-RP (s⁻¹)</td>
<td>3.47 ± 0.4</td>
<td>3.65 ± 0.4</td>
<td>3.50 ± 0.4</td>
<td>3.19 ± 0.3</td>
</tr>
<tr>
<td>Global Sr-RM (s⁻¹)</td>
<td>3.23 ± 0.4</td>
<td>3.49 ± 0.4 *</td>
<td>3.27 ± 0.5</td>
<td>2.91 ± 0.3</td>
</tr>
</tbody>
</table>

Global S-L: global peak systolic longitudinal strain; Global S-CP: global peak systolic circumferential strain at the level of the papillary muscle; Global S-CM: global peak systolic circumferential strain at the level of the mitral valve; Global S-RP: global peak systolic radial strain at the level of the papillary muscle; Global S-RM: global peak systolic radial strain at the level of the mitral valve; T2P: time to peak global systolic strain; Sr: strain-rate. * P < 0.05 statistically significant difference when compared to control subjects ¥ P < 0.05 statistically significant difference when compared to previous VAS category; determined by two-way analysis of variance with Bonferroni correction for multiple comparisons.
Figure 19 - Bar plot of time to peak systolic strain (T2P) of study subjects in different directions of deformation according to VAS severity

Linear regression analyses showed weak, but statistically significant, correlations between 2DSTE parameters and peak-to-peak pressure gradients. For each global 2DSTE variable multiple linear regression analysis was performed on demographic, anthropometric and conventional echocardiographic parameters. This invariably resulted in $P$ values $> 0.05$, indicating no additional effect, besides age and VAS severity, on global peak systolic strain (-rate) and time to global peak systolic strain values.
4.5 DISCUSSION

The rationale for intervention in children with moderate to severe congenital valvular aortic stenosis is obvious when the patient is symptomatic and intervention/surgery most likely improves the outcome of this subset of patients. The decision is controversial when the patients are asymptomatic, which is often the case in the pediatric population, even in case of severe stenosis. Although the risk for sudden death might be overrated in historical retrospective studies, their natural history has not been well described. Data to indicate intervention are entirely based on pressure gradients in analogy with the data in adults, indicating that when transvalvular gradients are more than 50 mmHg peak-to-peak an increased risk for myocardial damage is present.

Patients with valvular aortic stenosis are subjected to long-standing pressure overload of the left ventricle often leading to hypertrophy, fibrosis and myocardial dysfunction. The pathophysiology of left ventricular dysfunction caused by pressure or volume overload is only partially understood. In aortic stenosis, the decrease in effective valve area results in a pressure gradient across the aortic valve and a corresponding increase in afterload with a subsequent escalation in left ventricular wall stress. The increased wall stress in turn stimulates the development of concentric ventricular hypertrophy, an adaptation process in order to (try to) normalize the wall stress. However, concentric hypertrophy compromises ventricular tissue compliance as well as subendocardial (and sometimes coronary) perfusion, which could lead to ischemia. The above-mentioned changes, in combination with ischemia induced replacement of contractile tissue by fibrous tissue, lead to a limited functional capability of the ventricle. In adults, these alterations eventually cause heart failure characterized by a triad comprising (1) a cardiac abnormality, (2) exercise limitation and (3) neurohormonal activation, particularly elevated N-terminal pro brain natriuretic peptide (NT-pro BNP) levels. Patients with CHD manifest all the above-mentioned criteria that constitute the
chronic heart failure syndrome. It might therefore be more helpful to view heart failure as a continuum from asymptomatic ventricular dysfunction with modest neurohormonal activation to severe ventricular dysfunction with symptoms at rest and marked neurohormonal activation. Therefore, accurate detection of the first signs of ventricular failure in CHD might be an important key factor for optimal timing of intervention and hereby prevention of irreversible damage.

Unfortunately, diagnostic modalities such as conventional echocardiographic techniques, ECG and exercise testing, have failed to provide a reliable assessment of ventricular function in children with CHD, e.g., VAS. Doppler-derived peak velocity flow, which is commonly used to indicate VAS severity, is strongly dependent on the hemodynamic status. Furthermore, these velocity-based measurements do not provide any information on the impact of the stenosis on the condition of the myocardium. Other traditional echocardiographic methods, such as the one-dimensional M-mode technique (e.g., fractional shortening) and two-dimensional imaging (e.g., ejection fraction), are frequently used to evaluate left ventricular function in various (cardiac) conditions. Unfortunately, these methods are not always applicable to pediatric patients with congenital malformations. The cardinal reason for this is that they are based on geometric assumptions and overlook the complex characteristics of congenital heart disease. Furthermore; they provide no information about regional alterations in ventricular myocardial contraction. Even more importantly, in case of LV hypertrophy these conventional indices appear to be relatively insensitive to impaired myocardial performance. Left ventricular ejection fraction is frequently normal even in the presence of a severe pressure gradient across the stenotic valve and/or hypertrophy. One important reason for this is that ejection fraction is not merely a function of myocardial fiber shortening, but is also influenced by chamber’s morphology, especially relative wall thickness. When left ventricular wall thickness increases, endocardial contraction towards the cavity’s center can be obtained with lesser myocardial fiber shortening. Because of this reciprocal relation between wall thickness and the amount of
myocardial fiber shortening needed to cause a given displacement of the endocardium, the magnitude of systolic shortening measured at the endocardium does not directly reflect intramural shortening. In light of these findings it is of importance that reduced midwall shortening, but not endocardial shortening, predicted fatal and non-fatal cardiovascular events under pressure-overloaded conditions of the left ventricle.

The current reasons to intervene are based on extrapolated adult data. However, the adaptation mechanisms in children might be different compared to adults. In adults, it is evident that cardiac remodeling occurs early in the chronically pressure-overloaded heart. During childhood the LV has better compensation potentials as is suggested by LV retraining data in patients with congenitally corrected transposition. However, in children with VAS, the total amount of myocardial collagen was significantly increased despite normal left ventricular myocardial contractility and diastolic function assessed by means of standard echocardiographic techniques. Left ventricular remodeling was abnormal in about a quarter of the patients and none had more than mild hypertrophy despite significant fibrosis. These findings are in agreement with other studies that describe fibrosis and myocyte enlargement in patients with even modest aortic stenosis and normal conventional echocardiographic findings. This is of importance since fibrosis is not only a major determinant of diastolic and systolic cardiac function, but it is also one of the structural substrates for arrhythmia. As a result, fibrosis plays a major role in the occurrence of sudden cardiac death and the progression to heart failure. These findings together indicate that conventional echocardiography is adequate in detecting (late) global LV dysfunction, whereas it is not successful in the detection of early regional myocardial malfunction in (children with) VAS. In the present study, we did not detect LV hypertrophy. However, strain-rate parameters, which more closely reflect contractility, were decreased in all three principal directions of contraction.
In animal studies, early normalization of loading conditions led to an immediate complete recovery of myocardial velocities. In contrast, late normalization of loading conditions, even in the presence of normal conventional indexes of ventricular function, did not lead to a (complete) recovery of myocardial velocities. This is probably due to extensive alterations in the LV myocardial architecture leading to alterations in intrinsic myocardial contractility. These findings underscore the need for additional (preferably non-invasive) parameters to accurately detect deterioration of myocardial function. It would be desirable to be able to detect myocardial changes at an early stage to optimize the timing of intervention and hopefully prevent permanent damage of the cardiac muscle. Strain imaging has proven to be a sensitive marker for ventricular dysfunction. A previous report by Kiraly et al. showed that tissue Doppler strain imaging (TDI) indicated left ventricular dysfunction in longitudinal direction in children with various degrees of VAS and normal conventional indices of ventricular function (e.g., ejection fraction and shortening fraction). However, TDI is limited by its inherently one-dimensional nature and is strongly dependent on the angle of insonation.

2DSTE has emerged as a new index of local myocardial function. One important advantage is that 2DSTE is not limited by a dependency on the angle of insonation. Furthermore, the speckle pattern can be tracked in multiple directions, which allows for quantification of myocardial deformation in all three main directions of strain. 2DSTE can detect subtle changes in regional myocardial function in various myocardial diseases at an earlier stage when compared to traditional (echocardiographic) methods. Whether 2DSTE is able to detect (regional) myocardial dysfunction in children with congenital VAS has not been previously investigated. In adults, strain imaging by means of TDI and 2DSTE has proven to be more sensitive than conventional echocardiography for assessing myocardial dysfunction in isolated left ventricular pressure-overload. Strain and strain rate parameters seemed to relate to LV function and VAS severity. Furthermore, they appear to be superior to tissue velocity and conventional echocardiography in
detecting subtle changes in myocardial function after normalization of loading conditions.\textsuperscript{37} Data on 2D strain (-rate) measurements in pediatric patients with congenital VAS are still lacking.

The present study in children with congenital VAS has included asymptomatic subjects with various degrees of stenosis. Accordingly, it has covered different stages of VAS, thus providing insight into the development of functional abnormalities underlying the disease progression. Our main finding was a gradual deterioration of left ventricular function characterized by decreased peak systolic strain (-rate) measurements even in children with moderate VAS and normal conventional echocardiographic findings. We demonstrated that the decline in LV longitudinal systolic performance precedes that in other directions, which is in agreement with previous reports performed in pressure overloaded adults with hypertension.\textsuperscript{38,39} The decreased longitudinal systolic strain (-rate) may be ascribed to damage of subendocardial myocardial fibers, which are mainly responsible for longitudinal function. These fibers are more vulnerable to increased wall stress, stress-induced ischemia, and microvascular dysfunction accompanying left ventricular pressure overload.\textsuperscript{40} Peak tissue pressures in the subendocardium have been shown to exceed coronary perfusion pressures during one-third of the cardiac cycle. This increase in the extravascular component of coronary resistance is sufficient to cause cessation of blood flow through this area during systole. As a result, the subendocardium is poorly perfused at a time when its needs are greatest.\textsuperscript{41,42} Yet, the muscle fibers of the subendocardium are the longest in the ventricular wall and perform the greatest work in the development of tension. These two factors, the increased dependency of the subendocardium on diastolic perfusion and the increased energy requirements, account for the vulnerability of the subendocardial layer to ischemia.

The mechanisms underlying the maintenance of normal or supra-normal LV radial function in the early stages of myocardial affection by VAS may be related to the
augmented LV wall thickness. In addition, the radius of curvature of the circumferentially oriented myocardial fibers responsible for LV radial deformation is smaller than that of longitudinal ones, which might entail lower stress and, consequently delayed appearance of functional abnormalities. It is possible that the increase in radial strain demonstrated in mild cases of stenosis (partly) compensates for a reduction in longitudinal strain. In this light, a decline of radial strain in the more advanced stages of VAS could be regarded as a form of decompensation.

The decline in peak systolic strain (-rate) was not equally distributed over the different ventricular wall segments. In children with VAS, peak systolic strain (-rate) was most affected throughout the interventricular septum, especially its basal segment. A possible explanation for this finding may be found in its geometry. The interventricular septum, because of its flatter and more asymmetric contour with a larger radius of curvature in the longitudinal plane, is subjected to a greater increase in systolic wall stress than the lateral free wall. Consequently, both myocardial hypertrophy and a decrease in systolic function produced by the increase of afterload and subsequent exaggerated wall stress are predominantly seen in the interventricular septum, particularly in its basal part.

Time to peak systolic strain increased significantly with disease severity. Especially in patients with severe VAS, significantly more time elapsed to reach peak systolic strain. These results are in agreement with previous reports which described an abnormal pattern and timing of systolic deformation in adult pressure overloaded left ventricles, as well as in rats with hypertensive disease. As with peak systolic strain (-rate) measurements, time indices in our patient groups were most affected in the longitudinal plane and less affected in the radial plane. The prolonged duration of time to maximal deformation could be a consequence of progressive fibrosis. Previous studies have indicated that changes in passive mechanical characteristics caused by fibrosis and altered collagen deposition also affect deformation when active force is developed within a myocardial segment.
In contrast to others, we did not find a significant relation between left ventricular mass and 2DSTE indices in our study group. A possible explanation for this finding could be that, despite the presence of severe valvular aortic stenosis in some of the patients, our patient groups did not develop significant left ventricular hypertrophy.

Although we did find a statistically significant relation between transvalvular pressure gradients and 2DSTE parameters when analyzing the total group of pediatric VAS patients, 2DSTE parameters most likely more closely reflect myocardial systolic performance in the individual patient.

### 4.6 STUDY LIMITATIONS

We did not perform a validation study of the strain (-rate) observations using cardiac tagged magnetic resonance imaging studies or myocardial biopsies (for ethical reasons). Both investigations could be useful for validation of the suspected subclinical left ventricular systolic dysfunction and/or fibrosis. Since the clinical and prognostic implications of the identified abnormalities in myocardial deformation described in this study remain to be elucidated, their exact interpretation will require further confirmation through future prospective, follow-up studies.

### 4.7 CONCLUSION

The present study demonstrates that asymptomatic children with congenital VAS and preserved LV ejection fraction already exhibited decreased peak systolic myocardial strain (-rate) in all three directions (radial, circumferential and longitudinal), even in case of mild VAS. We found that the decline in LV longitudinal systolic performance precedes that in other directions. The 2DSTE parameters could
be useful for the early detection of subclinical LV myocardial dysfunction, which remains otherwise undetected by conventional measurements of systolic function. However, the prognostic implications of our findings remain to be assessed in future longitudinal studies.

4.8 REFERENCES


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Le coeur a ses raisons que la raison ne connaît pas

François Pascal
(1623-1662)
Chapter 5
Persistent reduction in left ventricular strain using two-dimensional speckle tracking echocardiography after balloon valvuloplasty in children with congenital valvular aortic stenosis

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5.1 ABSTRACT

Aim: Investigation of serial changes of myocardial deformation using two-dimensional speckle echocardiography (2DSTE) in children undergoing balloon valvuloplasty for congenital valvular aortic stenosis (VAS).

Methods: 37 children with isolated congenital VAS were enrolled in this study prospectively. Patients underwent echocardiographic evaluation at three instances: prior to balloon valvuloplasty, 6 months after intervention, and 3 years after intervention. Longitudinal, circumferential and radial peak systolic strain (S) values were determined, as well as systolic strain-rate (Sr), and the time to peak global systolic strain (T2P). Linear mixed statistical models were employed to assess the change in 2DSTE parameters after balloon intervention. With One Way Analysis of Variance, 2DSTE results at three years follow-up were compared with 2DSTE measurements in: (1) 74 healthy age-matched children (2) 76 children with uncorrected VAS whose severity of stenosis corresponded with residual stenosis of study subjects at three years follow-up.

Results: Global peak S and Sr measurements in all three directions were decreased prior to intervention as compared to healthy children. Global peak S and Sr measurements increased significantly (P<0.001) several months after balloon valvuloplasty and continued to increase at three years follow-up. However, at three years follow-up, global peak S and Sr in longitudinal and circumferential direction were significantly lower (P<0.001) when compared to both control groups. T2P measurements were significantly shorter at early follow-up when compared to measurements prior to intervention (P<0.05).
Conclusion: Shortly after balloon valvuloplasty for severe congenital VAS there is an improvement in systolic myocardial deformation. However, 2DSTE parameters do not return to normal at three years follow-up. These abnormalities in systolic deformation cannot be fully attributed to residual stenosis or aortic regurgitation.

5.2 BACKGROUND

Patients with congenital valvular aortic stenosis (VAS) are subjected to long-standing pressure overload of the left ventricle (LV), leading to alterations in LV architecture and myocardial dysfunction.\(^1\) Previous reports in both children and adults indicated that abnormal LV systolic function starts at an early stage of the disease with progressive reduction of regional and global deformation.\(^2,3\) However, those early alterations in myocardial function have proven to be difficult to detect in congenital VAS with conventional (echocardiographic) imaging modalities. Two-dimensional strain echocardiography or speckle tracking echocardiography (2DSTE) has emerged as a relatively new index of regional and global myocardial function. The spectrum of potential clinical applications is very wide. Due to its ability to differentiate between active and passive deformation of myocardial segments, to quantify intraventricular dyssynchrony and to evaluate individual components of myocardial function such as longitudinal myocardial shortening, 2DSTE is able to detect myocardial dysfunction at an early stage.\(^4,5,6,7\) Previous studies have indicated that 2DSTE is more sensitive than conventional echocardiography in detecting early myocardial dysfunction in VAS.\(^8,9,10,11\) Two-dimensional strain parameters have been shown to be abnormal even in children with asymptomatic, mild congenital aortic stenosis.\(^3\) In adults with degenerative VAS, these abnormal alterations in (regional) deformation appear to be only partially reversible after aortic valve replacement (AVR).\(^10,11,12,13\) In children with congenital isolated VAS, the treatment of choice is valvuloplasty, either surgical or balloon valvulotomy. The reversibility of systolic myocardial dysfunction after valvulotomy in children with congenital VAS has not
been established by means of 2DSTE. The aim of the present study was twofold. First, to assess the effects of balloon valvuloplasty on myocardial deformation assessed with 2DSTE in all three directions of deformation (longitudinal, circumferential and radial) in children with isolated congenital VAS. Second, in case of persistent abnormal 2DSTE findings at three years follow-up we aimed to determine whether or not these abnormalities could be attributed to a residual valvar stenosis.

5.3 MATERIAL and METHODS

5.3.1 STUDY POPULATION

All asymptomatic children with severe isolated congenital VAS visiting the outpatient clinic at the Children’s Heart Centre Nijmegen between May 2005 and May 2009, who were scheduled for balloon valvuloplasty, were prospectively analyzed for their eligibility for inclusion in the present study. Inclusion criteria consisted of an isolated congenital VAS (e.g., thickening of the valve or functional bicuspid aortic valve), accompanied by a repeated Doppler-derived peak systolic instantaneous aortic flow velocity of at least 4.0 m/s (which corresponds to a transvalvular pressure gradient of more than 64 mmHg) at subsequent visits. Peak LV-to-peak-aortic pressure gradient assessed during catheterization under general anesthesia, had to be confirmative of aortic stenosis (according to the ACC/AHA guidelines 2006 of the management of patients with valvular heart disease). Previous reports have indicated that general anesthesia results in a declined stroke volume and lower pressure gradient estimations. Exclusion criteria were: (1) moderate or severe aortic regurgitation (i.e. > grade 1), (2) (a history of repeated) abnormal cardiac rhythm such as (supra)ventricular tachycardia, (3) previous balloon and/or surgical valvulotomy, (4) acute or chronic illness at the time of echocardiographic evaluation, (5) metabolic and/or genetic syndrome. Demographic and anthropometric characteristics, including age and gender were
collected at the same time the echocardiographic study was performed. A complete
history, physical examination, ECG, as well as (2DST) echocardiographic examination
were performed at three times: (1) within one month prior to balloon valvuloplasty,
(2) 6 months after balloon intervention, (3) at three years follow-up.

5.3.2 CONTROL GROUPS

1. Subjects who were routinely referred for echocardiographic evaluation of
an asymptomatic, innocent heart murmur or for screening purposes
between May 1, 2005 and November 1, 2009, were retrospectively
analyzed for their eligibility for inclusion in the study to serve as a control
group. Subjects with structural (congenital) heart disease, abnormal
cardiac rhythms and/or (a past history of) chronic or acute illness were
excluded. A group of 74 healthy age-matched children was included and
examined to provide normal reference values for conventional
echocardiographic and 2DSTE parameters.

2. A total of 76 children, consisting of a cohort of 45 children with mild,
isolated congenital VAS (peak systolic instantaneous flow velocity between
2.5 and 3.0 m/s) and a cohort of 31 children with moderate, isolated
congenital VAS (peak systolic instantaneous flow velocity between 3.0 and
4.0 m/s) without prior surgical or balloon intervention were previously
analyzed and described by our research group. In the present study, their
2DSTE results were compared with 2DSTE findings in children with residual
aortic stenosis at late follow-up after balloon intervention.

This study was approved by the Local Ethics Committee and conforms to the
Declaration of Helsinki. Each participant and/or their parents gave consent.
5.3.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

All subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography and a local research protocol previously described by Mavinkurve-Groothuis et al.\textsuperscript{18,19} Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) phased-array transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). The choice for a S3 or a S5 transducer depended on the age and posture of the child. Quantification of cardiac chamber size, ventricular mass and systolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography.\textsuperscript{18} Left ventricular systolic function was characterized using fractional shortening (FS), ejection fraction (EF), pulsed-wave Doppler-derived left ventricular myocardial performance index (or Tei index) and rate-corrected velocity of circumferential fiber shortening (VCFc).\textsuperscript{20,21,22} Ejection fraction was calculated using the modified Simpson’s rule. The obtained values of left ventricular mass (corrected for BSA) were compared with those previously described in healthy children.\textsuperscript{23,24}

5.3.4 2DSTE DATA ACQUISITION

Two-dimensional multiframe B-Mode (grayscale) images were obtained from the apical four-chamber (4C), the parasternal midcavity short-axis view (at the level of the papillary muscle: PM) and parasternal basal short-axis view (at the level of the mitral valve: MV). A sector scan angle of 30 to 60 degrees was chosen and frame rates of 60 to 90 Hz were used since these rates are considered to be optimal for 2D speckle tracking.\textsuperscript{7} Data were stored at the same frame rate as the acquisition frame rate. Preferably, images from five cardiac cycles triggered by the R wave of the QRS
complex were digitally saved in cineloop format. Offline strain analysis was performed using speckle tracking software for echocardiographic quantification (EchoPAC 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). The timing of aortic valve closure and mitral valve opening with respect to peak strain and peak systolic strain were manually obtained, using single gated pulsed-wave (PW-)Doppler or continuous-wave (CW-)Doppler blood flow velocity images of the left ventricular outflow tract. For these measurements, special care was taken to keep the heart rate in the same range as during the 2D grayscale imaging used for 2D strain calculations. Endomyocardial borders of the left ventricle were manually tracked within the end-systolic frame. The second, epicardial tracing was generated by the EchoPAC software and, when necessary, manually adjusted to cover the whole myocardial wall. The tracking algorithm then followed the myocardial speckles through the cardiac cycle. Tracking was accepted only if both visual inspection as well as EchoPAC software indicated adequate tracking. The software automatically divided the cross-sectional image into six segments, which were named and identified according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. The left ventricular segments to be analyzed were the apical, middle and basal segments of the septal and the lateral wall of the 4C view, as well as the anteroseptal, anterior, lateral, posterior, inferior and septal segments of the basal (MV) and midcavity (PM) short-axis views. Strain curves of the three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for averaging the strain curves of three consecutive cycles. The Q-Q interval was determined from the electrocardiogram signal to obtain cardiac cycle length. Cardiac cycles with lengths more than ten percent different from the mean length of the three cardiac cycles were excluded from averaging and thus for further analysis. Myocardial longitudinal, radial and circumferential strain values were obtained, as well as peak systolic strain-rate and the time to reach peak systolic strain. To determine global strain (-rate), the strain (-rate) values of the six segments were averaged for the four-chamber as well as for the short-axis views. All
2DSTE analyses were performed by the same investigator (K.A.M.) to avoid interobserver variability. Intraobserver variability scores have been previously described by our research group.\textsuperscript{19,26} Strain values are dimensionless and are expressed as percentages (%). Strain-rate is the time derivative of strain and is expressed as per second (s\textsuperscript{-1}). Time to peak systolic strain is expressed as milliseconds (ms). Negative strain values reflect shortening/thinning, while positive strain values reflect lengthening/thickening.

\textbf{5.3.5 STATISTICAL ANALYSIS}

All normally distributed demographic, anthropometric, conventional echocardiographic and 2D strain values are expressed as mean ± standard deviation (SD), all non-normally distributed data as median and range. Conventional echocardiographic as well as 2DSTE measurements derived from the children with severe VAS prior to intervention were compared with those acquired at intermediate and late follow-up. The change in each standard echocardiographic finding as well as each 2DSTE parameter was reported using linear mixed models and Bland-Altman plots. Since it is possible that changes in 2DSTE results after intervention (especially at late follow-up) could be (partly) biased by maturational changes, all 2DSTE results were compared with pediatric reference values established by our research group.\textsuperscript{26} For all individual 2DSTE results of each study subject (pre-intervention and at follow-up), the deviation from normal was calculated (2DSTE result of the individual patient minus mean reference value of 2DSTE parameter at the same age). The change in deviation from normal for each 2DSTE parameter at follow-up was determined by using linear mixed models. 2DSTE measurements at late follow-up were compared with respective findings in both control groups: (1) a cohort of 74 healthy age-matched children and (2) 76 children with mild (45) or moderate (31) isolated congenital VAS without prior surgical or balloon intervention. For the latter comparison, VAS patients at late follow-up were
divided according to residual VAS prior to further analysis. None to mild VAS was defined as a peak systolic flow velocity < 3.0 m/s, moderate VAS between 3.0 and 4.0 m/s, whereas patients with a systolic peak jet velocity of more than 4.0 m/s were considered to have severe VAS. Subsequently, global peak systolic strain, global peak systolic strain-rate and T2P measurements were compared with respective findings in children with corresponding peak systolic flow velocity measurements but no prior balloon intervention as well as healthy age-matched children. To determine their relation, one-way analysis of variance (ANOVA) was used with the Bonferroni correction for multiple comparisons. To determine the relation between individual 2DSTE parameters and aortic regurgitation at late follow-up, one-way analysis of variance (ANOVA) was performed. Relations between pre-interventional conventional echocardiographic and 2DSTE parameters and respective post-interventional findings were expressed in terms of linear regression analyses. All 2DSTE parameters were normally distributed. P-values less than 0.05 were considered to indicate significance. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).

5.4 RESULTS

5.4.1 STUDY POPULATION

A total of 51 children who underwent a balloon valvuloplasty to relieve pressure overload due to severe congenital VAS was identified from our outpatient clinic for possible inclusion in the present study. Fourteen of those patients were subsequently excluded: in two cases VAS was accompanied by another congenital heart defect, three patients suffered from moderate to severe aortic regurgitation, eight patients had a past history of valvuloplasty prior to the present valvuloplasty, and one patient had been diagnosed with Turner syndrome. Finally, 37 pediatric patients aged 0 to 15 years
were eligible for inclusion in the study. Their Doppler-derived peak instantaneous systolic flow velocity across the aortic valve prior to intervention was on average 4.8 m/s, corresponding to a pressure gradient across the stenotic valve of 91 mmHg. The peak-to-peak gradient at catheterization was 64 mmHg (range 51 – 75 mmHg). All catheterizations were performed under general anesthesia. Of those 37 patients, one patient underwent balloon valvuloplasty less than one year prior to the end of the inclusion period and therefore no information regarding three years follow-up findings are available for this particular patient.

5.4.2 HISTORY, PHYSICAL EXAMINATION AND ECG

History and physical examination pre-intervention were unremarkable with the exception of a systolic murmur. All were asymptomatic and in New York Heart Association functional class I. ECG findings did not reveal abnormal cardiac rhythm, conduction disorders or signs of ischemia. There were no T-wave abnormalities indicative of cardiac strain at rest. In five patients ECG findings were suggestive of left ventricular hypertrophy, with increased LV forces and leftward deviation of the QRS axis. Echocardiography confirmed the presence of LV hypertrophy in three of these patients. At intermediate follow-up (5.8±1.5 months after balloon intervention) and late follow-up (3.4±0.7 years after balloon intervention) patients were free from cardiac symptoms. As expected, several anthropometric characteristics of the study subjects were statistically significant different (P<0.05) at three years follow-up when compared to pre-interventional measurements by linear mixed models. These small, but statistically significant differences can be fully explained by maturational changes. Patient demographic characteristics and anthropometric parameters pre-intervention and at follow-up are listed in Table 18. Demographic and anthropometric characteristics of control subjects and study subjects at three years follow-up are described in Table 19. There were no statistical significant differences in anthropometric parameters between the various groups of children under investigation (e.g., children with severe VAS at three years follow-up
after balloon valvuloplasty, healthy control subjects, VAS patients without prior intervention).

Table 18 – Demographic and anthropometric characteristics (mean ± standard deviation or median and range) of study subjects pre-intervention and at follow-up

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Pre-intervention (n=37)</th>
<th>Intermediate Follow-up (n=37)</th>
<th>P value</th>
<th>Late Follow-up (n=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25 (68%)</td>
<td>25 (68%)</td>
<td>-</td>
<td>25 (68%)</td>
<td>-</td>
</tr>
<tr>
<td>Age (y)</td>
<td>6.7 (0.0 – 15.5)</td>
<td>7.3 (0.3 – 16.3)</td>
<td>0.52</td>
<td>10.3 (2.5–19.4)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>114 ± 42</td>
<td>121 ± 39</td>
<td>0.35</td>
<td>139 ± 30</td>
<td>0.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>26.5 (2 – 65)</td>
<td>30.4 (5 – 66)</td>
<td>0.38</td>
<td>38.8 (12 – 73)</td>
<td>0.14</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.90 ± 0.51</td>
<td>0.99 ± 0.51</td>
<td>0.37</td>
<td>1.20 ± 0.46</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.1 ± 2.9</td>
<td>17.5 ± 3.9</td>
<td>0.58</td>
<td>18.3 ± 4.5</td>
<td>0.45</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>99 ± 32</td>
<td>95 ± 32</td>
<td>0.45</td>
<td>86 ± 21</td>
<td>0.38</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>64 ± 10</td>
<td>67 ± 9</td>
<td>0.17</td>
<td>74 ± 9</td>
<td>0.002*</td>
</tr>
<tr>
<td>Syst.BP (mmHg)</td>
<td>91 ± 13</td>
<td>94 ± 11</td>
<td>0.30</td>
<td>103 ± 13</td>
<td>0.003*</td>
</tr>
<tr>
<td>Diast.BP (mmHg)</td>
<td>51 ± 10</td>
<td>54 ± 9</td>
<td>0.13</td>
<td>60 ± 8</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

BMI: body mass index; HR: heart rate; BSA: body surface area; Diast.BP: diastolic blood pressure; MAP: mean arterial blood pressure; Syst.BP: systolic blood pressure. * P < 0.05 when compared with the same parameter at the prior examination by linear mixed models.
Table 19 – Demographic and anthropometric characteristics (mean ± standard deviation or median and range) of study subjects and control groups at late follow-up

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Late follow-up</th>
<th>Late follow-up</th>
<th>No prior intervention</th>
<th>No prior intervention</th>
<th>Healthy control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None-Mild residual stenosis</td>
<td>Moderate residual stenosis</td>
<td>Mild VAS</td>
<td>Moderate VAS</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>17</td>
<td>19</td>
<td>45</td>
<td>31</td>
<td>74</td>
</tr>
<tr>
<td>Male</td>
<td>11 (65%)</td>
<td>12 (64%)</td>
<td>31 (69%)</td>
<td>23 (74%)</td>
<td>50 (68%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>10.2 (3 – 18)</td>
<td>10.3 (3 – 19)</td>
<td>10.2 (2 – 18)</td>
<td>9.0 (0 – 17)</td>
<td>10.3 (3 – 19)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>140 ± 30</td>
<td>139 ± 31</td>
<td>137 ± 30</td>
<td>133 ± 30</td>
<td>141 ± 30</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>37.4 (12 – 68)</td>
<td>40.0 (14 – 73)</td>
<td>36.2 (9 – 75)</td>
<td>31.0 (7 – 86)</td>
<td>38.7 (13 – 70)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.19 ± 0.44</td>
<td>1.22 ± 0.48</td>
<td>1.20 ± 0.44</td>
<td>1.13 ± 0.45</td>
<td>1.21 ± 0.47</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.6 ± 3.1</td>
<td>18.9 ± 5.4</td>
<td>17.9 ± 3.2</td>
<td>18.1 ± 3.6</td>
<td>17.8 ± 2.9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>79 ± 19</td>
<td>83 ± 23</td>
<td>81 ± 20</td>
<td>83 ± 22</td>
<td>79 ± 16</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>76 ± 7</td>
<td>73 ± 10</td>
<td>75 ± 9</td>
<td>76 ± 10</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Syst.BP (mmHg)</td>
<td>106 ± 11</td>
<td>101 ± 14</td>
<td>103 ± 13</td>
<td>100 ± 11</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>Diast.BP (mmHg)</td>
<td>61 ± 7</td>
<td>59 ± 9</td>
<td>60 ± 8</td>
<td>61 ± 11</td>
<td>60 ± 6</td>
</tr>
</tbody>
</table>

BMI: body mass index; BSA: body surface area; Diast.BP: diastolic blood pressure; HR: heart rate; MAP: mean arterial blood pressure; Syst.BP: systolic blood pressure.

5.4.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional echocardiographic findings are shown in Table 20. Prior to intervention, echocardiographic findings indicated significant left ventricular hypertrophy in three patients. At that time, mild aortic regurgitation was present in 11 out of 37 patients. Conventional systolic echocardiographic indices were normal in all echocardiographic examinations performed prior to intervention. At intermediate and three years follow-up, none of the patients had significant left ventricular hypertrophy. Mild aortic regurgitation was present in 19 out of 37 patients, whereas moderate aortic regurgitation was revealed in two patients. Post-
intervention, conventional functional echocardiographic findings did not indicate systolic left ventricular dysfunction and were all within normal limits. Each conventional echocardiographic finding of the pre-interventional examination was similar to respective echocardiographic findings at follow-up as determined by linear mixed models. Peak instantaneous systolic flow velocity across the aortic valve and the Bernoulli equation derived peak pressure gradient across the stenotic valve were the only conventional parameters that changed after balloon valvuloplasty. Both declined significantly ($P<0.001$) within 6 months after balloon valvuloplasty and remained stable at three years follow-up.
Table 20 – Conventional echocardiographic findings (mean ± standard deviation or median and range) of study subjects and significance (P-value) of change during follow-up after intervention

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre-intervention (n=37)</th>
<th>Intermediate Follow-up (n=37)</th>
<th>P value</th>
<th>Late follow-up (n=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak velocity (m/s)</td>
<td>4.8 (4.1 – 6.2)</td>
<td>3.2 (2.1 – 4.1)</td>
<td>&lt;0.001*</td>
<td>3.1 (1.9 – 3.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Peak pressure (mmHg)</td>
<td>91 (67 – 154)</td>
<td>42 (18 – 68)</td>
<td>&lt;0.001*</td>
<td>39 (14 – 61)</td>
<td>0.49</td>
</tr>
<tr>
<td>Mitral A velocity</td>
<td>0.59 ± 0.12</td>
<td>0.58 ± 0.12</td>
<td>0.72</td>
<td>0.57 ± 0.14</td>
<td>0.88</td>
</tr>
<tr>
<td>Mitral E velocity</td>
<td>1.00 ± 0.17</td>
<td>0.98 ± 0.17</td>
<td>0.50</td>
<td>1.03 ± 0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Mitral EA Ratio</td>
<td>1.75 ± 0.41</td>
<td>1.74 ± 0.39</td>
<td>0.91</td>
<td>1.85 ± 0.33</td>
<td>0.27</td>
</tr>
<tr>
<td>LVETc (s)</td>
<td>0.30 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.83</td>
<td>0.30 ± 0.02</td>
<td>0.76</td>
</tr>
<tr>
<td>VCFc (circ/s)</td>
<td>1.30 ± 0.14</td>
<td>1.29 ± 0.10</td>
<td>0.49</td>
<td>1.27 ± 0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>Tei index</td>
<td>0.39 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.64</td>
<td>0.39 ± 0.02</td>
<td>0.58</td>
</tr>
<tr>
<td>FS</td>
<td>0.40 ± 0.04</td>
<td>0.39 ± 0.02</td>
<td>0.16</td>
<td>0.38 ± 0.03</td>
<td>0.22</td>
</tr>
<tr>
<td>LVEF biplane</td>
<td>0.74 ± 0.04</td>
<td>0.73 ± 0.05</td>
<td>0.36</td>
<td>0.72 ± 0.05</td>
<td>0.92</td>
</tr>
<tr>
<td>LVM (g) z-score</td>
<td>68.1 ± 37 + 0.85</td>
<td>71.2 ± 39 + 0.54</td>
<td>0.88</td>
<td>84.9 ± 42 - 0.03</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>(-0.30 to +2.45)</td>
<td>(-0.76 to +1.70)</td>
<td></td>
<td>(-1.14 to +1.33)</td>
<td></td>
</tr>
<tr>
<td>LVM/BSA (g/m²)</td>
<td>75.7 ± 18</td>
<td>71.9 ± 14</td>
<td>0.52</td>
<td>70.8 ± 16</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Peak velocity: Doppler-derived peak instantaneous systolic flow velocity over the aortic valve; Peak pressure: peak pressure gradient across aortic valve; LVETc: left ventricular ejection time corrected for heart rate; VCFc: heart-rate corrected velocity of circumferential fiber shortening; Tei index: left ventricular myocardial performance index; FS: fractional shortening; LVEF biplane: left ventricular ejection fraction measured by modified Simpson’s method; LV mass/BSA: left ventricular mass corrected for body surface area. * P < 0.05 when compared with the same echocardiographic parameter at the prior examination by linear mixed models.
5.4.4 MYOCARDIAL STRAIN PARAMETERS

Tracking was feasible in 98% of all segments of the four chamber view, in 97% of all segments in the short axis view at the level of the papillary muscle and in 93% of all segments in the short axis view at the level of the mitral valve. The 2DSTE findings prior to intervention and at follow-up are presented in Table 21 and Figure 20-22. One important observation was a strong, statistically significant change in 2DSTE parameters after balloon valvuloplasty. At intermediate follow-up, global peak systolic strain in all three directions (longitudinal, circumferential and radial) improved significantly when compared to pre-interventional measurements by means of linear mixed models. Peak systolic strain in all three directions continued to change thereafter. At three years follow-up peak systolic strain in all three direction was significantly improved when compared to findings at intermediate follow-up. Time to global peak systolic strain values at intermediate follow-up were significantly shorter in radial direction when compared to pre-interventional measurements. On the contrary, T2P values at three years follow-up were significantly longer (in all three directions of contraction) when compared to findings at intermediate follow-up and T2P results acquired prior to intervention. The latter finding could be the effect of maturational changes in heart rate (prolongation of R-R interval) since regression analysis showed a strong, statistically significant, relation between T2P indices and R-R interval ($R^2 0.65; P < 0.0001$).

Global peak systolic strain-rate in longitudinal and circumferential direction improved significantly at intermediate follow-up when compared to pre-interventional measurements. At three years follow-up global peak systolic strain-rate results were similar to those acquired at intermediate follow-up in all three directions of deformation.
Global SL: Global peak systolic longitudinal strain (%); 0 (red): baseline data acquired in VAS patients prior to balloon valvuloplasty; 1 (blue): data acquired in VAS patients following balloon valvuloplasty at six months follow-up; 2 (green): data acquired in VAS patients at three years follow-up; normal (yellow): data acquired in healthy controls. * $P < 0.05$ when compared with the adjacent data group.
Global SCP: Global peak systolic circumferential strain at the level of the papillary muscle (%); 0 (red): baseline data acquired in VAS patients prior to balloon valvuloplasty; 1 (blue): data acquired in VAS patients following balloon valvuloplasty at six months follow-up; 2 (green): data acquired in VAS patients at three years follow-up; normal (yellow): data acquired in healthy controls. * $P < 0.05$ when compared with the adjacent data group.
Figure 22 - Box and Whisker plots of global peak systolic strain values

Global SCP: Global peak systolic radial strain at the level of the papillary muscle (%); 0 (red): baseline data acquired in VAS patients prior to balloon valvuloplasty; 1 (blue): data acquired in VAS patients following balloon valvuloplasty at six months follow-up; 2 (green): data acquired in VAS patients at three years follow-up; normal (yellow): data acquired in healthy controls. * $P < 0.05$ when compared with the adjacent data group.
Table 21 - 2DSTE findings (mean ± standard deviation) of study subjects

<table>
<thead>
<tr>
<th>2DSTE parameter</th>
<th>Pre-intervention (n=37)</th>
<th>Intermediate follow-up (n=37)</th>
<th>Late follow-up (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global SL (%)</td>
<td>-14.7 ± 1.0</td>
<td>-16.2 ± 1.6 *</td>
<td>-17.2 ± 1.2 ¥</td>
</tr>
<tr>
<td>Global SC-P (%)</td>
<td>-18.2 ± 2.2</td>
<td>-19.4 ± 2.0 ¥</td>
<td>-20.7 ± 1.4 ¥</td>
</tr>
<tr>
<td>Global SC-M (%)</td>
<td>-16.0 ± 1.3</td>
<td>-17.7 ± 1.8 *</td>
<td>-18.5 ± 1.6</td>
</tr>
<tr>
<td>Global SR-P (%)</td>
<td>51.7 ± 2.5</td>
<td>53.3 ± 2.6 ¥</td>
<td>54.8 ± 2.0 ¥</td>
</tr>
<tr>
<td>Global SR-M (%)</td>
<td>49.2 ± 2.5</td>
<td>51.0 ± 2.6 ¥</td>
<td>52.5 ± 2.2 ¥</td>
</tr>
<tr>
<td>T2P global SL (ms)</td>
<td>374 ± 58</td>
<td>363 ± 48</td>
<td>393 ± 31 ¥</td>
</tr>
<tr>
<td>T2P global SC-P (ms)</td>
<td>347 ± 60</td>
<td>335 ± 52</td>
<td>366 ± 30 ¥</td>
</tr>
<tr>
<td>T2P global SC-M (ms)</td>
<td>363 ± 55</td>
<td>353 ± 45</td>
<td>375 ± 22 ¥</td>
</tr>
<tr>
<td>T2P global SR-P (ms)</td>
<td>354 ± 55</td>
<td>324 ± 37 ¥</td>
<td>344 ± 23 ¥</td>
</tr>
<tr>
<td>T2P global SR-M (ms)</td>
<td>401 ± 51</td>
<td>352 ± 52 ¥</td>
<td>353 ± 41</td>
</tr>
<tr>
<td>Global SrL (s⁻¹)</td>
<td>-0.9 ± 0.1</td>
<td>-1.1 ± 0.2 *</td>
<td>-1.1 ± 0.1</td>
</tr>
<tr>
<td>Global SrC-P (s⁻¹)</td>
<td>-1.4 ± 0.2</td>
<td>-1.6 ± 0.2 ¥</td>
<td>-1.6 ± 0.2</td>
</tr>
<tr>
<td>Global SrC-M (s⁻¹)</td>
<td>-1.0 ± 0.3</td>
<td>-1.2 ± 0.3 ¥</td>
<td>-1.2 ± 0.3</td>
</tr>
<tr>
<td>Global SrR-P (s⁻¹)</td>
<td>3.1 ± 0.5</td>
<td>3.4 ± 0.4</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Global SrR-M (s⁻¹)</td>
<td>2.9 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

SL: global peak systolic longitudinal strain; SC: global peak systolic circumferential strain; -P: the level of the papillary muscle; -M: at the level of the mitral valve; SR: global peak systolic radial strain; T2P: time to global peak systolic strain; SrL: global peak systolic longitudinal strain-rate; SrC: global peak systolic circumferential strain-rate; SrR: global peak systolic radial strain-rate; * P < 0.0001 when compared with the same 2DSTE parameter at the prior examination by linear mixed models; ¥ P < 0.05 when compared with the same 2DSTE parameter at the prior examination by linear mixed models.
DEVIATION FROM NORMAL 2DSTE REFERENCE VALUES:

All 2DSTE results were compared with pediatric reference values to correct for possible bias caused by maturational changes. For all individual 2DSTE results of the study subjects (pre-intervention and at follow-up), the deviation from normal was calculated (2DSTE result of the individual patient minus mean reference value of 2DSTE parameter for age). The deviation from normal for all individual strain parameters was significantly \((P<0.0001)\) reduced at intermediate follow-up. At three years follow-up, there was no significant further reduction in deviation from normal observed and similar values as at intermediate follow up were found (Table 22 and Fure 23-25). The decline in longitudinal systolic strain as well as longitudinal systolic strain rate prior to intervention was more pronounced in segments of the interventricular septum, especially its basal parts. Global peak systolic longitudinal strain was on average 6.8% ± 1.0 lower in the interventricular septum (7.2% ± 0.9 in the basal part) and 5.0% ± 1.2 lower in the lateral free wall, when compared to healthy age matched children \((P<0.001)\). In contrast to longitudinal deformation, were circumferential and radial strain (-rate) values equally affected throughout the interventricular septum and left ventricular lateral wall segments.

In the individual patient with aortic stenosis under investigation, pre-interventional global peak systolic strain was abnormal (below 5\(^{th}\) percentile of normal according to previously mentioned reference values) in 97% of patients for longitudinal strain, 65% of patients for circumferential strain at the level of the papillary muscle and 68% of patients for circumferential strain at the level of the mitral valve.\(^26\) Global peak systolic radial strain was abnormal at the level of the papillary muscle in 24% of the patients versus 16% of patients at the level of the mitral valve.

As for the post-intervention 2DSTE measurements at three years follow-up, 97% of patients persisted to show an abnormal peak systolic longitudinal deformation, 38% of patients for circumferential strain at the level of the papillary muscle and 36% of
patients for circumferential strain at the level of the mitral valve. Global peak systolic radial strain was abnormal at the level of the papillary muscle in 10% of the patients versus 8% of the patients at the level of the mitral valve.

Figure 23 – Deviation from normal reference values for age of global peak systolic strain values (%) pre-intervention and at follow-up
Figure 24 – Deviation from normal reference values for age of time to global peak systolic strain values (ms) pre-intervention and at follow-up

Figure 25 – Deviation from normal reference values for age of global peak systolic strain rate values (s⁻¹) pre-intervention and at follow-up
Table 22 – Deviation from normal reference values according to age of each 2DSTE parameter (absolute values: mean ± standard deviation)

<table>
<thead>
<tr>
<th>Deviation from reference value (Δ)</th>
<th>Pre-intervention (n=37)</th>
<th>Intermediate follow-up (n=37)</th>
<th>Late follow-up (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Global SL (%)</td>
<td>5.8 ± 0.9</td>
<td>4.5 ± 1.3 *</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>Δ Global SC-P (%)</td>
<td>3.5 ± 0.8</td>
<td>2.6 ± 1.2 *</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>Δ Global SC-M (%)</td>
<td>4.0 ± 1.0</td>
<td>2.5 ± 1.3 *</td>
<td>2.3 ± 1.2</td>
</tr>
<tr>
<td>Δ Global SR-P (%)</td>
<td>-3.1 ± 1.0</td>
<td>-1.8 ± 0.9 *</td>
<td>-1.4 ± 0.5</td>
</tr>
<tr>
<td>Δ Global SR-M (%)</td>
<td>-3.0 ± 1.3</td>
<td>-1.3 ± 0.6 *</td>
<td>-1.0 ± 0.4</td>
</tr>
<tr>
<td>Δ T2P global SL (ms)</td>
<td>40 ± 8</td>
<td>22 ± 10 *</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>Δ T2P global SC-P (ms)</td>
<td>26 ± 9</td>
<td>9 ± 4 *</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>Δ T2P global SC-M (ms)</td>
<td>25 ± 7</td>
<td>11 ± 5 *</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Δ T2P global SR-P (ms)</td>
<td>18 ± 4</td>
<td>8 ± 3 *</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>Δ T2P global SR-M (ms)</td>
<td>36 ± 13</td>
<td>15 ± 6 *</td>
<td>-5 ± 2 *</td>
</tr>
<tr>
<td>Δ Global SrL (s⁻¹)</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.2 *</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Δ Global SrC-P (s⁻¹)</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.2 *</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Δ Global SrC-M (s⁻¹)</td>
<td>0.5 ± 0.3</td>
<td>0.3 ± 0.3 *</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Δ Global SrR-P (s⁻¹)</td>
<td>-0.4 ± 0.5</td>
<td>-0.1 ± 0.4 *</td>
<td>-0.1 ± 0.5</td>
</tr>
<tr>
<td>Δ Global SrR-M (s⁻¹)</td>
<td>-0.3 ± 0.5</td>
<td>-0.1 ± 0.4</td>
<td>-0.2 ± 0.6</td>
</tr>
</tbody>
</table>

SL: global peak systolic longitudinal strain; SC: global peak systolic circumferential strain; -P: the level of the papillary muscle; -M: at the level of the mitral valve; SR: global peak systolic radial strain; T2P: time to global peak systolic strain; SrL: global peak systolic longitudinal strain-rate; SrC: global peak systolic circumferential strain-rate; SrR: global peak systolic radial strain-rate; * P < 0.0001 when compared with the same 2DSTE parameter at the prior examination by linear mixed models; ¥ P < 0.05 when compared with the same 2DSTE parameter at the prior examination by linear mixed models.
THREE YEARS FOLLOW-UP 2DSTE FINDINGS COMPARED WITH THOSE IN CONTROL GROUPS:

The 2DSTE findings in study subjects at three years follow-up compared with those found in both control groups are shown in Table 23. Determined with One Way Analysis Of Variance (ANOVA), each global peak systolic strain and global peak systolic strain-rate parameter at three years follow-up was significantly lower ($P<0.001$) in longitudinal and circumferential direction when compared to both healthy age-matched controls and age-matched children with an equal degree of stenosis but no prior intervention. Radial 2DSTE parameters at three years follow-up as well as T2P values in all three directions were similar to those found in both control groups. There was no statistically significant relation between global peak systolic strain (-rate) results or T2P values and aortic regurgitation as assessed by means of One Way Analysis of Variance ($P \approx 0.72$). The relation between various conventional echocardiographic parameters and longitudinal and circumferential strain indices determined at three years follow-up was assessed by means of regression analysis (Table 24). LVEF and FS at baseline appeared not to be related to strain (-rate) measurements at follow-up. On contrast, peak flow velocity at baseline and follow-up showed strong, statistically significant ($P \approx 0.001$), relationship with deformation at three years follow-up. The reduction of peak flow velocity (pre-interventional measurement minus peak flow velocity at three years follow up) was statistically significant related to the increment in peak systolic strain (difference in peak systolic strain between pre-interventional assessment and post-interventional assessment at three years follow up): $R^2 \approx 0.41$ for global SL ($P <0.001$), $R^2 \approx 0.38$ for global SC-P ($P <0.001$), and $R^2 \approx 0.36$ for global SC-M ($P <0.001$).
Table 23 - 2DSTE findings (mean ± standard deviation) of study subjects at late follow-up and control groups

<table>
<thead>
<tr>
<th>2DSTE parameters</th>
<th>Mild VAS</th>
<th>No prior intervention</th>
<th>P value</th>
<th>Late follow-up</th>
<th>P value</th>
<th>Healthy control subjects</th>
<th>P value</th>
<th>Moderate residual stenosis</th>
<th>P value</th>
<th>Late follow-up</th>
<th>P value</th>
<th>Moderate VAS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>45</td>
<td>17</td>
<td>74</td>
<td>19</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. patients with AR</td>
<td>10 (22%)</td>
<td>10 (59%)</td>
<td>-</td>
<td>11 (58%)</td>
<td>6 (19%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak velocity (m/s)</td>
<td>2.8 ± 0.2</td>
<td>1.00</td>
<td>2.6 ± 0.3</td>
<td>-</td>
<td>3.5 ± 0.3</td>
<td>1.00</td>
<td>3.4 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global SL (%)</td>
<td>-19.5 ± 1.2</td>
<td>&lt;0.001</td>
<td>-17.8 ± 1.2</td>
<td>&lt;0.001</td>
<td>-21.6 ± 1.4</td>
<td>&lt;0.001</td>
<td>-16.7 ± 1.0</td>
<td>&lt;0.001</td>
<td>-18.4 ± 1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global SC-P (%)</td>
<td>-22.9 ± 1.5</td>
<td>&lt;0.01</td>
<td>-21.0 ± 1.3</td>
<td>&lt;0.001</td>
<td>-23.3 ± 2.0</td>
<td>&lt;0.001</td>
<td>-19.8 ± 1.5</td>
<td>&lt;0.01</td>
<td>-21.1 ± 2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global SC-M (%)</td>
<td>-20.9 ± 1.5</td>
<td>&lt;0.01</td>
<td>-19.3 ± 1.5</td>
<td>&lt;0.001</td>
<td>-21.2 ± 2.1</td>
<td>&lt;0.001</td>
<td>-17.8 ± 1.2</td>
<td>0.22</td>
<td>-19.0 ± 1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global SR-P (%)</td>
<td>57.4 ± 2.3</td>
<td>0.87</td>
<td>55.4 ± 2.2</td>
<td>1.00</td>
<td>55.9 ± 5.5</td>
<td>1.00</td>
<td>54.3 ± 1.7</td>
<td>0.94</td>
<td>56.2 ± 2.7</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Global SR-M (%)</td>
<td>54.8 ± 2.8</td>
<td>1.00</td>
<td>53.6 ± 2.2</td>
<td>1.00</td>
<td>53.7 ± 5.7</td>
<td>0.57</td>
<td>51.6 ± 1.6</td>
<td>1.00</td>
<td>53.3 ± 2.9</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T2P global SL (ms)</td>
<td>368 ± 26</td>
<td>0.37</td>
<td>390 ± 31</td>
<td>0.79</td>
<td>373 ± 31</td>
<td>0.08</td>
<td>396 ± 28</td>
<td>1.00</td>
<td>383 ± 25</td>
<td></td>
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<tr>
<td>T2P global SC-P (ms)</td>
<td>342 ± 33</td>
<td>0.81</td>
<td>360 ± 28</td>
<td>1.00</td>
<td>355 ± 38</td>
<td>0.83</td>
<td>371 ± 32</td>
<td>0.34</td>
<td>349 ± 31</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2P global SC-M (ms)</td>
<td>350 ± 32</td>
<td>1.00</td>
<td>365 ± 32</td>
<td>1.00</td>
<td>357 ± 36</td>
<td>0.06</td>
<td>380 ± 23</td>
<td>1.00</td>
<td>366 ± 29</td>
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<td></td>
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<tr>
<td>T2P global SR-P (ms)</td>
<td>324 ± 27</td>
<td>0.22</td>
<td>341 ± 22</td>
<td>1.00</td>
<td>339 ± 36</td>
<td>1.00</td>
<td>345 ± 23</td>
<td>1.00</td>
<td>339 ± 24</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T2P global SR-M (ms)</td>
<td>342 ± 27</td>
<td>1.00</td>
<td>349 ± 22</td>
<td>1.00</td>
<td>341 ± 43</td>
<td>0.43</td>
<td>356 ± 44</td>
<td>1.00</td>
<td>354 ± 23</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global SrL (s01)</td>
<td>-1.3 ± 0.2</td>
<td>&lt;0.01</td>
<td>-1.2 ± 0.1</td>
<td>&lt;0.001</td>
<td>-1.4 ± 0.1</td>
<td>&lt;0.001</td>
<td>-0.9 ± 0.1</td>
<td>&lt;0.001</td>
<td>-1.1 ± 0.1</td>
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</tr>
<tr>
<td>Global SrC-P (s01)</td>
<td>-1.9 ± 0.1</td>
<td>&lt;0.01</td>
<td>-1.7 ± 0.2</td>
<td>&lt;0.01</td>
<td>-1.9 ± 0.1</td>
<td>&lt;0.001</td>
<td>-1.4 ± 0.2</td>
<td>&lt;0.01</td>
<td>-1.7 ± 0.2</td>
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<td></td>
</tr>
<tr>
<td>Global SrC-M (s01)</td>
<td>-1.5 ± 0.2</td>
<td>&lt;0.01</td>
<td>-1.3 ± 0.1</td>
<td>&lt;0.01</td>
<td>-1.5 ± 0.2</td>
<td>&lt;0.001</td>
<td>-1.2 ± 0.3</td>
<td>1.00</td>
<td>-1.3 ± 0.2</td>
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</tr>
<tr>
<td>Global SrR-P (s01)</td>
<td>3.6 ± 0.4</td>
<td>1.00</td>
<td>3.5 ± 0.5</td>
<td>1.00</td>
<td>3.5 ± 0.4</td>
<td>0.36</td>
<td>3.2 ± 0.5</td>
<td>0.29</td>
<td>3.5 ± 0.4</td>
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<td></td>
</tr>
<tr>
<td>Global SrR-M (s01)</td>
<td>3.5 ± 0.4</td>
<td>0.06</td>
<td>3.1 ± 0.6</td>
<td>1.00</td>
<td>3.2 ± 0.4</td>
<td>0.35</td>
<td>2.9 ± 0.6</td>
<td>0.22</td>
<td>3.3 ± 0.5</td>
<td></td>
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</tr>
</tbody>
</table>

Peak velocity: peak instantaneous systolic flow velocity over the aortic valve; AR: aortic regurgitation; SL: peak systolic longitudinal strain; SC: peak systolic circumferential strain; -P: at the level of the papillary muscle; -M: at the level of the mitral valve; SR: peak systolic radial strain; T2P: time to peak systolic strain; SrL: peak systolic longitudinal strain-rate; SrC: peak systolic circumferential strain-rate; SrR: peak systolic radial strain-rate; *P* values with respect to pair wise comparison of the means of two adjacent groups, resulting from one-way ANOVA with Bonferroni correction.
Table 24 - Coefficient of determination

<table>
<thead>
<tr>
<th>Echocardiographic parameter</th>
<th>Global SL Late Follow-up</th>
<th>Global SC-P Late Follow-up</th>
<th>Global SC-M Late Follow-up</th>
<th>Global SrL Late Follow-up</th>
<th>Global SrC-P Late Follow-up</th>
<th>Global SrC-M Late Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak velocity at T0</td>
<td>R² 0.57</td>
<td>R² 0.46</td>
<td>R² 0.42</td>
<td>R² 0.59</td>
<td>R² 0.47</td>
<td>R² 0.38</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
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<tr>
<td>LVM at T0</td>
<td>R² 0.18</td>
<td>R² 0.15</td>
<td>R² 0.21</td>
<td>R² 0.17</td>
<td>R² 0.20</td>
<td>R² 0.22</td>
</tr>
<tr>
<td></td>
<td>P 0.010</td>
<td>P 0.017</td>
<td>P 0.006</td>
<td>P 0.015</td>
<td>P 0.007</td>
<td>P 0.004</td>
</tr>
<tr>
<td>LVEF at T0</td>
<td>R² 0.02</td>
<td>R² 0.08</td>
<td>R² 0.03</td>
<td>R² 0.03</td>
<td>R² 0.06</td>
<td>R² 0.04</td>
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<tr>
<td></td>
<td>P 0.404</td>
<td>P 0.087</td>
<td>P 0.360</td>
<td>P 0.301</td>
<td>P 0.142</td>
<td>P 0.235</td>
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<tr>
<td>FS at T0</td>
<td>R² 0.001</td>
<td>R² 0.007</td>
<td>R² 0.002</td>
<td>R² 0.03</td>
<td>R² 0.05</td>
<td>R² 0.03</td>
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<tr>
<td></td>
<td>P 0.873</td>
<td>P 0.632</td>
<td>P 0.783</td>
<td>P 0.353</td>
<td>P 0.200</td>
<td>P 0.331</td>
</tr>
<tr>
<td>Peak velocity at T0 minus</td>
<td>R² 0.65</td>
<td>R² 0.51</td>
<td>R² 0.48</td>
<td>R² 0.66</td>
<td>R² 0.53</td>
<td>R² 0.44</td>
</tr>
<tr>
<td>Peak velocity at T2</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Peak velocity at T2</td>
<td>R² 0.37</td>
<td>R² 0.29</td>
<td>R² 0.28</td>
<td>R² 0.36</td>
<td>R² 0.30</td>
<td>R² 0.22</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.004</td>
</tr>
</tbody>
</table>

Global SL: global peak systolic longitudinal strain; Global SC: Global peak systolic circumferential strain; -P: at the level of the papillary muscle; -M: at the level of the mitral valve; Global SrL: Global peak systolic longitudinal strain rate; Global SrC: Global peak systolic circumferential strain rate; peak velocity: Doppler-derived peak instantaneous systolic flow velocity over the aortic valve; LVM; left ventricular mass; LVEF: left ventricular ejection fraction measured by modified Simpson’s method; FS: fractional shortening; T0: baseline (pre-intervention); T2: late follow-up; R²: coefficient of determination assessed by means of linear regression analysis.


5.5 DISCUSSION

Congenital isolated VAS is one of the most common forms of congenital heart disease with an estimated incidence of 3.8 per 10,000 live births and an increasing prevalence with age, since certainly not all cases are diagnosed at time of birth. Previous reports suggest that congenital VAS is a progressive disease with an overall 25-year survival rate of 85% after the diagnosis has been made and a worrisome incidence of sudden cardiac death.27,28

Although these figures might be overrated, lifelong, periodic cardiac evaluation and monitoring is clearly indicated. The rationale for intervention in children with moderate to severe congenital valvular aortic stenosis is obvious when the patient is symptomatic and (surgical) intervention most likely improves the outcome of this subset of patients. The decision is controversial when the patients are asymptomatic, which is often the case in the pediatric population, even in case of severe stenosis. In addition to symptomatic status, current criteria to indicate intervention are entirely based on the pressure gradient in analogy with adults.27 Thus, indicating that when transvalvular peak pressure gradients exceed 50 mmHg, an increased risk for myocardial damage is present.

Unfortunately, adequate monitoring of myocardial function and remodeling in children with congenital VAS imposes a challenge. Conventional diagnostic imaging modalities such as standard echocardiographic techniques, have failed to provide a reliable assessment of ventricular function in this subset of patients. Although they are appropriate to detect global LV dysfunction at advanced stages of cardiac disease, they are not able to identify subclinical (regional) systolic myocardial abnormalities.

Previous reports on adults with degenerative VAS have clearly illustrated that strain imaging more carefully reflects myocardial systolic function in VAS.9,29,30,31,32 Strain
parameters seemed to relate to LV function as well as to the severity of stenosis. Furthermore, they have proven to be superior to tissue velocity imaging and conventional echocardiography in detecting subtle changes in myocardial function after intervention and are predictive of (post-operative) morbidity and mortality.\textsuperscript{8,11,13,33,34,35}

Several studies in adults with degenerative VAS aimed to assess the changes in regional myocardial function due to aortic valve surgery. At short-term follow-up after AVR, improvement of strain values was evident immediately after intervention. This early recovery in global and regional LV function within the first 24 hours after surgery suggests an important negative impact of increased afterload on regional systolic function. Although regional and global myocardial deformation improved, strain parameters did not normalize at short-term follow-up.\textsuperscript{10,13,36}

Concerning the long-term effects of AVR on the recovery of systolic ventricular performance in adults, most studies are indicative of incomplete recovery with a persistent decline in strain parameters.\textsuperscript{10,11}

The latter findings are in agreement with an animal study in which rats were exposed to pathological pressure-overload of the left ventricle. In these animals, abnormal regional myocardial velocities were detected as well as significantly increased collagen density. These abnormalities were present despite normal conventional indices of systolic ventricular function. Subsequently, early normalization of loading conditions led to an immediate and complete recovery of myocardial velocities. In contrast, late normalization of loading condition did not lead to a (complete) recovery, probably due to extensive alterations in the LV myocardial architecture leading to alterations in intrinsic myocardial contractility.\textsuperscript{37,38}

Weidemann \textit{et al.} described similar findings in adult VAS patients in a Tissue Doppler imaging study. Their data suggested that aortic valve replacement fails to
reduce the degree of replacement fibrosis. Patients without fibrosis experienced the greatest clinical functional improvement, which was accompanied by an increased myocardial deformation. However, in patients with myocardial fibrosis both functional status and abnormal deformation appeared not to be reversible after AVR. These findings suggest that improvement of regional deformation and clinical outcome is limited by permanent myocardial alterations such as fibrosis (which in turn is related to severity and duration of disease).\textsuperscript{11}

Information on (regional) myocardial systolic dysfunction in children with congenital VAS is limited. A study by Kiraly et al. was the first to indicate a decline in regional deformation in various degrees of congenital VAS as assessed by means of Tissue Doppler Imaging.\textsuperscript{39} Recently, a study performed by our research group showed that 2DSTE parameters were significantly related to the severity of congenital valvular aortic stenosis, with abnormal longitudinal deformation even in patients with mild stenosis.\textsuperscript{3} Whether these signs of myocardial dysfunction are reversible by normalization of loading conditions has not been elucidated. Myocardial reaction to pressure overload can be quite different in children, especially of younger ages, when compared to adults. The myocardial cells of young children have yet some multiplication potential and vascular growth can more flexibly follow the hypertrophy process.\textsuperscript{40} It is possible therefore that in children the elasticity of the heart wall may be preserved and permanent myocardial alterations will only develop after a relatively long period of time. Thus, findings in adults with degenerative VAS cannot be expected to be the same in children with congenital VAS. We aimed to assess the reversibility of changes in (regional) myocardial systolic deformation as determined by means of 2DSTE in this subset of patients.

\textit{The present study} revealed significant decreased deformation indices in nearly all VAS patients prior to balloon valvuloplasty when compared to healthy subjects. The decline in peak systolic longitudinal deformation was not equally distributed over the different ventricular wall segments. Peak systolic longitudinal strain (-rate) was
most affected throughout the interventricular septum, especially its basal segment. A possible explanation for this finding may be found in its geometry. The interventricular septum, because of its flatter and more asymmetric contour with a larger radius of curvature in the longitudinal plane, is subjected to a greater increase in systolic wall stress than the lateral free wall. Consequently, both myocardial hypertrophy and a decrease in systolic function produced by the increase of afterload and subsequent exaggerated wall stress are predominantly seen in the interventricular septum, particularly in its basal part. Conventional echocardiographic parameters to assess systolic function prior to intervention were within normal limits. Several months after balloon valvuloplasty significant improvement of 2DSTE parameters in all three principal directions of deformation was observed. When corrected for maturational changes, these changes remained statistically significant. The deviation from normal reference values for all individual strain parameters was significantly reduced at six months follow-up. The improved myocardial deformation, observed following balloon valvuloplasty, is probably at least partially caused by a reduced pressure-overload and a decline of its negative effects on myocardial function. However, when 2DSTE results at three years follow-up were corrected for maturational changes with use of normal reference values, no statistically significant further reduction in deviation from normal could be identified. These findings indicate that recovery of left ventricular systolic deformation in children with severe congenital VAS primarily occurs in the first months after balloon valvuloplasty. Thereafter, no significant further improvement can be anticipated using two-dimensional strain parameters.

Although there is substantial recovery of myocardial deformation after balloon intervention, our results indicate incomplete recovery of longitudinal and circumferential myocardial function. Global peak systolic strain values and global peak systolic strain-rate in the longitudinal and circumferential plane were significantly lower at three years follow-up when compared with healthy age-matched children. More importantly, these respective strain values at three years
follow-up were also significantly lower when compared to age-matched children with an uncorrected aortic valve stenosis similar to the residual stenosis in our study subjects. These latter results could indicate fixed structural myocardial alterations (e.g., myocardial replacement fibrosis) of at least the subendocardial layers of the myocardium, which are mainly responsible for longitudinal function.11,43 These findings are in agreement with other strain imaging studies that have indicated that impairment of longitudinal, and to a lesser extend circumferential function, precedes radial dysfunction in VAS as well as other conditions characterized by a pressure overload of the left ventricle.3,10,31,34,42,44 The reason for this is that these subendocardial fibers are more vulnerable to increased wall stress, stress-induced ischemia, and microvascular dysfunction accompanying left ventricular pressure overload.45 In contrast, myocardial function in radial direction as well as most of the time to global peak systolic strain values fully normalized after balloon valvuloplasty. Neither left ventricular ejection fraction, nor fractional shortening prior to intervention, was predictive of systolic deformation indices at three years follow-up. In contrast, peak transvalvular velocity across the stenotic valve prior to balloon valvuloplasty as well as the extent to which the pressure gradient was reduced after intervention were significantly related to peak systolic strain parameters at three years follow-up.

**5.6 STUDY LIMITATIONS**

Post-interventional echocardiographic examinations revealed significantly more frequent aortic regurgitation which is a well recognized complication following aortic valvuloplasty. Theoretically the higher incidence of aortic regurgitation can also account for incomplete recovery of myocardial performance as assessed by 2DSTE. However, with multiple regression analysis such a relation was not identified, perhaps due to the limited number of patients. It is uncertain whether the persistent abnormalities in myocardial systolic deformation at late follow-up
after balloon valvuloplasty are the result of irreversible myocardial alterations due to longstanding pressure overload or merely part of the congenital heart defect where developmental abnormalities result in alterations in functional capacity. Whether earlier timing of intervention might influence the recovery is beyond the scope of our study. The clinical and prognostic implications of the identified persistent abnormalities in myocardial deformation described in this study remain to be elucidated. Longer follow-up studies will hopefully provide an answer to these questions. The relation between decreased myocardial strain (rate) parameters and myocardial fibrosis could not be sustained by myocardial biopsies due to ethical reasons. Furthermore, at the time of the present study, tagged MRI in young children was not available for research purposes. However, previous studies have clearly indicated the relation between persistent abnormal deformation and replacement fibrosis.\textsuperscript{11,37} Although myocardial replacement fibrosis due to chronic pressure-overload affects systolic as well as diastolic ventricular function, we did not investigate left ventricular diastolic function by means of 2DSTE. At present, the optimal frame rate for speckle tracking appears to be 60-90 FPS. As a consequence, two-dimensional strain echocardiography is less suitable for the measurement of shorter events such as deformation during the diastolic rapid filling phase, especially in children in whom heart rate is known to be relatively high.\textsuperscript{46}

\textbf{5.7 CONCLUSION}

Echocardiographic examination after balloon valvuloplasty for severe VAS in children shows a reduction of transvalvular pressure gradients and normal myocardial performance as assessed with conventional echocardiographic indices of left ventricular systolic function. However, there is an incomplete recovery of pre-interventional, decreased, systolic two-dimensional strain parameters. The latter might be indicative of (fixed) myocardial alterations. The clinical and prognostic
implications of persistent abnormal deformation in the longitudinal and circumferential direction remain to be elucidated by long-term follow-up.

5.8 REFERENCES


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Health consists of having the same diseases as one's neighbors.

Quentin Crisp
Engelse auteur (1908-1999)
Chapter 6
Early detection of myocardial dysfunction in children with mitochondrial disease

An ultrasound and two-dimensional strain echocardiography study

Karen A. Marcus, Marlieke E. Barends, Eva Morava-Kozicz, Ton Feuth, Chris L. de Korte, Livia Kapusta

MITOCHONDRION
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6.1 ABSTRACT

*Background:* Myocardial dysfunction in children diagnosed with mitochondrial disease is an ominous sign and has been associated with substantial increased mortality rates. Early detection of cardiac involvement would therefore be desirable. Two dimensional strain echocardiography (2DSTE) has proven to be more sensitive than conventional echocardiography for the detection of early myocardial dysfunction in various conditions.

*Aim:* To determine left ventricular systolic function in children with mitochondrial disorders by means of physical examination, electrocardiography (ECG), conventional echocardiography and 2DSTE.

*Methods:* A total of 27 children with established mitochondrial disease and 54 age-matched control subjects underwent cardiac evaluation. Global longitudinal, circumferential and radial peak systolic strain (S) values were determined as well as global peak systolic strain-rate (Sr) and the global time to peak systolic strain (T2P). One-way analysis of variance was performed to assess the influence of the presence of mitochondrial disease on conventional echocardiographic and 2DSTE outcomes.

*Results:* Conventional echocardiographic findings did not indicate systolic left ventricular dysfunction. Global peak S, Sr and T2P measurements in all three directions were significantly lower in children with mitochondrial disease (*P*<0.001) when compared to controls.

*Conclusion:* 2DSTE detects alterations in myocardial systolic function in children diagnosed with mitochondrial disease, whose conventional echocardiographic findings did not indicate ventricular systolic dysfunction.
6.2 BACKGROUND

Mitochondrial oxidative phosphorylation (OXPHOS) defects are the most common inborn errors of metabolism, with a prevalence of 1 : 5000.\(^1\) The OXPHOS system consists of five enzyme complexes and uses the trans-membrane proton gradient to produce adenosine triphosphate (ATP).\(^2\) Mutations in either nuclear or mitochondrial genes encoding structural or facilitating mitochondrial proteins cause a suboptimal production of ATP, affecting mostly organs with a high energy demand.\(^2\)

The clinical presentation of mitochondrial disorders is extremely variable, ranging from isolated myopathies to complex multisystem syndromes.\(^3\) The heart, being a highly energy-dependent tissue, is frequently affected by mitochondrial dysfunction. The identification of cardiac involvement in (children suspected of) mitochondrial disorders is important, since mitochondrial cardiomyopathies (MIC) are a diagnostic criterion in several mitochondrial syndromes.\(^4\) The need for timely identification of MIC is further illustrated by its adverse clinical course attributed to progressive biventricular dysfunction which is associated with a substantial increased mortality rate.\(^5,6\) MIC is usually identified by means of conventional echocardiographic examination. With use of this modality, MIC is diagnosed in 20-25\% of children with mitochondrial disease.\(^5,7\) Unfortunately, these conventional echocardiographic techniques provide no information about regional alterations in ventricular myocardial contraction and have proven to be relatively insensitive to impaired myocardial performance in various (primary or secondary) cardiac conditions.\(^8\) Occult LV dysfunction in children with mitochondrial disease could have important implications for morbidity and mortality. Therefore, it would be clinically valuable to detect such subclinical dysfunction.

Two-dimensional strain echocardiography (2DSTE) has emerged as a relatively new index of regional and global myocardial function. This ultrasound-based modality investigates regional as well as global myocardial deformation (e.g., stretching and
shortening versus thickening and thinning of the myocardial wall). The spectrum of potential clinical applications is very broad. Due to its ability to differentiate between active and passive deformation of myocardial segments, to quantify intraventricular dyssynchrony and to evaluate individual components of myocardial function such as longitudinal myocardial shortening, 2DSTE is able to detect myocardial dysfunction at an early stage. Previous studies have indicated that 2DSTE is more sensitive than conventional echocardiography for detecting early myocardial dysfunction in a wide variety of clinical disorders. However, the value and applicability of 2DSTE in the evaluation of ventricular systolic myocardial function in children with mitochondrial disease have not been elucidated.

We hypothesized that abnormal regional myocardial systolic function in children with mitochondrial disease can be detected, even before the overt manifestation of cardiomyopathy and heart failure. The aim of this study is to introduce 2DSTE as a useful technique for the early detection of such subtle alterations in myocardial function.

6.3 MATERIAL and METHODS

6.3.1 STUDY POPULATION

Between December 2008 and December 2009 we prospectively included children who were previously diagnosed with mitochondrial disease, visiting the outpatient clinic of the Institute for Metabolic, Genetic and Endocrine Disorders of the Radboud University Nijmegen Medical Centre, The Netherlands. Mitochondrial disease had been established according to the following protocol. Patients with a clinically and metabolically suspected oxidative phosphorylation disorder were first evaluated based on the Mitochondrial Disease Criteria (MDC). The biochemical and genetic characteristics were consecutively obtained by using standard diagnostic protocols.
The MDC score is a screening instrument for mitochondrial disease, which has a high specificity in distinguishing between mitochondrial and other multisystem disorders in children. Following the first outpatient visit, all children undergo a standard diagnostic protocol of multiple investigations including anthropometric measurements, electrocardiogram, chest X-ray, electroencephalogram, brain auditory evoked potential, visual evoked potential, sensory evoked potentials and a cranial magnetic resonance imaging prior to the muscle biopsy. Serial lactic acid measurements, pyruvic acid levels, blood gas, serum acyl-carnitine, amino acid and urine organic acid profiles are analysed in all children. The MDC score is calculated based on clinical symptoms, metabolic alterations and abnormal neuro-imaging features. A score of 2-4 suggest a “possible mitochondrial disorder”, 5-7 are comparable with a “probable mitochondrial disorder” and score 8-12 confirm the diagnosis “definite mitochondrial disorder”.

According to the standard clinical practice patients with an MDC score above 4 are admitted for surgical muscle and skin biopsy under generalized anesthesia. Parallel with routine immune-histological and electron-microscopic analysis, oxidation ratios, ATP-production from pyruvate oxidation and the activity of pyruvate dehydrogenase complex (PDHc) and the respiratory enzyme complexes I-V are measured in the fresh muscle sample. ATP-production from pyruvate oxidation and the activity of the respiratory enzyme complexes I-V are also determined in fibroblasts according to the methods described previously. Based on the complex deficiencies, histological findings and the clinical features appropriate genetic studies are initiated.
The diagnosis mitochondrial disease was confirmed when one of the following criteria was met:

1. A mitochondrial disease score of (more than) eight.
2. An identified deficiency of at least one of the respiratory chain enzyme complexes:\textsuperscript{16}
   - OXPHOS complex deficiency in muscle biopsy and/or in fibroblast culture.
   - Enzyme histochemical evidence of OXPHOS complex deficiency in muscle.
3. A significantly decreased ATP synthesis rate from pyruvate oxidation in fresh muscle biopsy specimen (≤ 20 nmol/h/mU CS; normal reference values: 42-81 nmol/h/mU CS).\textsuperscript{16}
4. The presence of a known pathogenic mutation of mitochondrial DNA or nuclear DNA, together with compatible clinical symptoms.

Exclusion criteria consisted of: (1) congenital heart disease (2) valvular stenosis or regurgitation (3) acute illness at the time of echocardiographic evaluation. Demographic and anthropometric characteristics, including age and gender were collected at the same time the echocardiographic study was performed. A complete history, physical examination, ECG as well as echocardiographic examination were performed as part of hospital policy.

6.3.2 CONTROL GROUP

Subjects who were routinely referred for echocardiographic evaluation of an asymptomatic, innocent heart murmur or for screening purposes between May 1, 2005 and November 1, 2009, were retrospectively analysed for their eligibility for inclusion in the study to serve as a control group. Subjects with structural
(congenital) heart disease, abnormal cardiac rhythms and/or (a past history of) chronic or acute illness were excluded.

6.3.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

All subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography and a local research protocol previously described by Mavinkurve-Groothuis et al. Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). Quantification of cardiac chamber size, ventricular mass and systolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography. Left ventricular systolic function was characterized using fractional shortening (FS), ejection fraction (EF), left ventricular myocardial performance index, or Tei index, end-systolic wall stress (ESWS) and rate-corrected velocity of circumferential fiber shortening (VCFc). Ejection fraction was calculated using the modified Simpson’s rule. The PW-Doppler-derived myocardial performance index (Tei index) was calculated as: Tei = (iso-volumetric contraction time + iso-volumetric relaxation time) / ejection time. Left ventricular end-systolic wall stress was calculated using the modified formula of Rowland and Gutgesell. VCFc was calculated using the formula obtained from Colan and colleagues. Left ventricular mass (LVM) was calculated using the formula for estimation of LVM according to Devereux and Reichek and was subsequently indexed to body surface area (BSA).

6.3.4 2DSTE DATA ACQUISITION

Two-dimensional multiframe B-mode (grayscale) images were obtained in the apical four-chamber (4C), and parasternal midcavity short-axis view (at the level of the
papillary muscle: PM) and basal short-axis view (at the level of the mitral valve: MV). A sector scan angle of 30 to 60 degrees was chosen and frame rates of 60 to 90 Hz were used. Preferably images from five cardiac cycles triggered by the R wave of the QRS complex were digitally saved in cineloop format. Offline strain analysis was performed using software for echocardiographic quantification (EchoPAC 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). The timing of aortic valve closure and mitral valve opening with respect to peak strain and peak systolic strain were manually obtained, using single gated pulsed-wave (PW-)Doppler or continuous-wave (CW-)Doppler images of the left ventricular outflow tract. For these measurements, special care was taken to keep the heart rate in the same range as during the 2D grayscale imaging used for 2D strain calculations. Endomyocardial borders of the left ventricle were manually traced within the end-systolic frame. The second, epicardial tracing was generated by the computer algorithm and, when necessary, manually adjusted to cover the whole myocardial wall. The tracking algorithm then followed the endocardial and myocardial speckles through the cardiac cycle. Tracking was accepted only if both visual inspection as well as EchoPAC software indicated adequate tracking. The software automatically divided the cross-sectional image into six segments, which were named and identified according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association.24 The left ventricular segments to be analysed were the apical, middle and basal segments of the septal and the lateral wall of the 4C view, as well as the anteroseptal, anterior, lateral, posterior, inferior and septal segments of the basal (MV) and midcavity (PM) short-axis views. Strain curves of the three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for further analysis. The Q-Q interval was determined from the electrocardiogram signal to obtain cardiac cycle length. It is known that the duration of the systolic phase of the cardiac cycle in rest does not change with small changes in heart rate, in contrast to the diastolic phase.25 Therefore, the diastolic phase of the three cardiac cycles was automatically extended and adjusted by the software package to the longest of the
three cardiac cycles. This intervention prevents a shift of the peak systolic strain with averaging of the three consecutive cardiac cycles. Cardiac cycles with lengths more than ten percent different from the mean length of the three cardiac cycles were excluded from averaging and thus for further analysis. Myocardial longitudinal, radial and circumferential strain values were obtained, as well as peak systolic strain-rate and the time to reach peak systolic strain. To determine global strain (-rate), the strain (-rate) values of the six segments were averaged for the 4C as well as the short-axis views. All 2D strain analyses were performed by the same investigator (K.A.M.) to avoid interobserver variability. Intraobserver (and interobserver) variability scores have been described by our research group previously. Strain values are dimensionless and are expressed as percentages. Strain-rate is the time derivative of strain and is expressed as s⁻¹. Time to peak systolic strain is expressed as milliseconds. Negative strain values reflect shortening/thinning, while positive strain values reflect lengthening/thickening.

6.3.5 STATISTICAL ANALYSIS

All normally distributed demographic, anthropometric, conventional echocardiographic and 2DSTE values are expressed as mean ± standard deviation (SD), non-normally distributed data as median and range. Demographic and anthropometric characteristics of the patient group were compared with those of the control group using one-way analysis of variance or Kruskal-Wallis nonparametric test when outcomes were not normally distributed. All demographic and anthropometric parameters were all normally distributed except for age. Conventional echocardiographic as well as 2DSTE measurements established in children with mitochondrial disease were compared with respective measurements acquired from healthy age-matched control subjects. The relation between the presence or absence of mitochondrial disease and individual conventional echocardiographic findings, as well as each global 2DSTE parameter (global peak systolic strain, global peak systolic strain-rate, time to global peak systolic strain in
longitudinal, circumferential and radial direction) was investigated using one-way analysis of variance (ANOVA) and linear regression analysis. The relation between the type of mitochondrial dysfunction (i.e., different types of respiratory chain enzyme complex deficiencies), as well as the presence of multiple enzyme complex deficiencies in the same patient and each 2DSTE parameter was assessed by one-way analysis of variance with Bonferroni correction for multiple comparisons. P-values less than 0.05 were considered to indicate significance. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).

6.4 RESULTS

6.4.1 STUDY POPULATION

In total 100 children referred to the outpatient clinic of the Institute for Metabolic, Genetic and Endocrine Disorders of the Radboud University Nijmegen Medical Centre, The Netherlands, for investigation of mitochondrial disease were evaluated for possible inclusion in the study. Of those children 73 were subsequently excluded: in 63 cases mitochondrial disease was not confirmed by subsequent investigations (MDC score was not diagnostic, or a mitochondrial dysfunction was detected, without a severely decreased ATP production; see diagnostic criteria), 10 children were diagnosed with a congenital heart defect or valvular disease. Finally, 27 pediatric patients aged 0 to 15 years with established respiratory chain dysfunction were eligible for inclusion in the study. Since this is a cross sectional study, patients were evaluated at various times after disease onset and diagnosis. The median time after diagnoses was 3.0 years (range 0.0 – 13.7 years). Clinical phenotype and diagnostic findings are described in Table 25.
Table 25 - Clinical phenotype and diagnostic findings of 27 children with established mitochondrial dysfunction

<table>
<thead>
<tr>
<th>Clinical phenotype</th>
<th>Myopathy</th>
<th>Encephalomyopathy</th>
<th>Encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>5 (18)</td>
<td>21 (78)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>MDC score ≥ 8</td>
<td>2</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Decreased ATP synthesis</td>
<td>3</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Pyruvate oxidation deficiency</td>
<td>4</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Complex I deficiency</td>
<td>3</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Complex II deficiency</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Complex III deficiency</td>
<td>2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Complex IV deficiency</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Multiple complex deficiencies</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Mt/nucl. DNA alterations</td>
<td>1</td>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

MDC score: mitochondrial disease criteria score; Mt/nucl. DNA mutation: mutation in mitochondrial or nuclear DNA

6.4.2 GENETIC RESULTS

Genetic analysis revealed alterations in the mitochondrial DNA in 5 patients. An unreported homoplasmic alteration was found in three siblings and their affected mother in the 12S ribosomal RNA gene (m.1008A>T) in combination with a polymorphism (m.14574C>T) in the gene encoding the NADH dehydrogenase 6 subunit (part of complex I). The pathogenicity of the alterations was not yet confirmed. Mitochondrial DNA analysis in a fourth patient revealed a homoplasmic mutation in 12S ribosomal RNA MTRN1 (m.01484C>T). In the fifth patient with an identified mitochondrial DNA mutation, genetic analysis revealed a point mutation in the ATPase 6 gene (MTATP6; m. 8993T>G) causing Leigh syndrome. Nuclear DNA mutations, resulting in mitochondrial dysfunction, were identified in two other patients. The first was an X-linked mutation in exon 10 of the pyruvate dehydrogenase complex (PDHc) E1 α-gene (PDHA1; 924G>T). The second patient in whom nuclear DNA mutations were identified, was finally diagnosed with a non-
mitochondrial mutation underlying the phenotypical and biochemical features of severe mitochondrial dysfunction due to compound heterozygous PLA2G6 gene mutations in exon 11 (c1445G>A) and exon 16 (c2222G>A), resulting in a progressive neurodegenerative disorder: the infantile form of dystonia parkinsonism (MIM 25660).

### 6.4.3 CONTROL GROUP

A group of 54 healthy age- and gender-matched children was included and examined to provide us with normal reference values for conventional echocardiographic and 2DSTE parameters.

### 6.4.4 HISTORY AND PHYSICAL EXAMINATION

History and physical examination of both patients diagnosed with mitochondrial disease and control subjects were not indicative of cardiovascular disease. None used medications for cardiac disease. Demographic and anthropometric findings in children with mitochondrial dysfunction were comparable to those found in control subjects using one-way analysis of variance. Patient demographic characteristics and anthropometric parameters are described in Table 26, together with the data of the control group.
Table 26 - Demographic and anthropometric characteristics (mean ± standard deviation or median and range) of patients with mitochondrial disease and control subjects

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Mitochondrial Disease (n = 27)</th>
<th>Control subjects (n = 54)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>19 (70%)</td>
<td>38 (70%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Age (y)</td>
<td>4.8 (0.8 – 15.3)</td>
<td>4.8 (0.7 – 15.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>107 ± 19</td>
<td>112 ± 26</td>
<td>0.38</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.6 ± 8</td>
<td>22.0 ± 7</td>
<td>0.22</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>0.74 ± 0.22</td>
<td>0.82 ± 0.33</td>
<td>0.26</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.5 ± 1.9</td>
<td>16.2 ± 1.8</td>
<td>0.14</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>103 ± 20</td>
<td>97 ± 21</td>
<td>0.10</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>68 ± 6</td>
<td>67 ± 7</td>
<td>1.00</td>
</tr>
<tr>
<td>Syst.BP (mmHg)</td>
<td>95 ± 8</td>
<td>94 ± 8</td>
<td>1.00</td>
</tr>
<tr>
<td>Diast.BP (mmHg)</td>
<td>55 ± 5</td>
<td>53 ± 5</td>
<td>1.00</td>
</tr>
</tbody>
</table>

BMI: body mass index; BSA: body surface area; Diast.BP: diastolic blood pressure; HR: heart rate; MAP: mean arterial blood pressure; Syst.BP: systolic blood pressure; characteristics of the patient group were compared with those of the control group using one-way analysis of variance or Kruskal-Wallis nonparametric test when outcomes were not normally distributed.

6.4.5 ECG FINDINGS

Electrocardiographic examination did not reveal abnormalities. There were no signs of ischemia or conduction disturbances.
6.4.6 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional systolic echocardiographic indices were normal in all children. In none of the children diagnosed with mitochondrial disease, echocardiographic findings indicated left ventricular hypertrophy and/or dilation. One-way analysis of variance did not indicate statistically significant differences in standard echocardiographic indices between patients and healthy controls. However, there was a trend towards lower left ventricular mass (corrected for body surface area as well as indexed to height) in children with mitochondrial disorders ($P < 0.06$ and $0.08$ resp.). Conventional echocardiographic findings are described in Table 27.
Table 27 – Conventional echocardiographic findings (mean ± standard deviation) of children with mitochondrial disease and healthy control subjects

<table>
<thead>
<tr>
<th>Conventional Parameters</th>
<th>Mitochondrial Disease (n = 27)</th>
<th>Control subjects (n = 54)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVPWd (mm)</td>
<td>4.7 ± 1.2</td>
<td>5.0 ± 1.4</td>
<td>0.35</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>4.3 ± 0.7</td>
<td>4.5 ± 1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>34 ± 5</td>
<td>36 ± 6</td>
<td>0.22</td>
</tr>
<tr>
<td>Mitral A velocity</td>
<td>0.55 ± 0.17</td>
<td>0.56 ± 0.11</td>
<td>0.98</td>
</tr>
<tr>
<td>Mitral E velocity</td>
<td>1.08 ± 0.13</td>
<td>1.07 ± 0.15</td>
<td>0.98</td>
</tr>
<tr>
<td>Mitral EA Ratio</td>
<td>1.96 ± 0.36</td>
<td>1.97 ± 0.41</td>
<td>0.97</td>
</tr>
<tr>
<td>LVETc (s)</td>
<td>0.32 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>1.00</td>
</tr>
<tr>
<td>VCFc (circ/s)</td>
<td>1.27 ± 0.13</td>
<td>1.27 ± 0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Tei index</td>
<td>0.38 ± 0.05</td>
<td>0.37 ± 0.02</td>
<td>0.55</td>
</tr>
<tr>
<td>FS</td>
<td>0.38 ± 0.03</td>
<td>0.38 ± 0.05</td>
<td>0.97</td>
</tr>
<tr>
<td>LVEF biplane</td>
<td>0.69 ± 0.04</td>
<td>0.70 ± 0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>ESWS (g/cm²)</td>
<td>41.6 ± 13</td>
<td>43.8 ± 12</td>
<td>0.45</td>
</tr>
<tr>
<td>LVM/BSA (g/m²)</td>
<td>45.7 ± 11</td>
<td>51.7 ± 13</td>
<td>0.06</td>
</tr>
<tr>
<td>LVM/height².⁷ (g/m².⁷)</td>
<td>27.7 ± 7</td>
<td>31.3 ± 8</td>
<td>0.08</td>
</tr>
</tbody>
</table>

LVPWd: diastolic left ventricular posterior wall thickness; IVSd: diastolic interventricular septal thickness; LVIDd: diastolic left ventricular diameter; LVETc: left ventricular ejection time corrected for heart rate; VCFc: heart-rate corrected velocity of circumferential fiber shortening; Tei index: left ventricular myocardial performance index; FS: fractional shortening; LVEF biplane: left ventricular ejection fraction measured by modified Simpson’s method; ESWS: end-systolic wall stress; LV mass/BSA: left ventricular mass corrected for body surface area; echocardiographic parameters of the patient group were compared with those of the control group using one-way analysis of variance.
Tracking was feasible in 98% of all segments of the 4 chamber view, in 98% of all segments in the short-axis view at the level of the papillary muscle and in 94% of all segments in the short-axis view at the level of the mitral valve.

The most important observations were statistically significant \((P < 0.001)\) differences of global peak systolic strain values and global peak systolic strain-rate measurements in all three directions between patients with mitochondrial disorders and healthy control subjects as determined by one-way analysis of variance. Linear regression analysis revealed that the presence or absence of mitochondrial dysfunction explained 21 to 62% of variation in global peak systolic strain and global peak systolic strain-rate values. Time to peak global systolic strain values was similar to those found in healthy children. Evaluation of time to peak systolic strain values in the different left ventricular segments did not indicate intraventricular dyssynchrony. There were no statistically significant differences in 2DSTE parameters between the various types of respiratory chain enzyme deficiencies. The presence of multiple complex deficiencies in the same patient did not influence 2DSTE values as assessed by one-way analysis of variance with Bonferroni correction for multiple comparisons. There was no relation between 2DSTE parameters and MDC-score.

In the individual patient with mitochondrial disease under investigation, global peak systolic strain was abnormal (below 5\(^{th}\) percentile of normal according to reference values established by our research group) in 56% of patients for longitudinal strain, 69% of patients for circumferential strain at the level of the papillary muscle and 46% of patients for circumferential strain at the level of the mitral valve.\(^{27}\) Global peak systolic radial strain was abnormal at the level of the papillary muscle in 15% of the patients versus 39% of patients at the level of the mitral valve. As for global peak systolic strain-rate, 44% of patients showed an abnormal longitudinal
deformation-rate, 65% of patients for circumferential strain rate at the level of the papillary muscle and 31% of patients for circumferential strain rate at the level of the mitral valve. Global peak systolic radial strain-rate was abnormal at the level of the papillary muscle in 73% of the patients versus 58% of the patients at the level of the mitral valve. Two-dimensional strain echocardiography findings in patients and healthy control subjects are described in Table 28.
Table 28 - 2DSTE-findings (mean ± standard deviation) of children with mitochondrial disease and healthy control subjects

<table>
<thead>
<tr>
<th>2DSTE parameters</th>
<th>Mitochondrial Disease (n = 27)</th>
<th>Control subjects (n = 54)</th>
<th>P value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global SL (%)</td>
<td>-17.9 ± 2.3</td>
<td>-21.2 ± 1.3</td>
<td>&lt;0.001*</td>
<td>0.44</td>
</tr>
<tr>
<td>Global SC-P (%)</td>
<td>-16.8 ± 2.7</td>
<td>-22.3 ± 2.3</td>
<td>&lt;0.001*</td>
<td>0.54</td>
</tr>
<tr>
<td>Global SC-M (%)</td>
<td>-16.4 ± 1.7</td>
<td>-20.5 ± 2.0</td>
<td>&lt;0.001*</td>
<td>0.51</td>
</tr>
<tr>
<td>Global SR-P (%)</td>
<td>51.7 ± 10</td>
<td>55.0 ± 7</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>Global SR-M (%)</td>
<td>43.1 ± 10</td>
<td>51.0 ± 6</td>
<td>&lt;0.001*</td>
<td>0.21</td>
</tr>
<tr>
<td>T2P global SL (ms)</td>
<td>346 ± 42</td>
<td>337 ± 41</td>
<td>0.37</td>
<td>-</td>
</tr>
<tr>
<td>T2P global SC-P (ms)</td>
<td>324 ± 46</td>
<td>321 ± 43</td>
<td>0.81</td>
<td>-</td>
</tr>
<tr>
<td>T2P global SC-M (ms)</td>
<td>336 ± 43</td>
<td>330 ± 43</td>
<td>0.61</td>
<td>-</td>
</tr>
<tr>
<td>T2P global SR-P (ms)</td>
<td>340 ± 39</td>
<td>333 ± 38</td>
<td>0.44</td>
<td>-</td>
</tr>
<tr>
<td>T2P global SR-M (ms)</td>
<td>379 ± 48</td>
<td>358 ± 54</td>
<td>0.09</td>
<td>-</td>
</tr>
<tr>
<td>Global SrL (s⁻¹)</td>
<td>-1.1 ± 0.1</td>
<td>-1.4 ± 0.1</td>
<td>&lt;0.001*</td>
<td>0.55</td>
</tr>
<tr>
<td>Global SrC-P (s⁻¹)</td>
<td>-1.5 ± 0.3</td>
<td>-1.9 ± 0.2</td>
<td>&lt;0.001*</td>
<td>0.37</td>
</tr>
<tr>
<td>Global SrC-M (s⁻¹)</td>
<td>-1.2 ± 0.2</td>
<td>-1.6 ± 0.2</td>
<td>&lt;0.001*</td>
<td>0.30</td>
</tr>
<tr>
<td>Global SrR-P (s⁻¹)</td>
<td>2.2 ± 0.7</td>
<td>3.5 ± 0.4</td>
<td>&lt;0.001*</td>
<td>0.62</td>
</tr>
<tr>
<td>Global SrR-M (s⁻¹)</td>
<td>2.2 ± 0.5</td>
<td>3.3 ± 0.4</td>
<td>&lt;0.001*</td>
<td>0.57</td>
</tr>
</tbody>
</table>

SL: peak systolic longitudinal strain; SC: peak systolic circumferential strain; -P: at the level of the papillary muscle; -M: at the level of the mitral valve; SR: peak systolic radial strain; T2P: time to peak systolic strain; SrL: peak systolic longitudinal strain-rate; SrC: peak systolic circumferential strain-rate; SrR: peak systolic radial strain-rate; * P < 0.05 determined by one-way analysis of variance and linear regression analysis; R²: coefficient of determination indicating the proportion of variability accounted for by the presence or absence of mitochondrial disease.
6.5 DISCUSSION

Mitochondrial disorders, although genetically diverse, share the common cellular consequences of decreased ATP production, increased reliance on anaerobic energy sources and increased production of reactive oxygen species. Since the extent of dysfunction is dependent upon the aerobic energy requirements of each tissue, highly energy-dependent tissues, such as the central nervous system and skeletal and cardiac muscle, are commonly involved. However, there is no strict correlation between the genetic background, biochemistry, system involvement and symptomatic status. The same biochemical and genetic defect can be associated with completely different phenotypes. This phenomenon, in some of the patients carrying mitochondrial DNA mutations, can be explained by unique features of mitochondrial disorders. A condition known as heteroplasmy allows mutated mitochondrial DNA to coexist with normal mitochondrial genomes in the same cell. Furthermore, different cells and tissues may contain vastly different percentages of mutant mitochondrial DNA. Together, these characteristics result in a remarkable phenotypic variation in expression, even within the same family. No obvious explanation exists however for the surprising phenotypic variability in most patients diagnosed with a mitochondrial disorder due to nuclear mutations. Due to this variable expression it is difficult to predict if and when cardiomyopathy will occur in a patient diagnosed with mitochondrial dysfunction. What we do know for certain, is such a patient to be at increased risk of developing myocardial malfunction.

The molecular events linking respiratory chain defects to cardiac hypertrophy have not been completely elucidated. It appears that multiple factors contribute to myocardial dysfunction. First, experimental models suggest that both energy derangements and increase of mitochondrial-derived reactive oxygen species are important steps in developing cardiac malfunction in mitochondrial disease. In addition, mitochondrial proliferation per se, a well-recognized compensatory
mechanism in mitochondrial disease, could interfere with sarcomere alignment and contraction through ultrastructural remodeling.\textsuperscript{33} Finally, mitochondrial disorders have been associated with angiopathy independently of the presence of cardiac hypertrophy. It has been speculated that mitochondrial proliferation leads to narrowing of the lumen of arterioles, which might be responsible for the ischemic findings observed by means of scintigraphy.\textsuperscript{34} Combined, these pathologic alterations lead to myocardial dysfunction which can present itself in various forms. Although concentric hypertrophic non-obstructive cardiomyopathy is observed most frequently, dilated and noncompaction cardiomyopathy have been reported.\textsuperscript{4,5,35,36}

Many cases of MIC are associated with both myopathy and neuropathy. Cardiac involvement rarely causes the initial symptoms of mitochondrial disease. In just 5-10% of children diagnosed with MIC, cardiomyopathy was the first sign of mitochondrial dysfunction.\textsuperscript{5,36} The onset of MIC as sole presenting symptom occurs predominantly early in childhood and is characterized by a rapid downward course. Frequently, these patients die before diagnosis can be made. In general, the median age at onset of cardiac symptoms in patients who presented with extracardiac symptoms is 5-24 months, ranging from birth to 27 years.\textsuperscript{5,6,36,37} It is important to note that myocardial dysfunction can develop long after the diagnosis mitochondrial disease has been made. Even when previous (traditional echocardiographic) investigations revealed no cardiac abnormalities.\textsuperscript{5}

Cardiomyopathy is a hallmark of several mitochondrial syndromes. Barth syndrome (MGA type II; MIM 302060) caused by defects of the TAZ gen presents with 3-methylglutaconic aciduria, mitochondrial dysfunction, cyclic neutropenia and dilated cardiomyopathy. Mutations of the \textit{DNAJC19} gene, also associated with 3-methylglutaconic aciduria, (MGA type V; MIM610198) is a syndrome of dilated cardiomyopathy and ataxia.\textsuperscript{4} In the clinically variable group of mitochondrial complex V (ATPase) deficiency, specific mutations in the mitochondrial-encoded \textit{MTATP6} (MIM 516060) and \textit{MTATP8} (MIM 516070) genes, or nuclear-encoded
TMEM70 gene (MIM 612418), share the same phenotypic features such as profound hypotonia, lactic acidemia, failure to thrive and hypertrophic cardiomyopathy.\textsuperscript{38,39} Cardiac involvement in patients diagnosed with other forms of mitochondrial disease has been reported in several series with varying prevalence.\textsuperscript{5,6,7,36,37,40,41} The frequency of MIC in the cited studies ranges from 14 to 40% of patients. Since there is some difficulty in comparing the results of these studies in light of possible ascertainment biases as a result of different inclusion criteria used, the true prevalence of cardiac involvement remains unclear. The same problem arises when estimating the prognosis of MIC once myocardial dysfunction has been identified, although most reports agree that MIC is associated with an adverse clinical course.\textsuperscript{5,6,35,37} The disease may be stable for many years, however, rapid deterioration and sudden cardiac death have been observed. In a study by Holmgren \textit{et al.} 10 of 17 children diagnosed with MIC died from a cardiac cause. Which is in agreement with other reports.\textsuperscript{5,35,37} This poor prognosis is further illustrated by a marked increased mortality rate in children with myocardial involvement when compared to those without cardiac manifestations. Holmgren \textit{et al.} reported that mortality increased from 26% to 71%, whereas Scaglia \textit{et al.} described an incline from 5% to 82% when myocardial dysfunction was present.\textsuperscript{5,6} These figures indicate that MIC is an ominous finding in children diagnosed with mitochondrial disease. Timely identification of deterioration of myocardial function is important since early and aggressive supportive therapy might improve the chances of survival.

Conventional diagnostic cardiac imaging modalities such as standard echocardiographic techniques are appropriate to detect global LV dysfunction at advanced stages of cardiac disease. However, they are often not able to identify subclinical systolic (regional) myocardial abnormalities in children with various conditions. Previous reports have illustrated that strain imaging more carefully reflects myocardial systolic function. With this study we aimed to assess myocardial systolic function in children with mitochondrial disease. Our results indicate
significant subclinical systolic myocardial dysfunction in the presence of normal conventional echocardiographic indices and normal electrocardiographic findings. In none of the patients mitochondrial cardiomyopathy was identified with conventional echocardiographic techniques. However, global peak systolic strain values and global peak systolic strain-rate measurements in all three directions were significantly lower in patients when compared to healthy age-matched children indicating abnormal myocardial deformation in the first cohort. Time to global peak systolic strain values in the patient group did not differ significantly from those found in control subjects.

The clinical and prognostic implications of the identified abnormalities in myocardial deformation described in this study remain to be elucidated. Since all patients were asymptomatic they were not treated for heart failure, e.g. with beta-blocker and/or ACE inhibitor. Currently we cannot predict the clinical course of the patient group under investigation whose echocardiographic findings suggest abnormal systolic deformation. Future longitudinal follow-up studies will hopefully provide an answer to this question. However, previous reports have indicated that abnormal myocardial deformation is associated with an increased risk of cardiovascular events in several other (cardiac) conditions, even in the absence of conventional functional echocardiographic abnormalities.42,43

6.6 STUDY LIMITATIONS

Abnormal global peak systolic strain (-rate) finding could not be related to specific enzyme defects or mitochondrial disease score. It is possible that the relatively small number of patients prevents the identification of such a relation.
6.7 CONCLUSION

Our results indicate subclinical systolic myocardial deformation abnormalities in all three directions of myocardial contraction (longitudinal, circumferential and radial) in children with mitochondrial disease in the presence of normal conventional echocardiographic indices. Since myocardial dysfunction in mitochondrial disorders is an ominous sign, these findings emphasize the need for repeated cardiac assessment as part of monitoring disease progression in patients with confirmed mitochondrial disorders. Our findings illustrate the additional value of 2DSTE in the cardiac assessment.

6.8 REFERENCES

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Twijfel is het begin van wijsheid

Rene Descartes
Frans filosoof (1596-1650)
Chapter 7
Cardiac evaluation in children with Prader-Willi syndrome

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7.1 ABSTRACT

Background: Prader-Willi syndrome (PWS) is associated with a substantial increased risk of cardiovascular death and morbidity, which most likely cannot be entirely attributed to obesity-related risk factors.

Aim: Cardiac evaluation in children with PWS based on history, physical examination, electrocardiography (ECG), conventional echocardiography and two-dimensional strain echocardiography (2DSTE).

Methods: Physical examination, ECG recordings and transthoracic echocardiograms were performed and evaluated in the Children’s Heart centre of the Radboud University Hospital Nijmegen, the Netherlands. In total 19 children diagnosed with PWS and 38 age-matched control subjects underwent cardiac evaluation. Main outcome measures consisted of physical characteristics, ECG findings, conventional echocardiographic indices and 2DSTE parameters. Longitudinal, circumferential and radial peak systolic strain (S) values were determined as well as peak systolic strain-rate (Sr) and the time to peak global systolic strain (T2P). Systolic deformation appeared more affected in case of maternal uniparental disomy.

Results: Abnormal ECG findings were detected in nine PWS patients. Mild structural cardiac abnormalities were found in two patients by echocardiography. Conventional echocardiographic findings did not indicate systolic left ventricular dysfunction, in contrast to 2DSTE examination. Global peak S and Sr measurements in all three directions of contraction were significantly lower in children with PWS ($P<0.001$) compared with healthy age-matched children, indicating abnormal left ventricular deformation. In two-thirds of the patients, 2DSTE revealed abnormal systolic deformation (peak systolic strain as well as strain rate). T2P values in PWS patients were similar to control subject.
Conclusion: Cardiac evaluation, including 2DSTE, detected alterations in myocardial systolic function in children diagnosed with PWS, whose conventional echocardiographic findings did not indicate ventricular systolic dysfunction. Since cardiovascular morbidity and mortality is substantial in PWS, especially adults, we emphasize the need for cardiac assessment in PWS.

7.2 BACKGROUND

Prader-Willi syndrome (PWS) was the first recognized human disorder related to genomic imprinting, which means that genes are expressed differentially based on the parent of origin. In PWS, symptoms result from failure of expression of paternally inherited genes in the PWS region of chromosome 15. It is characterized by a recognizable pattern of dysmorphic features and major neurological, cognitive, endocrine and behavioral disturbances. These disturbances include severe hypotonia and feeding difficulties in early infancy, followed by excessive eating and gradual development of morbid obesity when the child is older, unless eating is controlled by dietary restriction or behavior modification. Endocrine features of PWS are related to hypothalamic dysfunction and include hypogonadotrophic hypogonadism, central adrenal insufficiency and growth hormone deficiency resulting in short stature. Several reports and surveys within large cohorts indicate that PWS is associated with a high incidence of sudden death in both children and adults, suspected to be cardiopulmonary in origin. Traditionally, cardiovascular death in PWS has been attributed to obesity-related risk factors. Patel et al. described microcirculatory dysfunction and raised levels of the inflammatory marker high-sensitivity CRP in adult PWS patients, both of which are associated with coronary artery disease. Recently, awareness is rising that critical illness and sudden cardiac death in PWS may not be entirely caused by obesity alone. It has been postulated that the above mentioned endocrine disturbances associated with PWS, as well as structural and functional cardiac abnormalities contribute to
increased cardiovascular morbidity and mortality. However, the structural and functional changes that may occur in the heart or vasculature, predisposing PWS patients to myocardial dysfunction, remain to be elucidated. The few previous studies that have addressed this issue have focused on adults with PWS entirely. These reports describe decreased myocardial mass and lower ejective and chronotropic response to dobutamine when compared to healthy obese controls.\textsuperscript{5,7}

Two-dimensional strain echocardiography (2DSTE) has emerged as a relatively new index of regional and global myocardial function. The technique uses two-dimensional ultrasound multiframe images to determine myocardial deformation (i.e., strain) in all three principal directions (longitudinal, circumferential and radial direction) per cardiac segment over time. Due to its ability to differentiate between active and passive deformation of myocardial segments, to quantify intraventricular dyssynchrony and to evaluate individual components of myocardial function, 2DSTE is able to detect myocardial dysfunction at an early stage.\textsuperscript{9,10,11} Previous studies have indicated that 2DSTE is more sensitive than conventional echocardiography for detecting early myocardial dysfunction in a wide variety of clinical disorders.\textsuperscript{12,13,14,15} The aim of the present study was to assess cardiac anatomy and myocardial function in children with PWS.

\section*{7.3 MATERIAL and METHODS}

\subsection*{7.3.1 STUDY POPULATION}

All consecutive children previously diagnosed with Prader-Willi syndrome visiting the outpatient clinic of the Pediatric Endocrinology and Metabolism Department of the Radboud University Nijmegen Medical Centre, The Netherlands, between October 1, 2008 and May 31, 2010, were included in the study. DNA diagnostic testing was previously performed in all patients and confirmative of the diagnosis PWS. All patients showed the absence of a paternal methylation pattern by
methylation specific polymerase chain reaction (MS-PCR), or Southern blot analysis. In order to identify the underlying genetic defect, follow-up studies using FISH, microsatellite analysis or methylation sensitive multiplex ligation-dependent probe amplification were performed (MS-MLPA; ME028-B1 kit from MRC Holland; http://www.mrc-holland.com). A complete history, physical examination by a pediatric cardiologist, 12-lead ECG as well as echocardiographic examination were performed as part of the clinical evaluation.

7.3.2 CONTROL GROUP

A group of healthy, age- and gender-matched children were prospectively recruited to serve as a control group. These children underwent the same cardiac evaluation as the group of the children diagnosed with PWS. Informed consent was obtained from each participant and their parents.

7.3.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

All subjects underwent a detailed transthoracic echocardiographic examination in the left lateral position according to the recommendations of the American Society of Echocardiography and a previous described local research protocol previously. Every examination was performed at rest, without using sedation. Images were obtained with a 3.0-MHz (S3) or a 5.0-MHz (S5) phased-array transducer using a commercially available system, the Vivid 7 echocardiographic scanner (GE, Vingmed Ultrasound AS, Horten, Norway). The choice of an S3 or a S5 transducer depended on the age and posture of the child. Quantification of cardiac chamber size, ventricular mass and systolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography. Left ventricular systolic function was characterized using fractional shortening (FS), ejection fraction (EF), left ventricular myocardial performance index, or Tei index, end-systolic wall stress (ESWS) and rate-corrected velocity of circumferential fiber shortening (VCFc).
7.3.4 DSTE DATA ACQUISITION

Two-dimensional strain echocardiography measurements were performed according to a previous described protocol.\textsuperscript{18,23} In short: two-dimensional multiframe B-Mode images were obtained from the apical four-chamber, parasternal midcavity short-axis view (at the level of the papillary muscle: PM), and parasternal basal short-axis view (at the level of the mitral valve: MV). Offline strain analysis was performed using speckle tracking software for echocardiographic quantification (EchoPAC 6.1.0; GE Vingmed Ultrasound AS, Horten, Norway). Myocardial longitudinal, radial and circumferential peak systolic strain (-rate) values were obtained, as well as the time to reach peak systolic strain. To determine global strain (-rate), the strain (-rate) values of the six segments were averaged for each of the views. All 2D strain analyses were performed by the same investigator (K.A.M.) to avoid interobserver variability. Intraobserver variability scores as well as normal reference values have been described by our research group previously.\textsuperscript{18,23} Strain values are dimensionless and are expressed as percentages. Strain-rate is the time derivative of strain and is expressed as s\textsuperscript{-1}. Time to peak systolic strain is expressed as milliseconds. Negative strain values reflect shortening/thinning, while positive strain values reflect lengthening/thickening.

7.3.5 STATISTICAL ANALYSIS

All values are expressed as mean ± standard deviation (SD) or median and range when outcomes were not normally distributed. Demographic and anthropometric characteristics, as well as conventional and 2DSTE measurements of the patient group were compared with those of the control group with one-way analysis of variance or Kruskal-Wallis nonparametric test when outcomes were not normally distributed. The relation between PWS and individual conventional echocardiographic findings as well as each global 2DSTE parameter (global peak systolic strain, global peak systolic strain-rate, time to peak global systolic strain in
longitudinal, circumferential and radial direction) was investigated using one-way analysis of variance and multivariate analysis. The relation between the genetic defect (paternal deletion or maternal uniparental disomy), gender as well as growth hormone therapy (GHT) and each 2DSTE parameter was assessed by one-way analysis of variance and multivariate analysis. Two-dimensional strain echocardiography measurements in children with PWS were compared with previously described reference values.23 $P$-values less than 0.05 were considered to indicate significance. All statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, IL).

7.4 RESULTS

7.4.1 STUDY POPULATION

In total 19 children diagnosed with PWS by MS-PCR or Southern blot analysis were included. Follow-up studies using FISH, microsatellite analysis or MS-MLPA showed a deletion of the paternally inherited chromosomal 15q11.2-q13 region in 12 (63%) patients. In 7 (37%) others, maternal uniparental disomy 15 was detected.

7.4.2 CONTROL GROUP

A group of 38 healthy age- and gender-matched children was included and examined to provide us with normal reference values for conventional echocardiographic and 2DSTE parameters.

7.4.3 HISTORY AND PHYSICAL EXAMINATION

History and physical examination of patients diagnosed with PWS and control subjects, were not indicative of cardiovascular disease. None used medications for cardiac disease. Eleven patients received GHT (median duration GHT: 4.6 years;
range: 1.6 – 15.7 years). Of those patients treated with growth hormone, five patients also received thyroxine treatment. Eight PWS patients received neither GHT, nor other medication. All were free from hypertension. Demographic and anthropometric findings in children with PWS were similar to those found in control subjects. There were no statistically significant differences between both groups. An important observation was that body mass index (BMI) was increased in only one PWS patient (30.5 kg/m²: z-score 3). Patient demographic characteristics and anthropometric parameters are described in Table 29, together with the data of the control group.

7.4.4 ECG FINDINGS

Electrocardiographic examinations were normal in all control subjects. In the patient group, abnormalities were identified in nine PWS patients. High voltages in the left (two patients) and right (two patients) precordial leads, first degree atrioventricular block with PR interval lengthened beyond 0.2 s (one patient), pathological Q wave in lead III and aVF (one patient: Figure 26) and prolonged QT interval corrected for heart rate of 0.52 - 0.55 s (three patients). There were no ST segment or T-wave abnormalities detected. Electrocardiographic abnormalities were equally distributed among patients with uniparental disomy (4 patients) and patients with a deletion (5 patients).
Table 29 – Demographic and anthropometric characteristics (mean ± standard deviation or median and range) of patients with PWS and control subjects

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>PWS patients (n = 19)</th>
<th>Control subjects (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>12 (63%)</td>
<td>24 (63%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Age (y)</td>
<td>5.8 (0.3 – 19.9)</td>
<td>5.8 (0.3 – 19.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>120 ± 41</td>
<td>126 ± 44</td>
<td>0.64</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>30.0 (5 – 71)</td>
<td>27.8 (6 – 83)</td>
<td>0.89</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.04 ± 0.59</td>
<td>1.06 ± 0.61</td>
<td>0.92</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.9 (12.5 – 30.5)</td>
<td>17.9 (14.3 - 28.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.20 (-2 – 3)</td>
<td>-0.10 (-2 – 2)</td>
<td>0.19</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>95 ± 34</td>
<td>92 ± 30</td>
<td>0.77</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>70 ± 11</td>
<td>69 ± 11</td>
<td>0.69</td>
</tr>
<tr>
<td>Syst.BP (mmHg)</td>
<td>97 ± 14</td>
<td>96 ± 12</td>
<td>0.88</td>
</tr>
<tr>
<td>Diast.BP (mmHg)</td>
<td>57 ± 10</td>
<td>57 ± 9</td>
<td>0.71</td>
</tr>
</tbody>
</table>

BMI: body mass index; BSA: body surface area; Diast.BP: diastolic blood pressure; HR: heart rate; MAP: mean arterial blood pressure; Syst.BP: systolic blood pressure. Patient group and control subjects were compared using one-way analysis of variance or Kruskal Wallis nonparametric test when data were not normally distributed.
Figure 26 – Pathological Q waves in lead III and aVF in one pediatric PWS patient
7.4.5 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional echocardiographic findings are described in Table 30. Echocardiographic examination revealed an atrial septum defect of the ostium secundum type in one PWS patient accompanied by increased right ventricular internal diameters at both end-systole and end-diastole. In a second patient, a moderate-severe pulmonary valve stenosis was identified without right ventricular hypertrophy. Both patients were diagnosed with a deletion. Conventional echocardiographic indices to indicate ventricular systolic function were normal in all children with PWS as well as healthy, age-matched children. In none of the children echocardiographic findings were indicative of left and/or right ventricular hypertrophy. One-way analysis of variance did not indicate statistically significant differences in standard echocardiographic indices between patients and healthy controls. In children diagnosed with PWS, there was a statistically significant difference ($P < 0.003$) in left ventricular mass corrected for body surface area among patients who received GHT (n=11) compared to those without GHT (n= 8) with one-way analysis of variance ($63 \pm 11$ versus $45 \pm 7$ g/m$^2$ respectively).

7.4.6 MYOCARDIAL STRAIN PARAMETERS

Tracking was feasible in 98% of all segments of the four-chamber view, in 94% of all segments in the midventricular short-axis view and in 94% of all segments in the basal short-axis view. In one patient two-dimensional strain analysis was not possible since the quality of the B-Mode images was too low. Interestingly, global peak systolic strain values and global peak systolic strain-rate measurements in all three directions showed statistically significant ($P <0.001$) differences between patients with PWS and healthy control subjects as determined by one-way analysis of variance (Table 31).
Multivariate analysis revealed that the presence or absence of PWS explained 22 to 57% of variation in global peak systolic strain and global peak systolic strain-rate values. Time to peak global systolic strain values was comparable to those found in healthy children. There were no statistically significant differences in 2DSTE parameters between PWS patients who received GHT and those without growth hormone replacement as assessed by one-way analysis of variance. There were no gender differences identified. We did, however, detect statistically significant differences between 2DSTE parameters that could be attributed to different genotypes, i.e. maternal uniparental disomy or deletion. Although global peak systolic strain values and global peak systolic strain-rate measurements in all three directions were significantly lower in children with PWS when compared to healthy control subjects, irrespective of their specific genetic defect, radial systolic strain (strain-rate) parameters were more affected in PWS patients with maternal uniparental disomy (detected by means of one-way analysis of variance with Bonferroni correction for multiple comparisons). Global peak systolic radial strain and radial peak systolic strain-rate (both at the level of the papillary muscle and at the level of the mitral valve) were significantly ($P < 0.05$) lower in PWS patients with maternal uniparental disomy.

In the individual patient with PWS under investigation, global peak systolic strain was abnormal (below 5th percentile of normal according to reference values established by our research group) in 67% of patients for longitudinal strain, 78% of patients for midventricular circumferential strain and 67% of patients for basal circumferential strain. Global peak systolic radial strain was abnormal in 44% of the patients, both at the level of the papillary muscle and at the level of the mitral valve. Global peak systolic strain rate was abnormal in 72% of patients for longitudinal strain rate, 50% of patients for circumferential strain rate, both at the level of the papillary muscle and at the level of the mitral valve. Global peak systolic radial strain rate was abnormal in 77% of the PWS patients at the level of the papillary muscle and 72% of patients at the level of the mitral valve.
Table 30 – Conventional echocardiographic findings (mean ± standard deviation) of children with PWS and healthy control subjects

<table>
<thead>
<tr>
<th>Conventional Parameters</th>
<th>PWS patients (n = 19)</th>
<th>Control subjects (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVPWd (mm)</td>
<td>5.9 ± 1.8</td>
<td>5.5 ± 2.0</td>
<td>0.52</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>5.4 ± 1.5</td>
<td>5.0 ± 1.7</td>
<td>0.43</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>37 ± 10</td>
<td>39 ± 10</td>
<td>0.62</td>
</tr>
<tr>
<td>Mitral A velocity</td>
<td>0.62 ± 0.18</td>
<td>0.61 ± 0.19</td>
<td>0.87</td>
</tr>
<tr>
<td>Mitral E velocity</td>
<td>0.98 ± 0.09</td>
<td>0.97 ± 0.15</td>
<td>0.86</td>
</tr>
<tr>
<td>Mitral EA Ratio</td>
<td>1.70 ± 0.49</td>
<td>1.71 ± 0.46</td>
<td>0.96</td>
</tr>
<tr>
<td>LVETc (s)</td>
<td>0.31 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>VCFc (circ/s)</td>
<td>1.24 ± 0.20</td>
<td>1.27 ± 0.22</td>
<td>0.71</td>
</tr>
<tr>
<td>Tei index</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>FS</td>
<td>0.38 ± 0.05</td>
<td>0.37 ± 0.05</td>
<td>0.54</td>
</tr>
<tr>
<td>LVEF biplane</td>
<td>0.69 ± 0.05</td>
<td>0.67 ± 0.15</td>
<td>0.64</td>
</tr>
<tr>
<td>ESWS (g/cm²)</td>
<td>39 ± 8</td>
<td>43 ± 12</td>
<td>0.22</td>
</tr>
<tr>
<td>LVM/BSA (g/m²)</td>
<td>57 ± 13</td>
<td>56 ± 16</td>
<td>0.74</td>
</tr>
<tr>
<td>LVM/height².7 (g/m².7)</td>
<td>37 ± 12</td>
<td>32 ± 10</td>
<td>0.15</td>
</tr>
</tbody>
</table>

LVPWd: diastolic left ventricular posterior wall thickness; IVSd: diastolic interventricular septal thickness; LVIDd: diastolic left ventricular diameter; LVETc: left ventricular ejection time corrected for heart rate; VCFc: heart-rate corrected velocity of circumferential fiber shortening; Tei index: left ventricular myocardial performance index; FS: fractional shortening; LVEF biplane: left ventricular ejection fraction measured by modified Simpson’s method; ESWS: end-systolic wall stress; LV mass/BSA: left ventricular mass corrected for body surface area. Statistically significant differences between patient group and control subjects were assessed by one-way analysis of variance.
Table 31 - 2DSTE findings (mean ± standard deviation) of children with PWS and healthy control subjects

<table>
<thead>
<tr>
<th>2DSTE parameters</th>
<th>PWS (n = 18)</th>
<th>Control subjects (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global SL (%)</td>
<td>-17.3 ± 2.1</td>
<td>-21.2 ± 2.0</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SC-P (%)</td>
<td>-17.2 ± 1.8</td>
<td>-23.1 ± 2.8</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SC-M (%)</td>
<td>-16.1 ± 2.2</td>
<td>-20.7 ± 2.3</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SR-P (%)</td>
<td>46.2 ± 10</td>
<td>56.4 ± 6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Global SR-M (%)</td>
<td>44.8 ± 7</td>
<td>53.7 ± 6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>T2P global SL (ms)</td>
<td>341 ± 57</td>
<td>335 ± 69</td>
<td>0.79</td>
</tr>
<tr>
<td>T2P global SC-P (ms)</td>
<td>325 ± 55</td>
<td>315 ± 57</td>
<td>0.60</td>
</tr>
<tr>
<td>T2P global SC-M (ms)</td>
<td>342 ± 57</td>
<td>335 ± 54</td>
<td>0.68</td>
</tr>
<tr>
<td>T2P global SR-P (ms)</td>
<td>372 ± 62</td>
<td>360 ± 65</td>
<td>0.88</td>
</tr>
<tr>
<td>T2P global SR-M (ms)</td>
<td>379 ± 48</td>
<td>358 ± 54</td>
<td>0.56</td>
</tr>
<tr>
<td>Global SrL (s⁻¹)</td>
<td>-1.0 ± 0.2</td>
<td>-1.4 ± 0.1</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SrC-P (s⁻¹)</td>
<td>-1.3 ± 0.4</td>
<td>-1.9 ± 0.2</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SrC-M (s⁻¹)</td>
<td>-1.2 ± 0.2</td>
<td>-1.6 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Global SrR-P (s⁻¹)</td>
<td>2.5 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Global SrR-M (s⁻¹)</td>
<td>2.4 ± 0.4</td>
<td>3.2 ± 0.3</td>
<td>&lt;0.001 *</td>
</tr>
</tbody>
</table>

SL: peak systolic longitudinal strain; SC-P: peak systolic circumferential strain at the level of the papillary muscle; SC-M: peak systolic circumferential strain at the level of the mitral valve; SR-P: peak systolic radial strain at the level of the papillary muscle; SR-M: peak systolic radial strain at the level of the mitral valve; T2P: time to peak systolic strain; SrL: peak systolic longitudinal strain-rate; SrC-P: peak systolic circumferential strain-rate at the level of the papillary muscle; SrC-M: peak systolic circumferential strain-rate at the level of the mitral valve; SrR-P: peak systolic radial strain-rate at the level of the papillary muscle; SrR-M: peak systolic radial strain-rate at the level of the mitral valve; * P < 0.05 determined by one-way analysis of variance and multivariate analysis.
7.5 DISCUSSION

It is well recognized that pediatric as well as adult PWS patients are at substantial risk of sudden, premature death. A retrospective study, performed by Whittington et al. reported annual mortality rates of 3% across all ages and 7% in patients older than thirty years, compared to an overall annual mortality of 0.13% for the general population below 55 years. Cardiovascular illness is the main cause of death in adolescents and adults with PWS, whereas mortality in early childhood is predominantly related to respiratory and gastro-intestinal infections. In the latter group of young children with PWS, it has been postulated that (unrecognized) adrenal insufficiency could offer an explanation for increased mortality rates during relatively mild to moderate infections. In addition to fatal infections, life threatening circulatory complications, such as sudden cardiac death or progressive heart failure, are not uncommon in PWS throughout whole childhood. Despite the established substantial increased risk of cardiovascular morbidity and mortality in (pediatric) patients with PWS, cardiac structure and myocardial function has not been investigated previously in children diagnosed with PWS.

In the present study we conducted a thorough cardiovascular assessment in children with PWS. Electrocardiographic examination revealed mild abnormalities in nine out of nineteen PWS patients (47%). In four patients electrocardiographic findings were indicative of ventricular hypertrophy, which was not confirmed by means of echocardiography. This was not surprising, since it is well-known that electrocardiography is associated with a relatively low accuracy in assessing ventricular hypertrophy in children. In five other patients, electrocardiographic abnormalities necessitated future cardiac follow-up due to asymptomatic pathologic Q waves, a first degree atrioventricular block and a prolonged QT interval which could indicate pathologic myocardial alterations.
Conventional echocardiographic examination detected mild structural cardiac anomalies in two of the nineteen (11%) children diagnosed with PWS, both of which require long-term cardiac follow-up. One patient had a valvular pulmonary valve stenosis, whereas in another patient an atrial septum defect of the ostium secundum type was identified. In the latter patient, ECG findings indicated right ventricular hypertrophy, which was not confirmed by conventional echocardiography. Other morphological abnormalities were not detected. It is important to note that left ventricular mass (indexed for body surface area as well as height), left ventricular internal diameter as well as the interventricular septum thickness in PWS patients, irrespective of GHT, were equal to respective findings in healthy, age-matched children. These findings are in contrast to previous reports indicating a reduced left ventricular mass and left ventricular internal diameter in adult PWS patients and adults with growth hormone deficiency (GHD).\(^7,31,32,33\) We did however find a difference in left ventricular mass corrected for body surface area in children diagnosed with PWS when children who received GHT were compared to those who did not receive GH replacement. Left ventricular mass was significantly higher in the group of patients receiving GH substitution. This is in agreement with a study performed by Marzullo et al., which described that GHT in adult PWS patients resulted in an increased left ventricular mass.\(^34\)

Using conventional echocardiographic techniques to assess left ventricular systolic function, we did not identify myocardial dysfunction in children with PWS. All tested conventional systolic indices were within normal limits and similar to those found in age-matched control subjects. It is well known that standard echocardiographic techniques are appropriate to detect global LV dysfunction at advanced stages of cardiac disease. However, in children with various conditions these modalities have not been able to identify subclinical systolic (regional) myocardial abnormalities.

Previous reports have illustrated that strain imaging more carefully reflects myocardial systolic function.\(^11,12,13,15\) Our results indicate significant subclinical systolic myocardial dysfunction in the presence of normal conventional
echocardiographic indices in children diagnosed with PWS. Global peak systolic strain values and global peak systolic strain-rate measurements in all three directions were significantly lower in PWS patients compared with healthy, age-matched children. Time to global peak systolic strain values in the patient group did not differ significantly from those found in control subjects. Two-dimensional strain echocardiography measurements did not differ between boys and girls, nor did we identify a relation with GHT. It is possible that the number of children investigated in the present study is too small to detect such a relation. In contrast to electrocardiographic and conventional echocardiographic abnormalities, which were equally distributed among PWS patients with a deletion and maternal uniparental disomy, 2DSTE findings indicated more severe systolic dysfunction in PWS patients with uniparental disomy. Radial peak systolic strain (rate) measurement were significantly more affected in PWS patients with maternal uniparental disomy when compared to the group of patients in whom a deletion was detected. Interestingly, a previous report described an increased cardiovascular fatality rate in adult PWS patients with maternal uniparental disomy compared with fatality rates of PWS patients with a deletion. The reason for this (possible) increased risk of cardiovascular complications in PWS patients with maternal uniparental disomy is unknown. Smith et al. hypothesize that another gene on chromosome 15 (besides the q11-q13 region) is maternally imprinted and, when no paternal copy is present, leads to deleterious effects on cardiac function.

Together, our results indicate that structural and functional cardiac abnormalities are common in children diagnosed with PWS. Traditionally, cardiovascular fatalities in PWS have been ascribed to obesity-related complications, such as diabetes mellitus, sleep apnea, arterial hypertension, and coronary artery disease. This has prompted many investigators to recommend strict control on calorie intake in order to improve life expectancy. Currently, there is an increasing awareness that other factors, besides obesity, may contribute to poor health outcome and circulatory fatalities in PWS. The findings of the present study support the latter
hypothesis since cardiac abnormalities were frequently present despite the virtual absence of obesity in our PWS patients. 

In this context, GHD has been suggested as a possible contributing factor leading to increased risk of cardiovasculatory complications. Reduced GH secretion has been documented in both children and adults with PWS.\(^{37,38,39,40}\) It is known that patients with childhood-onset GHD often suffer both from structural cardiac abnormalities and functional myocardial impairment, that combine to reduce diastolic filling and impair left ventricular response to peak exercise.\(^{32}\) Experimental and clinical studies have provided evidence that both GH and its anabolic mediator, insulin-like growth factor-1 (IGF-1) are implicated in cardiac development and function.\(^{41,42}\) Left ventricular mass and LV diameter are reduced in GHD patients proportionately to the duration of GHD or to IGF-I levels.\(^{33}\) In large cohorts, cardiac dysfunction has been reported proportionately to the severity of GHD, with systolic and diastolic abnormalities affecting 45–78% of GHD patients.\(^{43}\) Another point that should not be disregarded in patients with GHD is the presence of endothelial dysfunction. GHD patients often have an increased vascular intima-media thickness and a higher occurrence of atheromatous plaques, that can further aggravate the hemodynamic conditions and contribute to increased cardiovascular risk.\(^{44}\) Epidemiological data suggest that adults with hypopituitarism have reduced life expectancy compared with healthy controls, with a greater than twofold increase mortality for cardiovascular disease.\(^{45}\) In patients with PWS, a GH/IGF-I-mediated control of cardiac risk has been reported. A study by Marzullo et al. described decreased cardiac mass and lower ejective and chronotropic response to dobutamine in adult PWS patients compared with healthy obese controls.\(^{7}\) Several lines of evidence have suggested that the cardiovascular abnormalities can be partially reversed by GH replacement therapy in GHD patients. The reversibility of cardiovascular abnormalities after GH replacement in PWS and its effect on circulatory-related mortality have not been investigated. However, it has been reported that one year of GHT in adult PWS patients results in improved LV mass without significant effects on cardiac function.\(^{34}\) A second factor, possibly contributing to circulatory
complications in PWS is autonomic dysfunction characterized by diminished parasympathetic nervous system activity. A disturbance in sympathovagal tone has been implicated in the development of arrhythmia and sudden death in different patient groups.

7.6 STUDY LIMITATIONS

This cross sectional study was conducted in a relatively small cohort of patients. Despite the small number of patients, statistical significant differences in systolic deformation between healthy individuals and patients with PWS were detected. The clinical and prognostic implications of the identified abnormalities in myocardial deformation described in this study remain to be elucidated. Currently we cannot predict the clinical course of the patient group under investigation whose echocardiographic findings suggest abnormal systolic deformation. Future longitudinal follow-up studies will hopefully provide an answer to this question. However, previous reports have indicated that abnormal myocardial deformation is associated with an increased risk of cardiovascular events in several other (cardiac) conditions, even in the absence of conventional functional echocardiographic abnormalities.

7.7 CONCLUSION

Our results indicate structural, electrocardiographic and subclinical systolic myocardial deformation abnormalities in all three directions (longitudinal, circumferential and radial) in children with PWS. Systolic myocardial function appears more affected in case of maternal uniparental disomy. Since cardiovascular morbidity and mortality is substantial in PWS, especially adults, we emphasize the
need for (repeated) cardiac assessment in PWS. Our findings indicate that 2DSTE could be of additional value.

7.8 REFERENCES


17. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18(12):1440-63.


Some remedies are worse than the disease itself.

(Lat. Graviora quaedam
sunt remedii perculs)

Publius Syrus
Remedia sancta

(Lat. Graviora quaedam
sunt remedii perculs)
Chapter 8
Myocardial 2D strain echocardiography and cardiac biomarkers in children during and shortly after anthracycline therapy for Acute Lymphoblastic Leukemia (ALL)

A prospective study

Annelies M. C. Mavinkurve-Groothuis, Karen A. Marcus, Milanthy Pourier, Jacqueline Groot-Loonen, Ton Feuth, Peter M. Hoogerbrugge, Chris L. de Korte, Livia Kapusta

Submitted.
8.1 ABSTRACT

Background: Anthracycline-induced cardiotoxicity can lead to severe heart failure and is a growing problem in survivors of childhood cancer.

Aim: The aim of this study was to investigate the additive value of cardiac biomarkers and myocardial 2D strain echocardiography in the assessment of cardiac function in children with Acute Lymphoblastic Leukemia (ALL) during and shortly after treatment with anthracyclines.

Methods: Cardiac function of 60 children with ALL was prospectively studied by means of cardiac Troponin T (cTnT) and N-Terminal-pro-brain natriuretic peptide (NT-pro-BNP) measurements, as well as conventional and myocardial 2D strain echocardiography. Cardiac assessment was performed at three instances: before start of anthracycline therapy (T=0), after three months (T=1) and after one year (T=2). Echocardiographic data were compared to respective measurements acquired from 60 healthy, age-matched controls.

Results: None of the patients showed clinical signs of cardiac failure. Fractional shortening (FS) remained normal. Cardiac function, as indicated by strain indices, decreased significantly during treatment and was significantly decreased compared to normal controls. cTnT levels were abnormal in 11% of the patients at T=1 and were significantly related to increased time to global peak systolic longitudinal strain at T=2 (P = 0.003). NT-pro-BNP levels were abnormal in 13% of patients at T=1 and in 20% at T=2. Absolute values of NT-pro-BNP increased throughout treatment in 59% of the patients. Predictors for abnormal cardiac biomarkers and myocardial 2D strain parameters at T=2 were cumulative anthracycline dose, z-score of the diastolic left ventricular internal diameter and FS at baseline, abnormal NT-pro-BNP at T=0 and T=1, and global peak systolic longitudinal strain (-rate) at T=1.
Conclusion: Cardiac biomarkers and myocardial 2D strain echocardiography are useful in the detection of subclinical early anthracycline-induced cardiotoxicity.

8.2 BACKGROUND

Cardiotoxicity is a well-known side effect of anthracycline therapy. Early-onset anthracycline-induced cardiotoxicity occurs during or within the first year after anthracycline treatment. It is a risk factor for the development of late-onset anthracycline-induced cardiotoxicity, which is known to cause serious cardiac health problems in a growing number of cancer survivors.1,2,3,4,5,6

Cardiac biomarkers are increasingly used for the detection of anthracycline-induced cardiotoxicity.7,8,9,10,11,12,13,14,15,16,17 Release of cardiac troponins is indicative for myocardial necrosis. Natriuretic peptides are released by the myocardium in response to volume and pressure overload and have shown to be sensitive markers of left ventricular dysfunction.7,8 The value of myocardial strain echocardiography in early-onset anthracycline-induced cardiotoxicity has been studied by tissue Doppler imaging and by means of 2D speckle tracking.15,16,17 These studies indicated abnormal myocardial deformation during anthracycline therapy and years after anthracycline therapy, with relatively preserved fractional shortening.15,16,17,18,19

In the present, prospective study, we aim (1) to document cTnT and NT-pro-BNP levels, as well as conventional and myocardial 2D strain echocardiographic parameters before, during and shortly after treatment with anthracyclines in children with newly diagnosed ALL, (2) to find predictors for abnormal cardiac function at one year after anthracycline treatment and (3) to compare cardiac performance of children with ALL after one year of anthracycline treatment to cardiac function of healthy controls.
8.3 MATERIAL and METHODS

8.3.1 STUDY POPULATION

Consecutive, newly diagnosed patients with childhood ALL and their parents/guardians were asked informed consent to participate in our study between January 2006 and March 2009. Exclusion criteria consisted of cardiac disease prior to therapy and renal failure.

The first cardiac evaluation was performed in the first week of ALL treatment, i.e. before the first anthracycline dose (T=0). The second cardiac evaluation was performed at the end of the induction phase of the ALL treatment, which is ten weeks after start of treatment and five weeks after the latest anthracycline dose (T=1). The third cardiac evaluation was performed one year after start of ALL treatment, which is at least two weeks after the last anthracycline dose (T=2). The study was approved by the local ethics committee.

8.3.2 CONTROL GROUP

As a control group, we included the conventional echocardiographic and 2D speckle tracking data of 60 healthy age-matched controls that were routinely referred for echocardiographic evaluation of an asymptomatic, innocent heart murmur or for screening purposes. Subjects with structural (congenital) heart disease or abnormal cardiac rhythms were excluded. Other exclusion criteria consisted of hypertension, chronic illness, recent acute illness or poor echocardiographic image quality.
8.3.3 ANTHRACYCLINE TREATMENT AS PART OF THE ANTILEUKEMIC THERAPY

Treatment according to the DCOG-ALL-10 protocol is based on the DCOG-ALL-8 protocol. Patients are stratified in three treatment groups; standard risk (SR), medium risk (MR) and high risk (HR) patients, largely based on response to treatment.\textsuperscript{20} The total duration of treatment is two years, but shorter for HR patients who are eligible for stem cell transplantation (SCT). At T=1, the cumulative anthracycline dose of all patients is 120 mg/m\textsuperscript{2}. At T=2, SR patients have received a cumulative anthracycline dose of 120 mg/m\textsuperscript{2}, MR patients 300 mg/m\textsuperscript{2} and HR patients a maximum cumulative anthracycline dose of 240 mg/m\textsuperscript{2} and an additional 52.5 mg/m\textsuperscript{2} of mitoxantrone and 18 mg/m\textsuperscript{2} of idarubicin, depending on (timing of) SCT. All SCT patients received total body irradiation (TBI).

8.3.4 CARDIAC BIOMARKERS

Blood samples were obtained from ALL patients at T=0, T=1 and T=2 from a central venous line. Cardiac TnT levels were assessed by the Elecsys Troponin T STAT Immunoassay, performed on a Modular E immunoassay analyzer (Roche Diagnostics, Mannheim, Germany).\textsuperscript{21} A level of ≤ 0.01 ng/ml was defined as normal. NT-pro-BNP was measured using an electrochemiluminescence immunoassay, using the NT-pro-BNP kit (Roche Diagnostics, Mannheim, Germany). Normal values for children were based on age-dependent reference values (97.5\textsuperscript{th} percentile).\textsuperscript{22}

8.3.5 CONVENTIONAL ECHOCARDIOGRAPHY

Transthoracic echocardiography in left lateral position was performed at rest, without using sedation. Images were obtained with a 3.0-MHz and a 5.0-MHz transducer, depending on the age and weight of the study subjects, using a Vivid 7 echographic scanner (GE, Vingmed Ultrasound, Horten, Norway). Frame rate was kept between 70 and 100 frames per second (fps).\textsuperscript{23,24} Quantification of cardiac
chamber size, left ventricular mass (LVM) and systolic and diastolic left ventricular function were measured in accordance with the recommendations for chamber quantification by the American Society of Echocardiography. Z-scores of left ventricular dimensions and LVM were calculated based on normal values of M mode measurements. The left ventricular systolic function was indicated using fractional shortening (FS). Left ventricular diastolic function was indicated using the E/A ratio, E/E’ ratio and the isovolumic relaxation time. Left ventricular mass was calculated according to the formula of Devereux.

8.3.6 2DSTE DATA ACQUISITION

Two-dimensional grayscale images were obtained from the parasternal apical 4-chamber and midcavity short-axis (at the level of the papillary muscle) views. A sector scan angle of 30-60 degrees was chosen and frame rates of 60-90 Hz were used since these rates are considered to be optimal for speckle tracking. Cine loops of three cardiac cycles triggered by the R wave of the QRS complex were digitally saved. Off-line analysis was performed using software for echocardiographic quantification (EchoPAC 6.1.0, GE Medical Systems, Horten, Norway). Separately, timing of aortic valve closure and mitral valve opening was obtained, using single gated pulsed Doppler or continuous wave Doppler images. These images were acquired just after the 2D strain grayscale images and special care was taken to ensure that the heart rate was in the same range. Myocardial segments were named and localized according to the statement of the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Manual tracking of the endomyocardial borders was performed at the end-systolic frame. An automatic generation of the second epicardial tracing was created by the software. The software automatically divided the image into six segments. Quality of the tracking was verified for each segment and adjusted when needed. Strain curves of three consecutive cardiac cycles and values of the manual timing were imported into a custom-made software package for further analysis. Cardiac cycles
with a length more than ten percent different from the mean length of the three cardiac cycles were excluded for further analysis. The average values of peak systolic longitudinal (SL), radial (SR) and circumferential (SC) strain and strain rate of the three imported curves were calculated by the custom made software. Global strain and strain rate were calculated by averaging the six segments of each view. Measurements of time to global peak systolic strain were expressed as a percentage of the duration of the complete cardiac cycle (i.e., R-R interval). Strain values are dimensionless and are expressed as percentages, whilst strain rate is denoted as s⁻¹. Negative strain (-rate) values reflect shortening/thinning, while positive strain values reflect lengthening/thickening. We recently showed that myocardial strain imaging with 2D echocardiography can produce scores with high interobserver, intraobserver and intrapatient reliability. Abnormal global peak systolic SL, -SR and -SC were defined as values below the 5th percentile of normal pediatric reference values according to age.

8.3.7 STATISTICAL ANALYSIS

Characteristics of the study population and biomarker levels are reported using median and range. Conventional echocardiographic and 2D strain parameters were summarized by using mean and standard deviation (SD). The biomarker levels were studied at T=1 and T=2 for available paired observations using the McNemar test for abnormal biomarkers levels and the Wilcoxon signed ranks test for the absolute biomarker levels. The echocardiographic data in the patient group were studied using Linear Mixed Models and Generalized Estimating Equations (GEE) to account for correlations between different points in time. Echocardiographic parameters of patients and controls were compared using the independent t-test. Using logistic regression analysis we searched for possible predictors at baseline and T=1 for abnormal cardiac biomarkers and 2D myocardial global SL, SR and SC at T = 2. We refrained from multiple regression analysis because of the small number of subjects.
Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 16.0 and SAS, version 8.2. A $P$-value less than 0.05 was considered to indicate significance.

**8.4 RESULTS**

**8.4.1 SUBJECT CHARACTERISTICS**

Seventy children and their parents were asked to give informed consent for participation in this study. Parents of four patients refused informed consent. One patient was excluded because of congenital heart disease (atrial septal defect). Consequently, 65 patients were included in the study. Before $T=1$, three patients altered treatment because of treatment related (non-cardiac) toxicity and two patients died (progressive disease and sepsis, respectively). Further analysis was done in the remaining 60 patients. Three patients died after $T=1$ (one sepsis and two relapses, respectively) and one patient was lost to follow-up by moving abroad. Parents of one SR patient refused echocardiography and biomarker sampling at $T=2$. In six patients, follow-up was not available at $T=2$. Six patients in the HR-group completed $T=2$. Four of them received the full dose of anthracyclines, two were eligible for SCT; one received a cumulative anthracycline dose of 120 mg/m$^2$, one a cumulative anthracycline dose of 120 mg/m$^2$ plus 18 mg/m$^2$ of idarubicin and 26.25 mg/m$^2$ of mitoxantrone. None of the patients showed clinical symptoms (e.g., rhythm disorders, acute heart failure) of acute anthracycline-induced cardiotoxicity and none of them used cardiac medication.

Hyperhydration, i.e., the infusion of 3000 ml/m$^2$/day of glucose 2.5%/NaCl 0.45%, as part of the supportive care to prevent tumor lysis syndrome was given in 95% of the patients at $T=0$, but in none at $T=1$ and $T=2$. The characteristics of ALL patients and the healthy controls are described in table 32.
Table 32 - Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Male gender (N)</td>
<td>37 (62%)</td>
<td>40 (67%)</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>6 (2.2-15.4)</td>
<td>5.8 (2.2-14.7)</td>
<td>0.09</td>
</tr>
<tr>
<td>BSA</td>
<td>0.87 (0.54-1.93)</td>
<td>0.81 (0.50-1.84)</td>
<td>0.07</td>
</tr>
<tr>
<td>HR</td>
<td>79 (53-122)</td>
<td>90 (53-122)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hb* level at diagnosis (mmol/l)</td>
<td>5.5 (2.2-9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLC† at diagnosis (*10⁹/l)</td>
<td>10.4 (0.7-348)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperhydration at T=0 (N (%))</td>
<td>57(95%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk stratification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR (n)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR (n)</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (n)</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cumulative anthracycline dose at the different time points

- **T=0** all patients: 0 mg/m²
- **T=1** all patients: 120 mg/m²
- **T=2** SR-patients: 120 mg/m²
  - MR-patients: 300 mg/m²
  - HR-patients (n=4), no SCT: 240 mg/m² + 18 mg/m² idarubicin + 52.5 mg/m² mitoxantrone
- **HR-patient (n=1), SCT:** 120 mg/m²
- **HR-patient (n=1), SCT:** 120 mg/m² + 18 mg/m² idarubicin + 26.25 mg/m² mitoxantrone

Values are expressed as median and range.

*Hemoglobin, †Total Leucocyte Count. ‡Mann-Whitney-U analysis.
8.4.2 CARDIAC BIOMARKERS

The biomarker levels at T=0, T=1 and T=2 are shown in Table 33. Levels of cTnT were normal in all patients before start of anthracycline treatment (T=0). At T=1, cTnT levels were abnormal in 11% of the patients (5/45) and normalized in all at T=2. In one MR-patient, cTnT became abnormal at T=2 (i.e. after a cumulative anthracycline dose of 300 mg/m²).

NT-pro-BNP levels were abnormal in 26% of the patients (12/46) at T=0. Ninety-five percent of the patients received hyperhydration at T=0, when plasma samples of NT-pro-BNP and cTnT were obtained. The median NT-pro-BNP level at T=0 was 13 pmol/l (range 2-185). Abnormal NT-pro-BNP levels were not significantly related to, but showed a tendency towards lower hemoglobin (Hb) levels, higher total leucocyte counts (TLC) and hyperhydration (data not shown). NT-pro-BNP levels were abnormal in 13% (6/45) at T=1 and in 20% of the patients (8/41) at T=2. Absolute NT-pro-BNP levels increased during treatment in 59% of the patients.

8.4.3 CONVENTIONAL ECHOCARDIOGRAPHIC PARAMETERS

Conventional echocardiographic parameters during treatment are shown in table 34. None of the patients had a FS below 28% at any point. In 23% of the patients FS decreased more than 10% between T = 1 and T = 2. As a group, FS decreased significantly after the first 120 mg/m² of anthracyclines (\(P <0.0001\)). Z-scores of left ventricular dimensions and LVM all changed significantly over time.

Table 35 describes the echocardiographic data of ALL patients at T=2 compared to that of healthy controls. Conventional echocardiographic parameters of patients showed significant lower FS, and increased IVRT compared to healthy controls. Z-scores of left ventricular dimensions and LVM were not significantly different from healthy controls.
Table 33 – Cardiac biomarkers in ALL patients during treatment with anthracyclines

<table>
<thead>
<tr>
<th></th>
<th>T = 0</th>
<th>T = 1</th>
<th>T = 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients (N)</strong></td>
<td>60</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td><strong>Number of biomarker samples (N)</strong></td>
<td>46</td>
<td>45</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Abnormal cTnT (%) [N]</td>
<td>0 (0)</td>
<td>11 (5)</td>
<td>2 (1)</td>
<td>0.2*</td>
</tr>
<tr>
<td>cTnT (ng/ml)</td>
<td>0.01</td>
<td>0.01 (0.01 – 0.04)</td>
<td>0.01 (0.01 – 0.02)</td>
<td>0.08†</td>
</tr>
<tr>
<td>Abnormal NT-pro-BNP (%) [N]</td>
<td>26 (12)</td>
<td>13 (6)</td>
<td>20 (8)</td>
<td>0.5*</td>
</tr>
<tr>
<td>NT-pro-BNP (pmol/l)</td>
<td>13 (2 – 185)</td>
<td>10 (1 – 45)</td>
<td>11 (1 – 68)</td>
<td>0.5†</td>
</tr>
</tbody>
</table>

Values are expressed as median and range, *McNemar test based on 37 paired available observations at T=1 and T=2, † Wilcoxon signed ranks test on 37 paired available observations at T=1 and T=2
Table 34 – Conventional echocardiographic

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T = 0</th>
<th>T = 1</th>
<th>T = 2</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>60</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>FS (%)</td>
<td>40 ± 5</td>
<td>36 ± 3</td>
<td>35 ± 3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>z-score LVIDd</td>
<td>0.14 ± 1.15</td>
<td>0.63 ± 0.82</td>
<td>0.48 ± 0.88</td>
<td>0.005</td>
</tr>
<tr>
<td>z-score LVPWd</td>
<td>-0.07 ± 1.14</td>
<td>-0.36 ± 0.88</td>
<td>-0.59 ± 1.28</td>
<td>0.04</td>
</tr>
<tr>
<td>z-score IVSd</td>
<td>-0.80 ± 1.05</td>
<td>-0.59 ± 0.98</td>
<td>-1.09 ± 1.05</td>
<td>0.02</td>
</tr>
<tr>
<td>z-score LVM</td>
<td>-2.23 ± 1.58</td>
<td>-1.61 ± 1.12</td>
<td>-2.20 ± 1.62</td>
<td>0.007</td>
</tr>
<tr>
<td>IVRT (msec)</td>
<td>72 ± 12</td>
<td>67 ± 9</td>
<td>71 ± 10</td>
<td>0.01</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.8 ± 0.6</td>
<td>1.8 ± 0.5</td>
<td>1.8 ± 0.6</td>
<td>0.80</td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>10.1 ± 4.8</td>
<td>12.4 ± 3.9</td>
<td>11.6 ± 5.7</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation. * P values with respect to differences between time points are based on linear mixed model analyses. FS = fractional shortening; LVIDd = diastolic left ventricular internal diameter; LVPWd = diastolic left ventricular posterior wall thickness; IVSd = diastolic intraventricular septum diameter; LVM = left ventricular mass; IVRT = isovolumic relaxation time
Table 35 – Conventional echocardiographic parameters in ALL patients at T = 2 compared to healthy controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Patients at T = 2</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>FS (%)</td>
<td>38 ± 4</td>
<td>35 ± 3</td>
<td>0.002</td>
</tr>
<tr>
<td>z-score LVIDd</td>
<td>0.51 ± 1.00</td>
<td>0.48 ± 0.88</td>
<td>0.86</td>
</tr>
<tr>
<td>z-score LVPWd</td>
<td>-0.34 ± 1.42</td>
<td>-0.59 ± 1.28</td>
<td>0.40</td>
</tr>
<tr>
<td>z-score IVSd</td>
<td>-0.92 ± 1.14</td>
<td>-1.09 ± 1.05</td>
<td>0.42</td>
</tr>
<tr>
<td>z-score LVM</td>
<td>-2.03 ± 1.38</td>
<td>-2.20 ± 1.62</td>
<td>0.56</td>
</tr>
<tr>
<td>IVRT (msec)</td>
<td>47 ± 5</td>
<td>71 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>2.0 ± 0.5</td>
<td>1.8 ± 0.6</td>
<td>0.03</td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>12.0 ± 5.0</td>
<td>11.6 ± 5.7</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation. * P values with respect to differences to ALL patients and controls are calculated using the independent t-test. FS = fractional shortening; LVIDd = diastolic left ventricular internal diameter; LVPWd = diastolic left ventricular posterior wall thickness; IVSd = diastolic intraventricular septum diameter; LVM = left ventricular mass; IVRT = isovolumic relaxation time

8.4.4 MYOCARDIAL STRAIN PARAMETERS

All myocardial 2D strain parameters altered during anthracycline treatment; a statistically significant decrease was observed for global SrL, global SR and global SC (P = 0.03, 0.004 and 0.01, respectively) and all time to peak systolic strain parameters increased significantly (P <0.001). Global SL, SrL and SR were inversely related to cumulative anthracycline dose (P = 0.004, 0.03 and 0.006, respectively). Patients with abnormal cTnT level at T=1 had a significant longer time to peak global SL at T=2 (P = 0.003).
In contrast, all myocardial global peak systolic strain (-rate) parameters were significantly decreased in patients compared to healthy controls, except for global SR. Time to peak global SL, SR and SC was significantly prolonged in patients compared to healthy controls ($P<0.0001$).

The 2DSTE parameters in ALL patients during treatment are shown in table 36. Table 37 describes the 2DSTE data of ALL patients at T=2 compared to that of healthy controls.

### 8.4.5 PREDICTORS FOR ABNORMAL CARDIAC BIOMARKERS AND MYOCARDIAL STRAIN PARAMETERS

Cardiac biomarkers and 2D myocardial global SL, SR and SC were defined as normal and abnormal at T=2 based on age-dependent reference values.

- Predictors for abnormal global SL at T=2 were cumulative anthracycline dose ($P<0.05$, OR associated with an increase of 100 mg/m$^2 = 2.25$, 95%CI [1.02-4.93]), LVIDd z-score at baseline ($P = 0.03$, OR = 1.95, 95%CI [1.05-3.62]) and FS at baseline ($P<0.05$, OR = 1.17, 95%CI [1.00-1.36]).

- Predictors for abnormal global SC at T=2 were global SL ($P=0.02$, OR = 1.33, 95%CI [1.05-1.68]) and global SrL ($P = 0.04$, OR = 24.96, 95%CI [1.22-510]) at T=1.

- Predictors for abnormal NT-pro-BNP at T=2 were an abnormal NT-pro-BNP at T=0 and T=1 ($P = 0.01$, OR = 11.0, 95%CI [1.6-73.5]).

- Predictors for a decreased FS of more than 10% at T=2 were FS ($P = 0.01$, OR = 1.5, 95%CI [1.1-2.0]), LVM z-score ($P = 0.01$, OR = 3.8, 95%CI [1.3-10.7] and z-score of the diastolic left ventricular septum ($P<0.01$, OR = 4.2 95%CI [1.6-11.1]) and z-score of LVPWd ($P = 0.004$, OR = 4.1, 95%CI [1.6-10.9] at T=1.
Table 36 - Strain parameters in ALL patients during treatment with anthracyclines

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T = 0</th>
<th>T = 1</th>
<th>T = 2</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>60</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Global SL (%)</td>
<td>-18.2 ± 3.1</td>
<td>-17.3 ± 3.6</td>
<td>-16.7 ± 5.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Global SR (%)</td>
<td>66.8 ± 12</td>
<td>53.5 ± 13</td>
<td>55.2 ± 16</td>
<td>0.004</td>
</tr>
<tr>
<td>Global SC (%)</td>
<td>-19.4 ± 4.3</td>
<td>-17.1 ± 3.7</td>
<td>-16.9 ± 3.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Global SrL (s⁻¹)</td>
<td>-1.4 ± 0.3</td>
<td>-1.3 ± 0.3</td>
<td>-1.2 ± 0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Global SrR (s⁻¹)</td>
<td>2.6 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>2.3 ± 0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Global SrC (s⁻¹)</td>
<td>-1.7 ± 0.3</td>
<td>-1.6 ± 0.3</td>
<td>-1.5 ± 0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Time to global SL (%)</td>
<td>43 ± 8</td>
<td>51 ± 6</td>
<td>48 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to global SR (%)</td>
<td>45 ± 8</td>
<td>53 ± 8</td>
<td>52 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to global SC (%)</td>
<td>43 ± 8</td>
<td>50 ± 7</td>
<td>48 ± 7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Abnormal global SL (%)</td>
<td>45</td>
<td>52</td>
<td>42</td>
<td>0.51 (GEE)</td>
</tr>
<tr>
<td>Abnormal global SR (%)</td>
<td>3</td>
<td>15</td>
<td>15</td>
<td>0.01 (GEE)</td>
</tr>
<tr>
<td>Abnormal global SC (%)</td>
<td>15</td>
<td>35</td>
<td>35</td>
<td>0.002 (GEE)</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation. * P values with respect to differences between time points are based on linear mixed model analyses or generalized estimating equations (GEE) analysis as indicated. Global SL: global peak systolic longitudinal strain; global SR: global peak systolic radial strain; global SC: global peak systolic circumferential strain; global SrL: global peak systolic longitudinal strain rate; global SrR: global peak systolic radial strain rate; global SrC: global peak systolic circumferential strain rate; time to global peak systolic strain is expressed as percentage of cardiac cycle (percentage of R-R interval).
Table 37 – Strain parameters in ALL patients at $T = 2$ compared to healthy controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Patients at $T = 2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>60</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Global SL (%)</td>
<td>-20.9 ± 1.3</td>
<td>-16.7 ± 5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Global SR (%)</td>
<td>54.3 ± 6</td>
<td>55.2 ± 16</td>
<td>0.60</td>
</tr>
<tr>
<td>Global SC (%)</td>
<td>-22.5 ± 2.1</td>
<td>-16.9 ± 3.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Global SrL (s$^{-1}$)</td>
<td>-1.3 ± 0.1</td>
<td>-1.2 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Global SrR (s$^{-1}$)</td>
<td>3.4 ± 0.4</td>
<td>2.3 ± 0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Global SrC (s$^{-1}$)</td>
<td>-1.9 ± 0.2</td>
<td>-1.5 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to global SL (%)</td>
<td>43 ± 3</td>
<td>48 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to global SR (%)</td>
<td>42 ± 4</td>
<td>52 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to global SC (%)</td>
<td>43 ± 4</td>
<td>48 ± 7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation. * $P$ values with respect to differences to ALL patients and controls are calculated using the independent $t$-test. Global SL: global peak systolic longitudinal strain; global SR: global peak systolic radial strain; global SC: global peak systolic circumferential strain; global SrL: global peak systolic longitudinal strain rate; global SrR: global peak systolic radial strain rate; global SrC: global peak systolic circumferential strain rate; time to global peak systolic strain is expressed as percentage of cardiac cycle (percentage of R-R interval).

8.5 DISCUSSION

Summary of study findings. In children with ALL treated with a relatively low dose of cumulative anthracyclines, cardiac function deteriorated over time as indicated by conventional and 2D strain echocardiography. Comparison of echocardiographic parameters of the patients at one year after anthracycline therapy with healthy controls showed that mainly deformation parameters were affected.
Biomarkers. Cardiac TnT levels were all normal before start of treatment (T=0) and became abnormal in 11% of patients after a cumulative anthracycline dose of 120 mg/m² (at T=1). Release of cTnT during therapy or directly after anthracycline therapy might reflect direct injury of cardiomyocytes. The present study shows that even after a cumulative anthracycline dose of 120 mg/m², which is considered to be a relatively safe dose, damage of the cardiomyocytes occurs as indicated by cTnT levels.\textsuperscript{34,35} The fact that most of the patients had their last anthracycline dose 6 months before T=2, might explain the absence of abnormal cTnT levels in all except for one patient at that moment. The absolute levels of the abnormal cTnT measurements in our patients were in the lower ranges. Measurement of high sensitive cTnT is a newer method to detect troponin release at even lower concentrations than that is detectable now.\textsuperscript{36} This might be an interesting future method to evaluate in our patients. Our results indicated that abnormal cTnT levels at T=1 were significantly related to increased time to reach the global peak systolic longitudinal strain at T=2 (P = 0.003). A recent study of Sawaya and colleagues showed a similar relation between cardiac Troponin I (cTnI) and longitudinal strain. They concluded that abnormal cTnI levels and longitudinal strain were predictive of cardiotoxicity in patients with breast cancer treated with anthracyclines and trastuzumab.\textsuperscript{37}

In our study, an interesting finding was the unexpected high percentage of abnormal NT-pro-BNP levels in patients (26%) before receiving anthracyclins. Our hypothesis is that in children with ALL, release of NT-pro-BNP might be addressed to commonly occurring symptoms as severe anemia, leucocytosis and to hyperhydration to prevent tumor lysis syndrome. A tendency towards lower Hb levels, higher leucocyte counts and hyperhydration was seen in our patients; however this relation was not statistically significant.

The number of patients with abnormal NT-pro-BNP levels increased throughout treatment from 13% at T=1 to 20% at T=2. Absolute values of NT-pro-BNP increased
throughout treatment in 59% of the patients. We recently showed that in a large group of asymptomatic long-term survivors of childhood cancer, none of them had abnormal cTnT levels 15 years after anthracycline treatment and concluded that assessment of cTnT levels does not contribute to the detection of subclinical late-onset anthracyline-induced cardiotoxicity. On the other hand, abnormal levels of NT-pro-BNP were detected in 13% of patients in this group and were related to cumulative anthracyline dose.\textsuperscript{38}

The predictive role of biomarkers in chemotherapy-induced cardiotoxicity has been reviewed before.\textsuperscript{39} These data indicate that increased troponin levels appears related to a high probability of major cardiac events within the first year of follow-up. Patients with a persistent normal troponin value would most likely not have cardiac complications, with a negative predictive value of 99%. The role of natriuretic peptides in the prediction of anthracycline-induced cardiotoxicity seemed less clear.\textsuperscript{39} Long-term follow-up of our patients might clarify this topic. Biomarkers reflecting fibrosis of the myocardium, such as galectin-3, might be of additional use in the assessment of anthracycline-induced cardiotoxicity.\textsuperscript{40}

\textit{Myocardial 2D strain}. None of the patients had clinical signs of acute and/or early-onset anthracycline-induced cardiotoxicity; all cardiac changes in our patients were subclinical. Although FS decreased significantly after 120 mg/m\textsuperscript{2} of cumulative anthracycline dose in our cohort, none of the patients had abnormal FS. FS decreased more than 10% in 23% of the patients. Predictors for a 10% decrease of FS were decreased z-scores of left ventricular dimensions and LVM. The cumulative anthracyline dose correlated negatively with global peak systolic longitudinal strain ($P = 0.004$) and global peak systolic longitudinal strain rate ($P = 0.03$) and global peak systolic radial strain ($P = 0.006$), but not with conventional echocardiographic parameters, including FS.
Increasing data suggest that systolic strain and especially strain rate are strong indices of left ventricular contractility.\textsuperscript{41,42} In addition, a growing number of studies report on the usefulness of myocardial 2D strain echocardiography in monitoring anthracycline-induced cardiotoxicity.\textsuperscript{15,16,17,18,19} In our previous study in long-term survivors of childhood cancer treated with anthracyclines, we also showed that strain parameters were lower as compared to healthy controls.\textsuperscript{16} Similar findings were reported by Tsai and colleagues, who studied cardiac function in long-term survivors of Hodgkin’s lymphoma.\textsuperscript{17} Interestingly, Cheung and colleagues reported impaired left ventricular myocardial deformation and mechanical dyssynchrony in ALL patients after more than one year of treatment as compared to healthy controls. They showed that synchronicity of myocardial deformation may provide additional insights into the alteration of left ventricular mechanics.\textsuperscript{19} In our present study, we also assessed time to reach global peak systolic longitudinal, radial and circumferential strain which was significantly longer as compared to healthy controls. The increased time to peak systolic strain seems to occur during anthracycline treatment. Reduced strain parameters and increased time to peak systolic strain, in the presence of reduced wall dimensions, can be attributable to loss of myocytes and increased collagen deposit, which may result in myocardial fibrosis. This phenomenon is already reported in adults with heart failure.\textsuperscript{43}

8.6 STUDY LIMITATIONS

Due to disease related complications, a complete follow-up of all patients was not possible. Abnormal cardiac function before start of treatment for ALL might not reflect the patients’ normal cardiac function, due to disease related anemia and leucocytosis and hyperhydration which might influence myocardial function.
8.7 CONCLUSION

Children with newly diagnosed ALL showed a decline of systolic and diastolic function during treatment with anthracyclines using conventional and myocardial 2D strain echocardiography. The use of cardiac biomarkers in combination with myocardial 2D strain echocardiography provides additive information in the assessment of cardiac function of children with ALL treated with anthracyclines.

8.8 REFERENCES


42. Nesbitt GC, Mankad S. Strain and Strain Rate Imaging in Cardiomyopathy. Echocardiography 2009;26(3):337-44.

La maladie principale de l’homme est la curiosité inquiète des choses qu’il ne peut savoir

Blaise Pascal

Frans wiskundige en filosoof (1623-1662)
Chapter 9

Summary, general discussion and future perspectives
9.1 SUMMARY & GENERAL DISCUSSION

Accurate knowledge of myocardial function is essential to the management of cardiac disease, and the quest continues for an optimal, quantitative technique to assess myocardial contractile function. Previous studies indicated that strain analysis can provide important additional information regarding quantification of myocardial function. Applications are found primarily in research, but also already in clinical settings.

The assessment of myocardial deformation might have potential benefits for patients in whom the cardiac anatomy obstructs quantification of ventricular function using M-mode or two-dimensional volumetric techniques. Investigating the myocardium directly renders the strain-based techniques geometry-independent, allowing the quantification of right ventricular performance as well as uni- or left ventricular systolic function in children with complex congenital heart disease. A second advantage of myocardial deformation techniques is that these are able to quantify regional myocardial function in addition to global cardiac function. Third, unlike velocity-based parameters, strain calculations are not affected by movement due to global cardiac motion within the chest (cardiac translation) nor by the effect (pulling or tethering) of surrounding segments on the segment of interest. This characteristic of strain discriminates passive movement from true shortening and stretching of the myocardial region under investigation.

A relatively new application to assess myocardial deformation, is known as two-dimensional strain echocardiography (2DSTE) or ‘speckle tracking’. This echocardiographic technique uses two-dimensional multi-frame B-Mode (grayscale) images to quantify strain and strain-rate.

In CHAPTER 2 and CHAPTER 3 we provide reference values for two-dimensional strain echocardiography parameters in children and young adults. The availability of
pediatric reference values is a mandatory prerequisite for its use in clinical practice. Previous studies were conducted in small cohorts of children and included peak systolic strain measurements in just one or two directions without providing information on the timing of peak systolic deformation. For these reasons, we aimed at evaluating left ventricular myocardial strain in all three principal directions of deformation (longitudinal, circumferential and radial) assessed with 2DSTE in a large, healthy pediatric and young adult cohort (birth to 40 years of age). Using 2DSTE we established reference values and determined the influence of age and growth on these 2DSTE parameters. Our results indicate that global peak systolic strain in all three directions is lowest on both ends of the age spectrum under investigation and reaches the maximum value around an age of 18 years. These findings illustrate the importance of using age-dependent reference values for the adequate interpretation of 2DSTE measurements, since left ventricular systolic deformation alters during growth and development.

Furthermore, we observed that deformation is not homogenously distributed throughout the left ventricular wall and interventricular septum. Successive contraction and relaxation of the ventricular myocardial band produces several fundamental movements of the left ventricle, such as narrowing and shortening (with torsion or twisting). Nonhomogenous deformation of the basal, mid and apical ventricular segments provides coordinated left ventricular contraction. This uneven distribution is illustrated by nonequal maximum systolic strain values among various left ventricular segments, as well as regional differences in the required time to reach the maximum deformation. These latter variations in timing of peak systolic strain between left ventricular myocardial segments are different for various age groups. Our findings indicate that aging results in a decreased extent of synchronicity of left ventricular systolic deformation, even after correction for differences in heart rate. The reference values provided in this study could be used to assess mechanical dyssynchrony in various congenital and acquired cardiac diseases. It is possible that 2DSTE indices could provide valuable additional
information on myocardial synchrony and systolic function which could be of assistance in selecting patients who might benefit from “cardiac resynchronization therapy” (CRT). The reason for this is that previous studies in adults have indicated that 2DSTE is a promising tool to predict which patients respond to CRT. Furthermore, in the pediatric age groups the currently used selection criteria for CRT, which are based on clinical status and QRS duration, appear not very suitable.

In the subsequent section we investigated the potential of 2DSTE in children with different congenital or inherited cardiac diseases.

In **CHAPTER 4** we evaluate two-dimensional strain echocardiographic findings in children diagnosed with isolated congenital valvular aortic stenosis. These children - without prior intervention to relief pressure overload of the left ventricle - suffered from various degrees of stenosis. Our findings demonstrate that asymptomatic children with congenital valvular aortic stenosis and preserved left ventricular ejection fraction already exhibited decreased peak systolic myocardial strain in all three principal directions of deformation (radial, circumferential and longitudinal), even in case of mild stenosis. We found that the decline in longitudinal systolic performance precedes that in other directions. These observations suggest that 2DSTE parameters could be useful for the early detection of subclinical left ventricular myocardial dysfunction, which remains otherwise undetected by conventional measurements of systolic function.

In **CHAPTER 5** we investigate the recovery of left ventricular myocardial systolic performance using conventional and two-dimensional strain echocardiography before and after balloon valvuloplasty for severe, isolated congenital valvular aortic stenosis. After balloon valvuloplasty we observed an incomplete recovery of left ventricular deformation that cannot be completely attributed to concurrent aortic regurgitation or residual stenosis. It is possible that these findings provide evidence of irreversible myocardial damage. The clinical and prognostic implications of
permanent abnormal deformation in longitudinal and circumferential directions remain to be elucidated. It is uncertain if earlier (surgical or catheter) intervention will lead to complete recovery of myocardial strain.

In **CHAPTER 6** we describe left ventricular myocardial performance assessed by means of physical examination, electrocardiography, conventional echocardiography and 2DSTE in a group of children with established mitochondrial disease. Our results indicate subclinical systolic myocardial deformation abnormalities in all three directions of deformation (longitudinal, circumferential and radial) in the presence of normal conventional echocardiographic indices. Since myocardial dysfunction in mitochondrial disorders is an ominous sign, these findings emphasize the need for repeated cardiac assessment as part of the monitoring of disease progression in patients with confirmed mitochondrial disorders. Our findings illustrate that 2DSTE could be of additional value in the cardiac assessment in children diagnosed with mitochondrial disease.

In **CHAPTER 7** we evaluate cardiac anatomy and left ventricular myocardial systolic function in children previously diagnosed with Prader-Willi syndrome. Prader-Willi syndrome is associated with a substantial increased risk at (sudden) cardiovascular death and morbidity, which cannot be entirely attributed to obesity-related risk factors. Cardiac structure and myocardial deformation has not been investigated previously in (pediatric) patients with Prader-Willi syndrome. Cardiac evaluation in the current study consisted of physical examination, electrocardiography, conventional echocardiography and 2DSTE. Our results indicate few minor structural (atrial septum defect and a pulmonary valve stenosis) as well as electrocardiographic abnormalities. However, subclinical systolic myocardial deformation abnormalities in all three directions were frequent. In approximately 70% of PWS patients under investigation, deformation appeared to be abnormal (below 5th percentile of previously established reference values according to age). Systolic myocardial deformation appeared more affected in case of maternal
uniparental disomy. Long-term follow-up studies in larger groups of patients are required to determine the clinical and prognostic implications of the identified abnormalities in myocardial deformation in pediatric Prader-Willi patients. Since cardiovascular morbidity and mortality is substantial in patients with Prader-Willi syndrome, especially in adults, we emphasize the need for (repeated) cardiac assessment. Our findings indicate that 2DSTE could be of additional value.

In CHAPTER 8 we describe how 2DSTE, in addition to Troponin T and N-Terminal-pro-brain natriuretic peptide assays, could be of assistance in the detection of early-onset anthracycline-induced cardiotoxicity. Cardiotoxicity is a well-known side effect of anthracycline therapy. Early-onset anthracycline-induced cardiotoxicity occurs during or within the first year after anthracycline treatment. It forms a risk factor for the development of late-onset anthracycline-induced cardiotoxicity, which is associated with serious cardiac morbidity and mortality in a growing number of cancer survivors. In the current study we prospectively evaluated myocardial performance using conventional echocardiographic modalities, 2DSTE and through the assessment of biomarkers (i.e., cardiac Troponin T and N-Terminal-pro-brain natriuretic peptide) before start, after three months and after one year of anthracycline therapy. The study population consisted of children recently diagnosed with acute lymphoblastic leukemia in addition to healthy, age-matched controls. Our results indicate that myocardial systolic function, as assessed with 2DSTE, declined significantly during anthracycline treatment and was significantly decreased compared to healthy, age-matched controls. Predictors for abnormal cardiac biomarkers and 2D myocardial strain parameters after one year of therapy were: cumulative anthracycline dose, diastolic left ventricular internal diameter (z-score), fractional shortening, cardiac biomarker levels and global peak systolic longitudinal strain(-rate) at baseline. These findings indicate that myocardial 2DSTE and cardiac biomarkers are useful in the detection of subclinical myocardial alterations indicative of early-onset anthracycline-induced cardiotoxicity.
9.2 FUTURE PERSPECTIVES

This relatively new method of 2D-strain imaging by using speckle tracking is still evolving through research and development. Currently, this technique is not yet considered part of a routine clinical echocardiographic examination. However, the amount of research using this technology is escalating and the clinical applications of this technique are likely to expand rapidly over the coming years.

In our experience, speckle tracking could provide valuable additive information on myocardial function in a variety of conditions. For example, in children with cardiac disease without overt signs of heart failure, since 2DSTE has proven to be able to identify subclinical myocardial dysfunction at an earlier stage when compared to conventional echocardiographic techniques. Second, since 2DSTE determines myocardial deformation independently of cardiac geometry, this technique may be especially useful for the evaluation of myocardial performance of ventricles with variable morphology (e.g., congenital heart disease). Third, the regional assessment of deformation is of potential value in ischemic heart disease. This may be important in congenital lesions where coronary artery anomalies are present or where, during cardiac surgery, the coronary artery flow was altered (e.g., re-implanted). In addition, 2DSTE could identify cardiac electromechanical dyssynchrony which, depending on the underlying substrate, can be treated with cardiac resynchronization therapy, even before dyssynchrony is accompanied by overt heart failure.

However, several unresolved issues remain which future research and development should address.
9.2.1 TECHNICAL ISSUES

IMAGE QUALITY
Adequate speckle tracking relies on the quality of grayscale B-mode images used for speckle tracking. For instance, acoustic shadowing or reverberations might influence strain estimates. Furthermore, since 2DSTE is influenced by signal noise, filtering algorithms are used. However, filtering represents a compromise between resolution and precision. If long windows are used or strain estimates of multiple small windows, the standard deviation of the estimates decreases. However, the resolution decreases also since the estimate represents a larger region.

In addition, in case of large ventricles it may be difficult to image the entire myocardium in one frame, especially the apical segments. However, apical foreshortening seriously affects the results of 2DSTE, and should therefore be minimized.

UNKNOWN SOFTWARE ALGORITHMS
To track speckles and to compute deformation values, filtering and search algorithms are used. The effects of these speckle tracking algorithms on the results represents a ‘black box’ and may vary from vendor to vendor, especially when radial strain is concerned. Differences among vendors are driven by the fact that 2DSTE analysis is performed on data stored in a proprietary scan line format, which cannot be analyzed by other vendor’s software. At present it is yet unclear how values from different scanners and software versions compare. This issue needs further investigation through cross-platform comparisons before 2DSTE can become a mainstream methodology. Currently there is a joint effort between the American Society of Echocardiography (ASE), European Association of Echocardiography (EAE), and the industry to address this issue.
9.2.2 STANDARDIZATION ISSUES

To enable the comparison of research outcomes as well as to facilitate clinical utility, standardization of 2DSTE examination is needed. For instance:

- What should be measured:
  - How many myocardial segments and which ones should be measured?
    Different vendors use various numbers of myocardial segments and different nomenclature. These differences among software vendors attribute to the difficulties in comparing 2DSTE results assessed with different software packages.
  - Peak strain (rate) and its timing during cardiac cycle is not uniform across publications. Peak strain can be measured as peak systolic strain, peak strain at end-systole (at time of aortic valve closure), or peak strain regardless of timing during cardiac cycle (in systole or diastole).

- Standardized reporting of measurements.
- An echocardiographic study that incorporates 2DSTE can yield a plethora of data points that need to be processed in the framework of busy clinical practice and synthesized into a coherent picture that is readily understood by the clinician who may not be familiar with these methods.

9.2.3 PROGNOSTIC IMPLICATIONS

It is now time to establish the usefulness of 2DSTE examination for clinical decision-making. Further research is needed to determine the sensitivity and specificity of the measurements obtained using this technique and to define its strengths and limitations in various cardiac conditions. In particular, whether the measured values correlate well with clinical outcome needs to be established in longitudinal interventional studies. A primary goal will be the definition of cut-off values for
medical decision-making and to correlate these with hard end-points. This will require larger prospective studies, as well as an effort to standardize 2DSTE examinations, address the problems listed above, and finally incorporate two-dimensional strain echocardiography into the realities of routine clinical practice.

9.3 TO CONCLUDE

What is clear is that further work is needed before this technique can be fully applied in the clinical setting, but we believe that the future appears bright for 2D-strain. To conclude: when asked ‘Strain, or no strain?’, our reply would be:

Yes, we “strain”!
Science is always wrong. It never answers a question without creating ten more.

George Bernard Shaw
Chapter 10

Samenvatting, discussie en toekomstperspectieven
10.1 SAMENVATTING & DISCUSSIE

Accurate kennis van het functionerend vermogen van de hartspier is van essentieel belang voor de behandeling van hartziekten. Tot op heden is het echter lastig gebleken om de cardiale functie in diverse patiëntengroepen op een betrouwbare wijze in kaart te brengen. De zoektocht naar een optimale techniek om de kwaliteit van het functioneren van de hartspier in mate en getal uit te drukken zet zich hiermee voort.

Uit voorgaand onderzoek is gebleken dat een relatief nieuwe techniek, genaamd ‘strain analyse’ belangrijke, toegevoegde informatie lijkt te bieden over de mate van functioneren van de hartspier. Deze techniek wordt momenteel al toegepast, hoewel vooralsnog voornamelijk in het kader van onderzoek.

Deze techniek, die de mate van vervorming (strain) van de hartspier bepaalt, zou met name van grote aanvullende waarde kunnen zijn bij patiënten met een afwijkende bouw en vorm van de hartkamer(s). De huidige echotechnieken om de cardiale functie weer te geven zijn namelijk niet goed toepasbaar in deze categorie patiënten. Aangezien door middel van strain analyses het functioneren van de hartspier onafhankelijk van zijn vorm bepaald kan worden, kan deze techniek ook toegepast worden in het geval van complexe aangeborene hartafwijkingen waarbij de bouw van de hartkamer(s) abnormaal is.

Een tweede voordeel van strain analyse is dat deze techniek naast de globale functie van de hartkamer(s) ook de regionale functie kan weergeven, iets wat tot op heden zeer moeilijk is gebleken. Ten derde, in tegenstelling tot parameters die gebaseerd zijn op snelheid, worden strain berekeningen (gebaseerd op vervorming van de hartspier) niet tot nauwelijks beïnvloed door globale bewegingen van het hart in de borstkas noch door het effect (trekkrachten) van omliggende weefsels op het gebied van de hartspier dat men wil onderzoeken. Deze kenmerken van strain
analyse zorgen ervoor dat deze techniek passieve bewegingen van de hartspier onderscheid van ware contractie (actieve beweging).

Een relatief nieuwe techniek om strain te bepalen is bekend als ‘twee-dimensionale strain echocardiografie (2DSTE) of speckle tracking’. De echocardiografische methode maakt gebruik van twee-dimensionale multiframe B-mode plaatjes om de mate en frequentie van vervorming van de hartspier te bepalen.

In HOOFDSTUK 2 en HOOFDSTUK 3 worden referentiewaardes voor 2DSTE parameters weergegeven in kinderen en jong volwassenen, welke tot op heden nog niet waren vastgesteld. De beschikbaarheid van referentiewaardes is een vereiste voor gebruik in de klinische praktijk. Voorgaand onderzoek op dit gebied werd uitgevoerd in kleine groepen kinderen en beschreef enkel strain bepalingen in één van twee richtingen zonder informatie te bieden over de timing van het bereiken van de maximale vervorming gedurende de hartcyclus. Om deze reden was ons streven om de normale vervorming van de linker hartkamer gedurende de hartcyclus in drie richtingen in kaart te brengen in een grote groep gezonde kinderen en jong volwassenen (0 tot 40 jaar) door middel van 2DSTE. Ons doel was om op deze manier referentiewaardes vast te stellen voor deze leeftijdscategorie en de invloed van leeftijd en groei te bepalen. Onze resultaten tonen aan dat de maximale vervorming van de linker hartkamer in alle 3 de richtingen het laagst is aan beide uiteinden van het onderzochte leeftijdsspectrum. De maximale waardes werden gevonden rond de leeftijd van 18 jaar. Zowel de zuigelingen als de dertigers vertoonden een lagere maximale vervorming in vergelijking met tieners. Deze resultaten onderstrepen het belang van het gebruik van leeftijdsspecifieke referentiewaardes voor een adequate interpretatie van 2DSTE bepalingen aangezien de mate vervorming lijkt te veranderen gedurende groei en ontwikkeling.

Daarnaast zagen we ook dat de mate en timing van vervorming van de hartspierwanden van de linker hartkamer niet overal gelijk is. Segmenten aan de basis van de kamer, in het midden en de apicale segmenten trekken in verschillende
mate en op verschillende tijdstippen samen. Hierdoor ontstaat een gecoördineerde contractie van de hartspier. Onze onderzoeksresultaten laten zien dat de onderlinge verschillen tussen de diverse segmenten van de linker hartkamer, met betrekking tot het tijdstip waarop de maximale vervorming wordt bereikt, niet voor alle leeftijdsgroepen gelijk is. Naar mate de leeftijd stijgt, neemt de mate van synchroniteit van deformatie af. Deze verschillen in synchronie tussen de diverse leeftijdsgroepen blijven ook bestaan na correctie voor variatie in hartfrequentie.

De referentiewaardes die in deze studie beschreven worden zouden gebruikt kunnen worden om de mate van mechanische dyssynchronie vast te stellen van de linker hartkamer in diverse cardiale aandoeningen. Het is zeer goed mogelijk dat 2DSTE parameters waardevolle, aanzienlijke informatie zouden kunnen bieden ten aanzien van myocardiale synchronie en systolische functie, hetgeen nuttig zou kunnen blijken bij de selectie van patiënten voor cardiale resynchronisatie therapie (CRT).

In de hierop volgende sectie hebben we de potentiele waarde van 2DSTE onderzocht bij het in kaart brengen van de cardiologische functie bij kinderen met verschillende aangeboren hartafwijkingen en erfelijke aandoeningen.

In **HOOFDSTUK 4** hebben we door middel van conventionele echotechnieken en met behulp van 2DSTE het functioneren van de linker hartspier in kaart gebracht bij een groep kinderen met een geïsoleerde aangeboren vernauwing van de aortaklep. Geen van deze kinderen had op het moment van onderzoek in het verleden een ingreep ondergaan (in een poging tot) om deze vernauwing te verhelpen. De ernst van vernauwing was niet uniform waardoor een breed spectrum van deze aandoening onderzocht kon worden. Onze bevindingen tonen aan dat de mate van vervorming van de linker hartkamer in deze kinderen afwijkend is in vergelijking met gezonde kinderen. Deze bevindingen werden gedaan ondanks het feit dat geen van deze kinderen klachten ondervond van de klepvernauwing en ondanks het feit dat de conventionele indices om het functioneren van de linker hartkamer weer te
geven niet afwijkend waren. Opvallend was de strain parameters afwijkend waren, zelfs in de groep met een milde vernauwing van de aortaklep. In het geval van een ernstige vernauwing van de aortaklep was de vervorming afwijkend in alle drie de richtingen van contractie, echter in het geval van milde vernauwing enkel in de lengterichting van de hart as (longitudinale). Deze resultaten tonen aan dat 2DSTE van toegevoegde waarde zou kunnen zijn bij het vroegtijdig opsporen van verminderd functioneren van de linker hartkamer welke door conventionele echotechnieken onopgemerkt zou blijven. Echter, de prognostische implicaties van deze bevindingen moeten nog worden vastgesteld door middel van toekomstig longitudinale onderzoek.

In HOOFDSTUK 5 onderzoeken we het herstel van de werking van de linker hartkamer door middel van conventionele echotechnieken en 2DSTE vóór en na correctie (ballondilatatie door middel van katheterisatie) van de drukbelasting van de linker hartkamer in kinderen met een ernstige vorm van aortaklep vernauwing. Na de ingreep zagen we een incompleet herstel van de linker ventrikel vervorming welke niet (geheel) toegeschreven kan worden aan een restant vernauwing dan wel het optreden van kleplekkage. Het is mogelijk dat deze bevindingen het gevolg zijn van onherstelbare schade die veroorzaakt is door langdurige blootstelling van de hartspier aan een verhoogde weerstand of dat het een uiting is van een intrinsiek aanlegstoornis. De klinische en prognostische implicaties van deze persisterend afwijkende vervorming moet nog worden bepaald in prospectief (interventie) onderzoek. Het is onzeker of eerder ingrijpen wel zal leiden tot compleet herstel.

In HOOFDSTUK 6 beschrijven we de functie van de linker hartkamer in kinderen met een aangetoonde stoornis in de energie huishouding (mitochondriële stoornis). De functie van de linker hartkamer hebben we in deze groep patiënten in beeld gebracht door middel van lichamelijk onderzoek, elektrocardiografie, conventionele echocardiografie en door middel van 2DSTE. Onze resultaten geven aan dat de vervorming van de linker hartspier in deze groep kinderen abnormaal is in alle drie
de richtingen zonder dat ze hiervan duidelijk merkbare klachten ondervinden. Het lichamelijk onderzoek, elektrocardiografisch en de conventionele echotechniek indices vertoonden geen afwijkingen. Aangezien het verminderd functioneren van de hartspier bij patiënten met een energie stofwisselingstoornis een omineus teken is en gepaard gaat met een sterk verminderde levensverwachting, onderstrepen deze bevindingen het belang van herhaaldelijk onderzoek van de hartspier functie in deze patiëntengroep. Het is zeer goed mogelijk dat 2DSTE hierbij van aanvullende waarde zou kunnen zijn.

In HOOFDSTUK 7 onderzoeken we de bouw (anatomie) en functie van de linker hartkamer in kinderen die eerder gediagnosticerd zijn met het Prader-Willi syndroom. Dit syndroom wordt geassocieerd met een substantieel verhoogd risico op (acuut) cardiovasculair overlijden en chronisch hart-en-vaat lijden. Voorheen schreef men dit voornamelijk toe aan het feit dat deze patiënten vaak obees zijn. Tegenwoordig is men echter steeds meer van mening dat obesitas-gerelateerde risicofactoren niet de volledige lading dekken. De cardiale anatomie en de mate van vervorming van de hartspier is nog niet eerder onderzocht bij kinderen die aan het Prader-Willi syndroom lijden. Ons doel was daarom om de bouw en functie van het hart bij deze groep kinderen in beeld te brengen. Dit hebben we gedaan door middel van lichamelijk onderzoek, elektrocardiografie, conventionele echocardiografie en 2DSTE. Met deze onderzoektechnieken toonden wij aan dat er in enkele gevallen sprake was van (milde) structurele hartafwijkingen en elektrocardiografische afwijkingen. Een abnormale vervorming van de hartspier van de linker hartkamer zonder dat deze patiënten hier (merkbaar) klachten van ondervonden kwam echter frequenter voor. In 70% van de patiënten was er sprake van een abnormale vervorming van de hartspier (<5e percentiel van de eerder vastgestelde referentiewaarden). De vervorming van de hartspier was afwijkend in alle drie de richtingen. Opvallend was dat de mate van vervorming vooral afwijkend was in het geval van maternale uniparentale disomie, wat een specifieke DNA afwijking is welke in sommige gevallen de genetische oorzaak is van het Prader-Willi
Prader-Willi syndroom. Lange termijn follow-up studies in grotere patiënten groepen zijn nodig om de klinische en prognostische betekenis van de aangetoonde afwijkingen te achterhalen. Echter gezien de cardiovasculaire morbiditeit en mortaliteit, zeker bij volwassenen, adviseren wij (herhaaldelijk) cardiaal onderzoek bij patiënten gediagnostiseerd met het Prader-Willi syndroom. Hierbij zou 2DSTE aanvullende informatie kunnen bieden ten aanzien van de werking van de hartspier.

In HOOFDSTUK 8 beschrijven we dat 2DSTE, in combinatie met Troponine T en N-Terminal-pro-brain natriuretic peptide bepalingen, gebruikt kan worden om in een vroeg stadium anthracycline geïnduceerde cardiotoxiciteit aan te tonen bij kinderen met acute lymfatische leukemie.

In de huidige, prospectieve studie evalueren we de myocardiale functie door middel van conventionele echotechnieken, 2DSTE en het bepalen van de serumconcentratie van biomarkers (Troponine T en N-Terminal-pro-brain natriuretic peptide) op 3 meetmomenten: voor de start van, na 3 maanden en 1 jaar na de start van anthracycline therapie. Onze bevindingen wijzen uit dat de functie van de linker hartkamer, weergegeven door 2DSTE parameters, achteruit gaat tijdens de behandeling met anthracyclines. Bovendien is de systolische functie van de linker hartkamer bij deze groep kinderen significant lager in vergelijking met gezonde leeftijdsgenoten. Voorspellende parameters voor afwijkende biomarker concentraties en abnormale strain indices na 1 jaar chemotherapie zijn: cumulatieve anthracycline dosis, diastolische interne diameter van de linker hartkamer, fractionele verkorting, biomarker concentratie en globale piek systolische longitudinale strain (-rate) vóór de start van anthracycline therapie. Deze resultaten geven aan dat 2DSTE en cardiale biomarkers gebruikt kunnen worden om subklinische veranderingen in de myocardiale functie aan te tonen, welke zouden kunnen duiden op anthracycline geïnduceerde cardiotoxiciteit.
10.2 TOEKOMSTPERSPECTIEVEN

Tweedimensionale echocardiografie is zich als techniek om de werking van de hartspier in kaart te brengen nog steeds aan het ontwikkelen door onderzoek en technische ontwikkelingen. Op dit moment kan deze techniek nog niet beschouwd worden als onderdeel van een routine echocardiografisch onderzoek. Echter, onderzoek op het terrein van 2DSTE neemt hand over hand toe en het is waarschijnlijk dat de klinische toepasbaarheid in de komende jaren zich verder zal uitbreiden.

Wij zijn van mening dat 2DSTE aanvullende informatie kan bieden met betrekking tot de functie van de hartspier in een groot scala aan cardiale ziektes. Bijvoorbeeld in kinderen met een primair of secundair gestoorde werking van het hart zonder overduidelijke tekenen van hartfalen. Het is in deze groep patiënten waarbij 2DSTE heeft aangetoond subklinisch abnormaal functioneren van de hartspier te kunnen detecteren voordat conventionele echotechnieken afwijkende waardes vertonen. Daarnaast zou 2DSTE van grote waarde kunnen zijn bij kinderen met een abnormale bouw van de hartkamer(s) aangezien deze techniek, in tegenstelling tot de huidige echotechnieken om de werking van de hartspier te kwantificeren, de mate en timing van vervorming bepaalt onafhankelijk van de geometrie. Aanvullend, biedt het feit dat 2DSTE het mogelijk maakt om niet alleen de globale hartspierwerking te detecteren maar ook de regionale verschillen hierin nieuwe mogelijkheden. Zo kan het in kaart brengen van de werking van de verschillende segmenten van de hartkamer(s) zeer nuttig zijn bij ischemische hartziekten. Voorbeelden hiervan bij kinderen zijn onder andere aangeboren afwijkingen van de kransslagaders en aangeboren hartafwijkingen waarbij gedurende de operatieve correctie hiervan de kransslagaders opnieuw geïmplanteerd zijn. Tot slot, zou 2DSTE cardiale elektromechanische dyssynchroniteit kunnen aantonen, hetgeen afhankelijk van de oorzaak behandeld zou kunnen worden met resynchronisatie therapie.
Dit alles tezamen evaluerend zou 2DSTE nieuwe inzichten kunnen leveren in het mechanisme van diverse cardiale aandoeningen en wellicht hiermee aanknopingspunten kunnen bieden voor het optimaliseren van diens behandelingen.

Echter, verschillende probleemstukken moeten hiervoor eerst een oplossing vinden door middel van toekomstig onderzoek en verdere technische ontwikkelingen.

### 10.2.1 TECHNISCHE PROBLEMEN

#### BEELDKWALITEIT

Een betrouwbare 2DSTE analyse is sterk afhankelijk van de beeldkwaliteit van de B-mode echobeelden. Zo kunnen reverberaties de resultaten van de 2DSTE bepalingen beïnvloeden. Om de invloed van ruis op de 2DSTE bepalingen te reduceren wordt gebruik gemaakt van filter algoritmes. Echter, het filteren van de echodata vormt een compromis tussen resolutie en standaard deviatie.

Daarnaast is de grootte van de hartkamer van belang. In het geval van een grote ventrikel kan het lastig blijken te zijn om de gehele hartkamer in beeld te krijgen. Dit geldt met name voor de apicaal gelegen segmenten. Echter als de apicale segmenten niet meegenomen worden in de 2DSTE analyse kan dit van significante invloed zijn op de verkregen resultaten. Het is dus van belang om (te pogen) de gehele hartkamer in beeld te krijgen.

#### ONBEKENDE SOFTWARE ALGORITMES

Bij speckle tracking en het uiteindelijk verkrijgen van strainwaardes wordt gebruik gemaakt van zoek- en filter algoritmes. Het exacte effect van deze algoritmes op de verkregen resultaten vertegenwoordigt een spreekwoordelijke ‘zwarte doos’ en kan variëren tussen de software van diverse fabrikanten. Dit geldt met name voor de bepaling van radiale strain (-rate). De verschillen tussen de diverse fabrikanten is
gebaseerd op het feit dat 2DSTE analyses verricht worden met behulp van data die opgeslagen zijn in een format dat eigendom is van desbetreffende firma en dat niet geanalyseerd kan worden door de software van een andere firma. Momenteel is het onduidelijk hoe de 2DSTE bepalingen van de diverse echoapparaten en software fabrikanten zich onderling verhouden. Dit aspect dient nader onderzocht te worden voordat 2DSTE een vast onderdeel van de dagelijkse praktijk gaat vormen. Op dit moment is er sprake van een gezamenlijke inzet van de “American Society of Echocardiography (ASE)”, “European Association of Echocardiography (EAE)” en de industrie om deze vraagstukken aan te pakken.

10.2.2 STANDAARDISATIE

Om vergelijking mogelijk te maken tussen de studieresultaten uit diverse onderzoeken en om de klinische toepasbaarheid te vergoten is standaardisatie van essentieel belang.

Eenduidigheid moet ontstaan over:

- Wat gemeten dient te worden:
  - Hoeveel segmenten en welke segmenten moeten gemeten worden?
    De diverse fabrikanten maken gebruik van een verschillend aantal te analyseren segmenten en verschillende nomenclatuur. Deze verschillen dragen bij aan de moeilijkheden die bestaan bij de onderlinge vergelijking van de verkregen onderzoeksresultaten.
  - Het moment waarop de maximale vervorming van de hartspier bepaald wordt is niet uniform. Onder maximale vervorming (piek strain) kan verstaan worden: maximale vervorming tijdens de systole, de vervorming aan het einde van de systole (op het moment waarop de aortaklep sluit) of maximale vervorming ongeacht het tijdstip tijdens de hartcyclus (systole of diastole).
- Ook dienen de resultaten op een gestandaardiseerde wijze gerapporteerd te worden, waardoor het inzichtelijker wordt.
- Aangezien een echocardiografisch onderzoek waar 2DSTE onderdeel van uitmaakt een veelheid aan aanvullende informatie biedt is het noodzakelijk dat deze uitkomstdata dusdanig vorm krijgen zodat ze passen in drukke dagelijkse klinische praktijk. De data dienen een coherent beeld te vormen dat gemakkelijk te begrijpen is voor een clinicus die wellicht nog weinig ervaring heeft met deze techniek.

**10.2.3 PROGNOSTISCHE BETEKENIS**

Het is nu van belang om de bruikbaarheid van 2DSTE voor het maken van klinische beslissingen alsmede de prognostische waarde van 2DSTE bevindingen vast te stellen. Toekomstig onderzoek is wenselijk om de sensitiviteit en specificiteit van 2DSTE bepalingen te onderzoeken in diverse aandoeningen en de sterke en minder sterke eigenschappen van deze techniek te bepalen. De correlatie tussen (herhaaldelijke) 2DSTE metingen en klinische uitkomstmaten op de langere termijn zal moeten onderzocht door middel van longitudinale interventie studies. Doel hierbij zal zijn om afkapwaardes voor strainparameters te formuleren en deze te correleren aan medisch inhoudelijke beslissingen en harde uitkomstmaten. Hiervoor zijn prospectief opgezette studies nodig, alsmede het standaardiseren van 2DSTE onderzoeksmethodes, moeten de bovengenoemde problemen aangepakt worden en dient 2DSTE uiteindelijk een plek te krijgen in de realiteit van de dagelijkse klinische praktijk.

**10.3 CONCLUSIE**

Het is duidelijk dat toekomstig onderzoek en verdere technische ontwikkelingen van belang zijn om deze techniek een plek te geven in de dagelijkse klinische praktijk.
Wij zijn echter van mening dat de perspectieven voor 2DSTE gunstig zijn en dat het een waardevolle aanvulling is op het echografisch onderzoek van het hart.
Feeling gratitude and not expressing it, is like wrapping a present and not giving it.
Chapter 11

Dankwoord
“Silent gratitude isn’t much use to anyone.” (G.B. Stern)

Nu het einde van dit wetenschappelijke avontuur nadert wil ik van de gelegenheid gebruik maken om eenieder te bedanken die mij opgeleid, gestimuleerd en bovenal gesteund heeft tijdens deze boeiende maar zeker ook uitdagende periode.

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Onze grootste overwinning is niet dat we nooit falen, maar dat we telkens als we struikelen weer opstaan

孔夫子 / Confucius
Chinees filosoof
(551 a.C. - 479 a.C.)
Chapter 12
Curriculum Vitae
Karen Marcus was born on the 21th of October 1980 in Oss. She obtained her high school diploma (cum laude) at the Titus Brandsma College in Oss in 1999. That year she entered Medical School in Maastricht (Maastricht University). Her medical degree was obtained (cum laude) in 2005, after which she worked as a resident at the pediatric department of the Maxima Medical Centre in Veldhoven. It was at this hospital where her training in Pediatrics commenced in 2006. October 2007 she continued her training in Pediatrics in the Radboud University Nijmegen Medical Centre. In 2009 she obtained the Sengers grant for her research. She completed her training in February 2012. Karen lives with her partner Vincent and their two sons: Thomas (January 9, 2010) and Max (November 17, 2011) in Vleuten.
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Chapter 14

List of publications


• Marcus KA, de Korte CL, Feuth T, Thijssen JM, Kapusta L. Abnormal two-dimensional strain echocardiography findings in children with congenital valvar aortic stenosis. Ultraschall in der Medizin/European Journal of Ultrasound 2011;


• Marcus KA, van Alfen-van der Velden JAAEM, Otten BJ, Weijers G, de Korte CL, Kapusta L.
  Cardiac evaluation in children diagnosed with Prader-Willi syndrome.

• Mavinkurve-Groothuis AMC, Marcus KA, Pourier M, Groot-Loonen J, Feuth T, Hoogerbrugge PM, de Korte CL, Kapusta L.

• Mavinkurve-Groothuis AMC, Groot-Loonen J, Marcus KA, Bellersen L, Feuth T, Bökkerink JP, Hoogerbrugge PM, de Korte CL, Kapusta L.
  Myocardial strain and strain rate in monitoring subclinical heart failure in asymptomatic long-term survivors of childhood cancer.