FETAL HAEMODYNAMICS IN MONOCHORIONIC TWINS

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ABSTRACT

Aim: To understand myocardial performance index (MPI)-conventional Doppler, MPI'-tissue Doppler imaging (MPI'-TDI) and aortic isthmus pulsatility index (AoI PI) normal trend in uncomplicated monochorionic diamniotic pregnancies, and to study the impact of fetal cardiac function on outcome of monochorionic (MC) twin pregnancies complicated by twin-to-twin transfusion syndrome (TTTS) in order to improve identification of cases and indications for therapy.

Materials and Methods: MC twin pregnancies examined between January 2009 and December 2012 at the University Hospital Spedali Civili of Brescia. 84 uncomplicated pregnancies (Group 1; controls) were studied and compared to 46 complicated pregnancies: 34 TTTS (Group 2), 10 selective intrauterine growth restriction (sIUGR; Group 3), 2 intrauterine deaths not for fetal anomaly or maternal condition (Group 4). Fetal heart Doppler studies assessing AoI PI, MPI-conventional Doppler and MPI'-TDI were performed three times between 18 and 24 weeks of gestation. Delivery records and pediatric discharge reports of the patients were reviewed after delivery.

Results: Fetal cardiac function was measured as MPI RV and LV, MPI' RV and LV, and AoI PI at a mean gestational age of 18.3 (range 17.1-20.5), 22.3 (21.1-23.5) and 24.3 (22.2-26.2) weeks. All the cardiac parameters were significantly different at 18 weeks in recipients versus controls except for MPI' RV, which was on the contrary the only cardiac parameter significantly different in donors. In recipients MPI RV, MPI LV, AoI PI and MPI' LV decreased significantly from 18 to 24 weeks' gestation. MPI'LV had the best sensitivity and negative predictive value in recipients at 18 weeks confirmed at 22 weeks. A significant improvement in recipients’ cardiac function towards mean values in controls was demonstrated after fetoscopic laser photocoagulation (FLP) therapy.

Conclusion: We described novel parameters of fetal cardiac function in uncomplicated monochorionic diamniotic pregnancies. We showed that in TTTS the donor’s cardiac
function is not grossly abnormal but in recipients it is abnormal at time of TTTS with normalization after FLP. We identified cardiac indices predictive of the subsequent development of TTTS, and suggest a possible role of these indices in the planning of monochorionic diamniotic pregnancy follow-up.
LIST OF ABBREVIATIONS

A = maximal velocity of the active diastolic filling (PW Doppler)
A’ = maximal velocity of the active diastolic filling (TDI)
AA = arterioarterial
AMM = anatomical M-Mode
(i)AREDF = (intermittent) absent or reversed end-diastolic flow
AoI = aortic isthmus
AV = atrio-ventricular
AV anastomosis = arteriovenous
CHD = congenital heart disease
CO = cardiac output
DV = ductus venosus
E = maximal velocity of the early passive diastolic filling (PW Doppler)
E’ = maximal velocity of the early passive diastolic filling (TDI)
HR = heart rate
ICT = isovolumetric contraction time
IRT = relaxation times
ECG = electrocardiography
EF = ejection fraction
EFW = estimated fetal weight
ET = ejection time
FLP = fetoscopy laser photocoagulation
FT = filling time
(s)IUGR = (selective) intrauterine growth retardation
LV = left ventricle
MAPSE = mitral annular plane systolic excursion
MC = monochorionic
MCA-PSV = middle cerebral artery peak systolic velocity
MPI = conventional Doppler myocardial performance index (o Tei Index)
MPI’ = TDI myocardial performance index (o Tei Index)
MRI = magnetic resonance imaging
PI = pulsatility index
PW = pulsed wave
RFA = radiofrequency ablation
RV = right ventricle
RVOTO = right ventricular outflow track obstruction
S = maximal velocity of the ejection (PW Doppler)
S’ = maximal velocity of the ejection (TDI)
SAPSE = septal annular plane systolic excursion
STIC = spatiotemporal image correlation
TAPS = twin anemia polycythemia sequence
TAPSE = tricuspid annular plane systolic excursion
TDI = tissue Doppler imaging
TRAP = twin reversed arterial perfusion or acardiac twinning
TTTS = twin to twin transfusion syndrome
UA = umbilical artery
VV = venovenous
Fetal cardiac dysfunction may be due to an intrinsic myocardial disease or to a secondary adaptive mechanism. The latter is particularly important because the heart seems to be a central organ in the fetal adaptive response to a variety of insults. Consequently, assessment of fetal cardiac function may be helpful in the diagnosis or monitoring of several fetal conditions. In addition, given the substantial evidence indicating the occurrence of programming of adult cardiovascular disease in fetal life, cardiac function assessment might help to predict perinatal and long-term cardiovascular outcomes.

Evaluating fetal cardiac function is particularly challenging. Fetal echocardiography was initially employed to detect structural anomalies, but its use in fetal cardiac function assessment has recently been proposed (Lee W 2008, Van Mieghem 2009a, Godfrey 2011, Huhta 2004, Rychik 2007, Crispi 2012). There are obvious difficulties in trying to measure cardiac function in a small, moving, and changing patient. An additional challenge is that fetal cardiac dysfunction is essentially subclinical. Fetuses rarely go into cardiac failure, and when they do, the outcome is generally dire, with very few exceptions. The results of cardiac examination in most fetuses in which cardiac function is of interest will be completely normal by child or adult cardiology standards. Thus classical indices used to determine the existence of cardiac failure in postnatal life are of little use in fetuses. Fortunately, adult cardiology has substantially developed in the last few years and a variety of new methods able to identify extremely subtle changes in cardiac function are now available (Crispi 2008, Hatem 2008, Van Mieghem 2009b, Barker 1989, Bjinens 2009, Gardiner 2006). Implementation of these technologies in the fetus is far from straightforward, but these advances have already shown highly promising results. More detailed evaluation of cardiac function will allow new pathophysiological insights into a number of fetal conditions and possibly new clinical applications (Crispi 2012).
Pathophysiology of cardiac function

The primary function of the heart is to eject blood in order to provide adequate perfusion of organs. The heart achieves this function by contracting its muscular walls around a closed chamber to generate sufficient pressure to eject blood from the ventricle through the aortic/pulmonary valve and into the aorta/pulmonary artery (systole). Adequate filling of the ventricle from the atria (diastole) is also essential (Guyton 2006). To maintain normal cardiac function, both systolic and diastolic processes must be preserved and time events must occur in a synchronized manner.

Cardiac cycle in normal conditions

The normal cardiac cycle involves five major phases. The first phases, considered together as the diastolic or ventricular filling stage, involve movement of blood from the atria into the ventricles (Guyton 2006). The next phases, or systolic period, involve the movement of blood from the ventricles to the aorta and the pulmonary artery. The phases are as follows:

(1) Isovolumetric relaxation phase: diastole starts after aortic/pulmonary valve closure with an isovolumetric relaxation period. While the myocardium starts to relax no blood enters or ejects from the ventricles and the intraventricular pressure drops.

(2) Early diastole: when ventricular pressure lowers the atrial pressure, the filling phase starts with the mitral/tricuspid valve opening and blood from the atria filling the ventricle in a passive manner.

(3) Atrial contraction period: the atria contract and complete the filling of the ventricle (late diastole).

(4) Isovolumetric contraction phase: systole is started by contraction of cardiomyocytes, which increases intraventricular pressure. This increase in pressure then opens the aortic/pulmonary valve (isovolumetric contraction time) while there is no change in volume.

(5) Ejection period: finally, when the ventricular pressure has increased sufficiently to open the aortic/pulmonary valves, the myocardium starts to deform and the blood is ejected from the ventricle.

These main components of the cardiac cycle define the main features of cardiac blood flow movement and myocardial motion and deformation (Guyton 2006, Crispi 2012).
**Definition of Heart Failure, Cardiac Dysfunction, and Remodeling**

Heart failure is defined as the inability of the heart to supply sufficient blood flow to meet the body’s needs (Jessup 2009). This is usually a late event that can be easily recognized by cardiomegaly, atrioventricular insufficiency, and fetal hydrops (Huhta 2004). Heart failure can also be quantified by measuring a significant decrease in cardiac output or ejection fraction (Jessup 2009).

However, in the initial stages of an insult, the heart usually manages to adapt and there is a long subclinical period of cardiac dysfunction before end-stage heart failure (Huhta 2004, Rychik 2007). During this period of cardiac adaptation, changes in cardiac function, as well as in the heart’s shape and size, can be measured. These changes are the heart’s attempt to adapt to the insult, a process known as cardiac remodeling.

**Determinants of fetal cardiac (dys-) function**

Changes in cardiac function and shape will depend mainly on the causal insult but are also determined by myocardial contractility, fiber orientation, tissue elasticity, heart geometry, segment interaction, loading conditions, electrical activation, and myocardial perfusion (Bjinens 2009). In the fetal heart, myocardial maturation and fetal blood circulation are also critical factors (Kiserud 2004). The most important determinants of fetal cardiac function are discussed below.

**Myocardial contractility**

Myocardial contractility is the intrinsic ability of cardiac muscle to develop force for a given muscle length and may be affected by genetic disposition to cardiac disease or by hypoxia. Myocardial contractility essentially conditions myocardial motion and deformation during systole (Bjinens 2009). If the velocities measured at all points within a moving object are the same, then the object will be described as having motion. If, on the other hand, different points within a moving object are moving at different velocities, then the object will exhibit deformation (Bjinens 2009) and alter its shape:

- Myocardial *motion* is defined as the distance covered by one point over a certain period of time and is determined by displacement (distance) and velocity (distance divided by time).
– Myocardial deformation is defined as the change in the length/thickness of a segment (two points) and is determined by strain (percentage of change) and strain rate (velocity of segment change).

When myocardial fibers contract, all segments deform and then the heart’s base moves toward the apex to eject blood (Bjinens 2009). Global longitudinal myocardial motion is usually measured at the mitral/tricuspid annulus as this fibrose area (with no intrinsic capacity for deformation) reflects the motion of all myocardial segments. Conversely, myocardial deformation should be assessed in a specific myocardial segment reflecting regional function.

**Fiber orientation**

Myocardial contraction is a complex three-directional motion involving longitudinal contraction, radial contraction, and rotation (circumferential axis) (Bjinens 2012). These components are mainly determined by the complex geometry of myocardial fibers and muscle band orientation (Anderson 2009, Sengupta 2008).

– **Longitudinal motion** consists of the movement of fibers from the apex to the base of the heart and is mainly determined by endocardial longitudinal fibers, which are those farthest from the epicardial blood supply and consequently the most sensitive under milder degrees of hypoxia. Therefore, longitudinal motion usually becomes abnormal in the very early stages of cardiac dysfunction.

– **Radial motion** is perpendicular to the epicardium and is determined by radial fibers mainly located in the mid part of the ventricular wall. Radial motion usually becomes abnormal in the late stages of fetal deterioration.

– The **circumferential axis** is perpendicular to both the longitudinal and the radial axes. In the left ventricle myocardial wall, the geometry of the myofibers changes smoothly from a right-handed helix in the subendocardium to a left-handed helix in the subepicardium such that the angle of the helix varies continuously from positive at the endocardium to negative at the epicardium. Therefore, the base and apex of the left ventricle rotate in opposite directions, leading to the twisting motion being described as ‘the wringing of a linen cloth to squeeze out the water’. Rotation and twisting have been shown to become abnormal in the very early stages of cardiac dysfunction. However, very few studies have
successfully assessed rotation in utero, and therefore its utility in fetal life remains to be elucidated (Crispi 2012).

Changes in myocardial maturation during in utero development
During gestation, maturational changes occur within the myocardium leading to changes in elasticity and contractility throughout the pregnancy (Sedmera 2011). Once the structural details have been organized during the embryonic period, the fetal heart continues to grow by cell division until birth, and continued growth thereafter is due to cell enlargement (Sedmera 2011). The density and compactation of myofibrils increases particularly in early pregnancy, but contractility and elasticity continue to improve during the second half of pregnancy. Changes in myocardial maturation should be taken into account when evaluating and interpreting fetal cardiac function.

Heart rate
HR influences cardiac performance because it is linearly related to cardiac output if stroke volume is held constant. Additionally, an increase in heart rate can increase the contractility and cardiac output. Relatively high fetal HR is responsible, in part, for higher cardiac output in comparison to the adult. However, rapid pacing of the fetal heart decreases stroke volume as filling time decreases. In normal pregnancy, the fetal HR decreases from 175-180 beats/min at 9-10 weeks to 145-150 beats/min at 15 weeks of gestation. The physiological range for baseline HR after this gestational age is 110-150 beats/min. However, significant variations of HR can be observed during fetal movements, breathing, or transient cord compression, the incidence of which may vary with gestational age. Under stressful situations, changes in the HR and/or force of contraction are needed to increase cardiac output and maintain tissue perfusion, as inability to do so will eventually lead to heart failure (Acharya 2006).

Ventricular loading
Volume and pressure loading conditions will determine cardiac function (Bjinens 2009):
– Preload is the muscle length prior to contractility and is dependent on ventricular filling or blood volume in end-diastole (Bjinens 2012, Guyton 2006). The most important determining factor for preload is venous return. Starling’s law of the heart states that, in
the non-failing heart, the increased length of the muscle fibers results in increased energy of contraction. In other words, increased end-diastolic volume causes increased stroke volume. Volume overload (e.g. due to fetal anemia, twin-to-twin transfusion syndrome, valve leakage, etc.) will mainly lead to heart dilatation to help the heart manage the increased blood volume more efficiently.

Afterload is the tension (or the arterial pressure) against which the ventricle must contract and depends on the maximum tension of the myocardial muscle mass in end-systole (Bjinens 2012, Guyton 2006). Afterload for the left ventricle is determined by aorta pressure, while afterload for the right ventricle is determined by pulmonary artery pressure. Pressure overload (e.g. due to valvular stenosis or TTTS) will mainly lead to myocardial hypertrophy in order to increase the contractile mass to overcome the elevated afterload (Opie 2006).

Stroke volume, the amount of blood ejected by the heart in a single beat, is principally determined by three factors: preload, afterload and contractility.

The fetal heart has very limited capacity to increase stroke volume by increasing end-diastolic filling pressure, the right ventricle even less than the left, as they are already operating at the top of their function curves. The Frank–Starling mechanism does operate in the fetal heart, which is particularly apparent during fetal arrhythmias. Adrenergic drive also shifts the function curve to increase stroke volume. However, increased heart rate may be the single most prominent mean of increasing cardiac output in the fetus (Godfrey 2012b).

Extracardiac constraints

The fetal heart has a limited ability to increase the amount of blood it pumps. The constraining effect of the pericardium, solid lungs, and chest wall appears to be a major factor in limiting the maximal stroke volume, particularly the left ventricular stroke volume, in the fetus (Acharya 2006).

Neurohumoral influences

Neurohumoral influences produce adjustments in heart rate to either maintain a constant homeostatic state or to alter cardiac output in response to stress. The nervous system is known to elicit beat-to-beat modifications in heart rate. Decrease in heart rate and
appearance of beat-to-beat variation in the second trimester probably reflects functional maturation of vagal parasympathetic control in the fetus. Unlike the adult, the fetal heart responds to hypoxia with bradycardia and hypertension which are abolished by carotid sinus denervation. Fetal heart rate is shown to decrease after an acute increase in systemic arterial pressure and this sensitivity of baroreceptors to changes in arterial pressure increases with advancing gestation. Combined carotid and aortic denervation or parasympathetic blockade with atropine abolishes this reflex. In the fetus, as in the adult, baroreflex control is influenced by hormonal systems although the extent to which they influence autonomic reflexes during fetal life is different than in the adult. Endogenous angiotensin II significantly contributes to a resetting of the arterial baroreflex early in life, whereas even high circulating levels of vasopressin have little effect (Acharya 2006).

**Ventricular interaction**

Ventricular interaction refers to interdependence of the right and left ventricular performance. This is related to anatomic association between the ventricles, i.e. they are encircled by common muscle fibers, share the interventricular septum, and are enclosed within a pericardial sac. In addition, shunting of blood through the foramen ovale and open ductus arteriosus leads to equalization of pressures between both sides of the fetal heart, as a result equally affecting the preload of both ventricles. However, due to the parallel arrangement of the fetal circulation (as explained below) the fetal heart appears to have the ability for selective regulation of ventricular output (Acharya 2006).

**Particularities of Fetal Circulation**

Understanding the particularities of fetal circulation is essential for adequate comprehension of fetal cardiac function changes in normal and pathological conditions. In contrast to postnatal life, the fetal systemic circulation is fed from the left and right ventricles *in parallel*, but with a small proportion of the right output being spared for the lungs (Kiserud 2004). The well-oxygenated blood is directed from the umbilical vein through the ductus venosus (DV) across the inferior vena cava, through the foramen ovale, left atrium, and ventricle and up the ascending aorta to join the low oxygenated blood in the descending aorta. Deoxygenated blood from the superior and inferior vena
cava is directed through the right atrium and ventricle, pulmonary trunk, and ductus arteriosus.

Additionally, the three shunts – DV, ductus arteriosus, and foramen ovale – are essential distributional arrangements, making fetal circulation a flexible and adaptive system for intrauterine life (Kiserud 2004). The haemodynamic properties and functional ranges of these shunts are important determinants of the development of the fetal heart and circulation during the second and third trimester.

In addition to the fetal shunts, the isthmus aortae has received increasing attention since it forms a watershed between the circulation of the upper body (including the brain) and that of the lower body (including the placenta) (Fouron 1994; Makikallio 2002). Another watershed is the section of the left portal vein situated between the main portal stem and the ductus venosus (Figure 1). This venous section normally directs umbilical blood to the right lobe of the liver. Under abnormal conditions, the flow may cease or be reversed, resulting in an increased admixture of splanchnic blood in the ductus venosus (Kiserud 2004).

Oxygen saturation (Kiserud 2004) gives a picture of distribution and blending of flows in the central fetal circulation. The lowest saturation is found in the abdominal inferior vena cava, and the highest in the umbilical vein. Interestingly, the difference between the left and right ventricle is only 10%, increasing to 12% during hypoxaemia.

With the two ventricles pumping in parallel to the systemic circulation, the pressure difference between the ventricles is minimal compared to postnatal life. Still, the difference in compliance of the great arteries and downstream impedance (upper body vs lower body and placenta) is visible in their pressure and velocity profiles. As already explained, some of the ‘stiffness’ of the fetal myocardium is attributed to the constraint of the pericardium, lungs and chest wall, all with low compliance since no air is introduced. However, with the shunts in operation and a metabolism capable of extracting oxygen at low saturation levels, the fetal heart appears to be a very flexible, responsive and adaptive structure (Kiserud 2004).
Traditionally, fetal cardiac function was assessed by measuring blood flow through conventional Doppler or cardiac morphometry in 2D or M-mode. More recently, direct assessment of myocardial motion and deformation has been proposed using tissue Doppler imaging (TDI) and 2D speckle tracking imaging. Lately, 4D spatiotemporal image correlation (STIC) has also been proposed to more accurately evaluate cardiac dimensions and volumes (Godfrey 2012a, Crispi 2012a).

The function of the heart during a complete cardiac cycle is conventionally assessed by parameters of systolic (contractile), diastolic (relaxation/stiffness), and global ventricular function. Cardiac function is typically assessed with a variety of techniques: direct measurement of cardiac dimensions (M-mode and B-mode ultrasound) or ventricular volumes (4D ultrasound) at different points of the cardiac cycle can be used to estimate cardiac output, as can measurement of blood flow (Doppler ultrasound) through vessels near to the heart, although these methods are often technically challenging; indirect indices involve qualitative assessment of blood flow, tissue excursion or time intervals during the cardiac cycle and are often easier to measure; investigation of arterial and venous Doppler measurements of the peripheral vasculature also provides an indirect means of assessing cardiac function (Tutschek 2011). A detailed list of the most common parameters and techniques used in the fetus is provided in Table 1 and described below.

Conventional Doppler

As the primary function of the heart is to eject blood in order to provide adequate perfusion of organs, blood flow assessment is a common approach to evaluate fetal cardiac function (Lee 2008, Crispi 2012a). Conventional Doppler allows to evaluate blood outflow (systole) and inflow (diastole) in the heart, as well as time events:

– Doppler measurement of flow through the outflow tracts reflects systolic function. This measurement can be multiplied by the area of the outflow tracts to calculate the stroke volume, the amount of blood ejected per heart beat (Guyton 2006). Combining this
information with the fetal heart rate allows **cardiac output** (volume per minute) to be estimated, which should normally be expressed as the cardiac index (cardiac output adjusted by fetal weight) (Guyton 2006, Hernandez-Andrade 2012). Cardiac output is a classical parameter to assess cardiac function but only becomes abnormal in the very late stages of deterioration, when the heart fails to adapt and insufficient blood is ejected to meet organ requirements (Hernandez-Andrade 2012a).

– The main Doppler indices used to evaluate **diastolic** function are the early distolic filling/atrial contraction (E/A ratios) and precordial vein pulsatility indices (described below in “Venous Flow Assessment”) (Lee 2008, Hernandez-Andrade 2012). Doppler allows evaluation of the blood flow filling the ventricle, which typically has a biphasic pattern reflecting E and A wave. The E-wave is the early, passive diastolic filling, which is dependent on ventricular wall relaxation. The A-wave is the active diastolic filling known as the ‘atrial kick’. Calculation of the **E/A ratio** essentially reflects ventricular filling (Jessup 2009) as E/A ratio express the relationship between the maximal velocities of the E and A waveforms of ventricular filling. The majority of ventricular filling occurs late in diastole and atrial contraction is a major contributor to this event. It is measured using pulsed-wave Doppler echocardiography, with the cursor set on or just below the AV valve (usually the mitral) in a four-chamber view. In normal fetuses, E/A ratios are usually < 1. However, there is a substantial increase in E/A ratio from approximately 0.5 at 13 weeks to 0.8 near term mainly due to the increase in E-wave velocity with advancing gestation. The increase in E-wave is thought to result from improved ventricular relaxation. This parameter is of little use in fetal life as it is strongly affected by respiratory and body movements, and a high fetal heart rate usually leads to temporarily fused E/A waves (Van Mieghem 2009a, Godfrey 2011). Another important limitation of this ratio is that impaired relaxation can be reflected by an increased, decreased, or pseudo normal value, hampering interpretation (Jessup 2009).

– Doppler is usually used to assess blood flow but can also be used to calculate **time periods** (Hernandez-Andrade 2012). Of great interest are **isovolumetric contraction time** (ICT), **isovolumetric relaxation time** (IRT) and **ejection time** (ET). The ICT is defined as the time elapsed from the start of contraction and the opening of the outflow valve with a mean duration of 28 ms (range 23-33). The IRT is defined as the time elapsed from the start of relaxation and the opening of the inflow valve, with a mean value of 34 ms (range...
The ET is the period between opening and closure of the semilunar valves, with a mean value of 175 ms (range 159–195) (Hernandez-Andrade 2012). Both ICT and IRT offer information on the first stages of the contractile and relaxation processes of the fetal heart. The ICT expresses the time that is necessary for the ventricle to increase its pressure from atrial to systemic level. The IRT expresses the time after all blood has been ejected and the semilunar valves are closed, the pressure is reduced, and the process of reuptake of calcium starts. A reduced calcium reuptake reflects a deteriorated cardiac function. These periods, particularly the isovolumetric relaxation time, become abnormal in the very early stages of dysfunction, reflecting an increase in the time required to properly relax the myocardium. In complicated pregnancies, the main parameter of the MPI being affected is the IRT. The ET by itself does not provide robust information on the cardiac status; it is its relationship with the isovolumetric times which provides important clinical information. In general, an increased IRT is accompanied by a reduced ET (Hernandez-Andrade 2012).

Time events can be displayed individually or as a composite parameter, such as the myocardial performance index (MPI), which takes several systolic and diastolic time events into account (Tei 1997, Hernandez-Andrade 2005). MPI can be calculated either by estimating the atrioventricular valve time over the ET ((AV-ET)/ET), where AV is ICT+ET+IRT, or by estimating the IVC and the IRT over the ET ((IVC+IRT)/ET) (Figure 2). Resulting values for the MPI have been shown to be relatively stable and vary slightly throughout gestation (mean MPI = 0.36; range 0.28–0.44). The ICT is the most stable parameter of the MPI. In complicated pregnancies, the main parameter of the MPI being affected is the IRT. In general, an increased IRT is accompanied by a reduced ET (Hernandez-Andrade 2012).

The MPI is considered a marker of global cardiac function and it has been shown to be a highly sensitive parameter of dysfunction (Cruz-Martinez 2011, Van Mieghem 2009c). The flow patterns are usually obtained with PW Doppler, but can also be obtained tissue Doppler imaging (TDI). To overcome technical limitation, the MPI has to be measured in the same waveform and the Doppler clicks of aperture and closure of the valves have to be used as landmarks for a better estimation of the time periods.
B-mode echocardiography

The key to functional cardiac assessment in the fetus is measurement of cardiac dimensions and their changes during the cardiac cycle that relate to cardiac function and output.

Individual chambers, measured using either B-mode or M-mode echocardiography, can be assessed in end-diastole and end-systole, estimated by the largest and smallest ventricular cavity size (Tutschek 2011).

M-mode

M-mode techniques are traditionally used in a transverse cardiac view to measure the difference in end-systolic and end-diastolic ventricular diameter and to calculate ejection fraction by applying the Teicholz formula (Godfrey 2012a). Ejection fraction is defined as the percentage of blood ejected in each heart cycle (stroke volume/end diastolic volume). Although ejection fraction is the essential parameter characterizing heart failure in adulthood (Jessup 2009), it is usually altered only in the late stages of deterioration as it mainly reflects ejection and radial function (Godfrey 2012a). As stroke volume, it is afterload-dependent.

It allows for calculation of the shortening fraction, the change in ventricular diameter between end diastole and end systole as a ratio of the end-diastolic diameter, which is a long-standing surrogate for function (Godfrey 2012b).

M-mode allows the identification of subtle abnormalities of cardiac rhythm and determination of the relative timing of cardiac events.

M-mode can be also applied in the long axis of the heart to evaluate tricuspid and mitral annular displacement (Figure 3), which have been proposed as sensitive markers of cardiac dysfunction as they reflect global longitudinal function (Gardiner 2006, Carvalho 2001). To study fetal atrioventricular annulus long-axis displacement, M-mode can be used as offline anatomic M-mode (AMM) or real-time conventional M-mode (MM) with similar values (Germanakis 2012). MAPSE (mitral annular plane systolic excursion), TAPSE (tricuspid annular plane systolic excursion) and SAPSE (septal annular plane systolic excursion) can be assessed with offline AMM in apical four-chamber view, placing the examination beam on the lateral mitral annulus, on the lateral tricuspid annulus and on the septum just below the offset, respectively.
**Tissue Doppler imaging**

While conventional echocardiographic techniques are based on blood flow, TDI uses frequency shifts in ultrasound waves to calculate myocardial velocity, which is characterized by a lower velocity and a higher amplitude (Sutherland 2006). TDI can be applied online to evaluate annular or myocardial velocities. Offline TDI analysis also allows deformation parameters (strain and strain rate) to be assessed:

- **Peak systolic strain and strain rate** assessed at each myocardial segment provide information on myocardial deformation and interaction with neighboring segments (Sutherland 2006). These parameters are also early markers of cardiac dysfunction (Yu 2007).

The application of color Doppler to TDI enables the assessment of **strain rate**, and, by mathematical derivation, myocardial **strain** itself. Strain is defined as percentage of change in the length/thickness of a segment, strain rate as velocity of segment change. These modalities have the advantage of directly measuring myocardial segments, as opposed to chamber-dimension changes, and thus should reflect myocardial contractility more accurately (Carvalho 2001, Crispi 2012a).

- **Peak velocities** evaluated at the mitral or tricuspid annulus reflect global systolic or diastolic myocardial motion and have been demonstrated to be an early and sensitive marker of cardiac dysfunction (Yu 2007, Comas 2010). Since the cardiac apex remains relatively stationary throughout the cardiac cycle, analysis of the motion of the valve annulus relative to the apex gives a good approximation of the longitudinal contractility of the ventricle. Pulsed-wave tissue Doppler examination of the valve annulus longitudinal motion gives three waveforms: **S'**, the velocity of the systolic downwards motion of the annulus towards the apex – a positive deflection waveform; **E'**, the velocity of the early diastolic movement away from the apex – a negative deflection waveform; **A'**, the velocity of the movement of the annulus associated with atrial contraction – a negative deflection waveform. The prime (') notation is used to differentiate from the E and A waveforms of mitral Doppler inflow velocities (Godfrey 2012b) (Figure 4).

S’ corresponds with ventricular systolic function. E’ corresponds with diastolic function, and has been shown to be less preload-dependent than the E/A profile. It can be combined with the mitral inflow, as the **E/E' ratio**, which is an even more sensitive measure of diastolic dysfunction. The A’ waveform has been shown to be more sensitive
than the AV valve inflow profile in detecting atrial mechanical dysfunction (Godfrey 2012b).

TDI can be used to calculate mitral, tricuspid and septal MPI at the level of the annulus. It has been reported recently that TDI is more sensitive than ‘conventional’ AV flow and MPI measurements in detecting systolic and diastolic dysfunction in particular fetal conditions such as IUGR.

Although TDI may provide valuable information on global and regional myocardial motion and deformation, the main disadvantages of this technique are that it can provide information about only one area of the myocardium at any one time as well as being very angle-dependent, i.e. only those areas of the myocardium that are parallel to the angle of insonation can be analyzed.

2D speckle tracking
Recent reports have described the use of non-Doppler technology. 2D speckle tracking techniques allow myocardial deformation to be quantified by using frame by frame tracking of bright myocardial areas (speckles) (Bijnens 2009).

2D speckle tracking requires post-processing and off-line analysis of 2D images and allows estimation of the EF as well as direct measurement of myocardial strain and strain rate (segmentally as well as for the whole chamber). Speckle tracking is usually coupled with an automated border recognition program, so that speckle tracking occurs within the context of the ventricle under investigation.

Speckle tracking essentially measures myocardial deformation (change of shape) as opposed to the point changes in velocities measured by TDI. Speckle tracking, which requires offline processing with dedicated software, is no better than is M-mode for measuring annular displacement techniques, which is readily performed on any modern ultrasound machine.

Despite its potential advantages, this is a recent technique that still requires validation for use in the fetal heart (Van Mieghem 2010a).

4D Spatiotemporal Image Correlation
4D STIC permits 3D reconstruction of the fetal heart over time. This technique is based on a sweep (volume data set) of the fetal heart containing a complete reconstructed cardiac
cycle. From this saved volume, any target region of interest can be obtained at any stage of the cardiac cycle (Godfrey 2011, Godfrey 2012a). 4D STIC has been proposed to measure ventricular volumes that allow more accurate estimation of the cardiac output and ejection fraction. The off-line analysis also allows mitral/tricuspid annulus displacement to be assessed. 4D STIC is a promising technique that requires further studies to improve its applicability in fetal cardiac function assessment (Godfrey 2011, Godfrey 2012a).

The most suitable parameters for assessing fetal cardiac function will mainly be determined by the cause of the dysfunction. Abnormal values of ejection fraction or cardiac output are usually found in the late stages of deterioration, and therefore more sensitive parameters have been proposed for earlier diagnosis and monitoring of fetal cardiac dysfunction. In most cases of cardiac dysfunction, diastolic parameters (such as DV or IRT) are the first to be altered, reflecting impaired relaxation and compliance due to a stiffer or less effective heart. Similarly, parameters reflecting longitudinal function (such as annular displacement or velocities) are typically affected in the early stages as compared to radial function (such as ejection fraction) (Crispi 2012a).

**Magnetic Resonance Imaging**

MRI, both in utero and ex utero, enables measurement and calculations of ventricular volumes and mass, as well as EF and CO/cardiac index. Unlike ultrasonographic techniques, MRI is not affected by maternal obesity or oligohydramnios, and image quality is not dependent on gestational age.

Since it does not rely on assumptions, but rather on true real-time measurements, it is useful for the examination of abnormal hearts that do not conform to the geometric models used in ultrasound techniques. Other advantages include better image quality and structural detail. Technical disadvantages include the cost of the technique, the relatively long duration of the examination (although this is reported to be as short as 15 minutes in some studies) and the lack of availability of both the technology and expertise to perform the examination. Some centers advocate using a sedative premedication to reduce fetal movements; however, as technology improves and study times shorten, this will no longer be required (Godfrey 2012b).
Venous flow assessment

Diastolic function can also be indirectly evaluated with Doppler assessment of the **precordial veins**, which reflect pressure changes in the right atrium and indirectly provide information on diastolic function of the right heart (Van Mieghem 2009a, Godfrey 2011). The ductus venosus (DV) is the most commonly used vessel in fetal medicine as it is known to reflect impaired relaxation and has been used in clinical practice as an early marker of disease (Baschat 2007).

Analysis of the flow (by PW Doppler) within venous channels contiguous with the right atrium (ductus venosus, inferior vena cava, hepatic veins and pulmonary veins), excluding the umbilical vein (UV) which is non-pulsatile from the end of the first trimester, gives a good approximation of the pressure gradients within the atrium itself. The major veins all exhibit a pulsatile flow waveform, representing changes in pressure during the cardiac cycle, with forward venous flow facilitated by low atrial pressures. Thus, at those points within the cycle where atrial pressure is lowest, forward venous flow will be maximal, and where atrial pressure is highest, venous flow will be minimal or even reversed. The normal waveform is the S-wave (maximal forward flow corresponds to ventricular systole, with rapid descent of the closed AV valves causing a drop in atrial pressure), v-descent (ventricular relaxation with rising AV valves, causing a temporary increase in atrial pressure), D-wave (early ventricular diastole, with blood rushing forward into the ventricles, causing a drop in atrial pressure) and a-wave (atrial systole, or atrial kick with pressure in atrium rising steeply) (Godfrey 2012b).

The most significant change in venous Doppler with cardiac dysfunction is reversal or absence of the a-wave, which portends serious consequences in cardiac pump function, with a subsequent daily risk of worsening fetal wellbeing and intrauterine death.

Another venous waveform with prognostic significance is pulsatile flow in the umbilical vein, which has been shown to correlate with the presence of myocardial dysfunction.

Various indices of venous flow profile have been devised. One of these, the **pulsatility index** for veins, is the peak systolic velocity minus the peak diastolic velocity, divided by the time-averaged maximum velocity.

Another way of examining cardiac function, as expressed in the venous system, is by analysis of the **vessel pressure waveform**. Mori et al. (2007) have shown that one can measure the changes in vessel diameter, providing a waveform that is equivalent to the
central venous pressure waveform, with ‘A’ and ‘V’ peaks, and ‘X’ and ‘Y’ troughs. Elements of the morphology of the waveform, in particular shortening of the A-X-V time and reduction in the X nadir, can be indicative of fetal cardiac dysfunction.

Arterial Flow Assessment

The aortic and pulmonary outflow tracts provide valuable information on the velocity and volume of blood ejected by either ventricle and allow afterload estimation of the cardiac function. Each recording is obtained at the emergence of the aortic or pulmonary arteries just after the semilunar valves. Many measures may be evaluated including: peak systolic velocity, acceleration time, velocity time integral, ejection time, pulsatility, and resistance indices (Hernandez-Andrade 2012). Both velocity measurements and vessel area can be obtained at this anatomical plane, allowing estimation of the right, left, and combined cardiac outputs.

The two outflow tracts represent the peripheral resistance of the vascular system. Each outflow provides blood flow to a different fetal region that might respond differently when a hypoxic insult is present. Blood ejected by the left ventricle is forwarded to the upper part of the fetal body and fetal brain. Changes in the tissue resistance of this region are reflected mainly in the left ventricle. Blood flow ejected by the right ventricle is mainly forwarded to the lower part of the fetal body, the pulmonary circulation, and the placenta. Increased placental resistance is also mainly expressed in the right ventricle.

Normal reference values have been reported by Groenenberg et al. (1991) who also showed a mean variation between operators of 5–7%.

The aortic isthmus forms a critical communication between the parallel circuits of the fetal right and left ventricles. Because of its unique position, blood flow from the right and left ventricular circuits has opposite effects on blood flow through the aortic isthmus. Therefore, aortic isthmus flow is a measure of the balance between the two ventricular circuits’ ejection force, duration, and volume, and their downstream impedance. In diastole, when the semilunar valves are closed, the direction of blood flow across the aortic isthmus is mainly affected by cerebral and placental vascular impedances. Under physiologic circumstances, the cerebral vascular impedance is higher than the placental vascular impedance throughout gestation. Therefore, in a normal fetus, regardless of the gestational age, blood flows forward through the aortic isthmus both in systole and
diastole (Acharya 2011). The aortic isthmus can be visualized and blood flow measured either in a longitudinal aortic arch view or in the 3-vessel view. Use of power Doppler may facilitate the visualization in difficult cases. In the longitudinal view, proper imaging of the aortic isthmus requires visualization of the origin of the left subclavian artery and the descending thoracic aorta. The sample volume should be placed in the aortic arch, close to where the aortic arch and the ductus arteriosus converge with the descending aorta, just distally to the origin of the left subclavian artery in order to obtain reliable waveforms (Figure 5).

The most commonly used parameters to describe aortic isthmus blood flow are the isthmic flow index and the pulsatility index (PI). Aortic isthmus Doppler velocimetry is likely to become an indispensable tool in the evaluation of fetal well-being. Another clinical utility of aortic isthmus blood flow measurement could be in the assessment of cardiac function in fetuses at risk of developing heart failure (Acharya 2011).

**Technical considerations when measuring cardiac function in the fetus**

Fetal heart evaluation is challenging due to the smallness of the fetal heart, the high heart rate, and limited access to the fetus far from the transducer. Fetal echocardiography requires specific training and expertise to acquire images and interpret the results. Several limitations should be taken into account when assessing fetal cardiac function (Table 2). These limitations are particularly important in techniques requiring offline analysis (4D STIC, color TDI, and 2D speckle tracking) (Crispi 2012a).

**Fetal position, movement, and size**

Several intrinsic particularities of the fetus such as its position, movements, and small size require expertise to acquire adequate images and may sometimes hamper complete evaluation. The fetus lies far down in the maternal abdomen and thus maternal adiposity, oligoamnios, or an anterior placenta may interfere with image quality. Fetal position changes constantly, requiring different angles to view the fetal heart. Optimal viewing can be impossible if the fetal spine is persistently in an anterior position, while evaluation of longitudinal or radial motion requires an apical/basal or transverse view, respectively. Both conventional and tissue Doppler are critically affected by the angle of acquisition, which should be as close to zero as possible (Sutherland 2006). Other techniques such as
4D STIC or M-mode are less angle dependent but a good angle is still required to obtain reliable results. Fetal corporal and respiratory movements may also interfere with the quality of acquisition. Additionally, the fetal heart is much smaller than the adult heart and varies with gestational age. Therefore, normality ranges throughout pregnancy are always required to calculate z-scores and standardize measurements. Some fetal conditions may affect heart size (e.g. leading to cardiomegaly) and therefore reference values adjusted by heart size or specific fetal biometries may be necessary to correctly adjust parameters that strongly depend on myocardial size (such as annular displacement or myocardial velocities) (Comas 2011).

The smallness of the fetal heart also reduces the accuracy of estimates of cardiac or vessel dimensions. This consideration is particularly important in parameters estimated on the basis of formulas that include several measurements (e.g. cardiac output), which show a relatively wide variability as the error induced by one inaccurate dimension is multiplied in the final calculation (Hernandez-Andrade 2012). Furthermore, heart size strongly limits any attempt to differentially evaluate the endocardial and epicardial layers within the myocardium, which is too thin to be assessed separately. All of these limitations warrant specific training and a critical mentality to properly acquire and interpret functional fetal echocardiography (Lee 2008).

Fetal heart rate and frame rate requirements
Proper acquisition, processing, and interpretation are even more critical in techniques requiring offline analysis, such as TDI or 2D speckle tracking (Comas 2012, Germanakis 2012). Software tools for offline analysis of deformation were initially designed for the adult heart with a low heart rate, fixed position, and electrocardiographic (ECG) co-registration (Comas 2012, Germanakis 2012).

Because of the restricted access to the fetal heart far down in the maternal abdomen, fetal ECG co-registration is impossible. ECG co-registration is critical to identify time events and, for example, to be able to assess postsystolic events. ECG co-registration is also mandatory for the correct functioning of offline cardiac software tools. Recent reports have proposed the use of dummy ECG by manual indication of time events based on the underlying M-mode (Willruth 2011) or 2D images (Crispi 2012b) in order to improve offline analysis of both TDI and 2D speckle tracking. Additionally, while the
required frame rate for proper offline analysis is reasonably well defined in the adult heart (Sutherland 2006), a higher frame rate would probably be necessary for the fetal heart (as the heart rate is about 2–3 times faster in fetuses than in adults) but optimal values remain to be defined. Poor quality acquisitions with a low frame rate or lack of ECG co-registration may lead to incorrect results. A clear example of inconsistent data is the disagreement in reports on longitudinal strain changes throughout gestation, which were described as increasing in the first studies performed with low frame rate acquisitions but were shown to decrease by recent studies using more appropriate methodology (Willruth 2011).

Although recent reports using acquisitions at a high frame rate and dummy ECG have improved the feasibility of these techniques (Willruth 2011, Crispi 2012b), several limitations such as the variable view of the fetal heart and the smallness of the heart (with potentially insufficient myocardium to allow analysis in early gestational ages) remain to be overcome.

**Differences between fetal and postnatal life**

Most echocardiographic techniques are derived from parameters previously developed and validated in the adult heart. However, unlike in the adult heart, changes in fetal cardiomyocyte maturation (myocardium stiffness and intrinsic contractility) and loading occur during gestation and within the myocardium. Additionally, the fetal circulation pattern differs from that in the adult, with a predominant right heart and both circulations being connected (Kiserud 2004). This pattern may also change during pregnancy, which may hamper the understanding of cardiac adaptation due to different insults (volume or pressure overload, hypoxia, cardiac compression, etc.) in utero. Therefore, all of these changes should be taken into account when interpreting the results of fetal echocardiography (Crispi 2012a).

**Lack of validation of techniques in the fetal heart**

Because invasive study of the fetal circulation is not feasible, most of the techniques used in fetal functional echocardiography have not been validated, limiting their interpretation. Additionally, there are discrepancies in the literature on many cardiac function parameters regarding methodology, normal values, and interpretation. For
example, measurement of MPI using either blood flow or valve clicks as landmarks leads to different normality values (Welsh 2012). Another example is the Teichholz formula for ejection fraction, which assumes a normal adult heart geometry, which the fetal heart cannot meet (Yagel 2009). Moreover, the E/E’ ratio has been demonstrated to correlate with intracavitary pressure at end-diastole, but its significance in fetal life is unknown. Therefore, the results of fetal echocardiography should be critically evaluated, taking into account gestational age and the methodology used. Finally, TDI and 2D speckle tracking techniques have been validated for deformation analysis in the adult heart by experimental settings including sonomicrometry (Sutherland 2006). However, no validation studies using invasive procedures can be performed to ascertain the real strain and strain rate values in the fetal heart during the maturation process. Despite these limitations, recent reports have demonstrated that deformation can be assessed in a reproducible manner when the appropriate methodology is employed (Willruth 2011, Crispi 2012b). However, many studies do not properly describe the methodology used or acknowledge potential limitations. Critical reading of all studies on fetal cardiac function, particularly of those using new technologies, is mandatory before accepting their results and conclusions.

As described above, fetal cardiac function assessment may have major limitations and therefore any technique or parameter proposed for its assessment should follow several steps for validation before being incorporated into clinical practice (Crispi 2012a). The first phase is to demonstrate feasibility and reproducibility in well-designed and conducted studies. Use of the proposed parameter following strict methodological criteria is also critical to ensure proper applicability. Then, the behavior of the parameter in normal fetal conditions (physiology), as well as in each clinical disease (pathophysiology), must be described before the technique or parameter can applied in clinical conditions.
Monochorionic (MC) twins account for 20% of spontaneous twin pregnancies and almost 5% occur as a result of medically assisted reproduction. In view of the rising rate of pregnancies in older women and the frequent recourse to assisted reproduction techniques, the incidence of MC twin pregnancies is increasing.

MC twins share the same placental mass across vascular anastomoses on the chorionic plate that allow blood to flow between the two fetuses.

They are therefore subjected to specific and serious complications responsible for severe perinatal complications. These complications manifest as a significant degree of intertwin discordance, either in fetal size, amniotic fluid volume, fetoplacental hemodynamics or structural defects. They include selective intrauterine growth restriction, fetal demise, neurological damage of the surviving twin if the co-twin dies during pregnancy, perinatal death, and haemodynamic discordance with either twin anemia polycythemia sequence (TAPS), twin reversed arterial perfusion or acardiac twinning (TRAP), or twin-to-twin transfusion syndrome (TTTS).

Complications of monochorionic twins are the most common reason for referral to a fetal therapy center. It is estimated that up to 10-15% of MC twins develop TTTS. More than half of all MC twins are complicated by some degree of pathologic condition resulting from their monochorionicity.

The diagnosis of some monochorionic twin anomalies, such as TRAP sequence, is relatively clearly defined. Other anomalies of monochorionic twinning, such as TTTS or unequal placental sharing, may have subtleties that are harder to differentiate at first glance, but the understanding of which make all the difference in terms of selecting the appropriate treatment. To further complicate diagnosis and appropriate management, many patients will have overlapping elements of multiple complications of monochorionic twinning.

Correctly diagnosing the specific abnormality that may occur in MC twins leads to optimal management protocols, counseling and treatment options.
In areas with access to invasive antenatal therapy, 90% of monochorionic twins diagnosed in the first trimester will survive. More specifically, in 85% of pregnancies both survive, in 7.5% one survives and in 7.5% there are no survivors. Most losses (80%) occur prior to 24 weeks and, as mentioned above, the extra loss in monochorionic twins is entirely due to complications of the shared circulation and for the most part to TTTS. Comparison with earlier series suggests that invasive fetal therapy increases the likelihood of at least one twin surviving, decreasing the double-loss rate (Lewi 2010).

**Placental vascular anatomy**

The placenta is designed to support one fetus. When two fetuses develop circulations within one placenta, there is no established or predictable pattern for the vasculature to follow. Understanding the angioarchitecture of how the two circulations interact within one placenta is the key to understanding the pathophysiology underlying the ensuing symptoms.

Counter to common perception, there is usually a significant amount of connectivity between the vasculature of each fetus even in uncomplicated monochorionic pregnancies. Unlike a dichorionic placentation, there is no embedded “barrier” to prevent the vessels from establishing anastomoses. Communication between the two circulations, however, does not equate with development of disease. Instead, development of disease depends, in large part, on the number and type (ie, arterial, venous) of intertwin vascular connections and the net direction of flow they create between the fetuses (Rand 2009).

**Normal angioarchitecture (paired vessels)**

Deoxygenated blood travels from the fetus to the umbilical cord by way of the two umbilical arteries, which wrap around the umbilical vein in a spiral. Once they reach the placenta’s umbilical cord insertion site, they travel along the surface of the placenta as a
pair and then dive down beneath the surface, where gas exchange occurs between them within a capillary network. After this capillary exchange, oxygenated blood enters the vein and then travels back up along the same route to the surface of the placenta so that it may make its way back to the umbilical cord (Figure 6). The unit that describes this path — artery entering into the placenta, travel toward a microvascular network, gas exchange, and return of the vein back to the surface of the placenta — is called a cotyledon. Normal angioarchitecture for a given twin is identifiable by a set of paired vessels, an artery and a vein, situated next to one another as they come out of (and return to) the umbilical cord insertion site and travel to the cotyledon. Such paired vessels belong to one twin’s circulation and do not represent communication between the twins but just a normal communication between a single fetus’s artery and vein (Rand 2009).

Abnormal angioarchitecture (unpaired vessels)

The hallmark of abnormal angioarchitecture in a monochorionic placenta is identification of unpaired vessels. A single artery emerges from the cord of one fetus and travels to a cotyledon alone (unpaired); rather than connecting to a vein that travels back to the fetus along the same path, however, it connects with a single unpaired vein from the other fetus, creating an arteriovenous (AV) anastomosis between the twins (Figure 7) (Rand 2009).

A variety of combinations exist in terms of vascular connections between the two fetal circulations. Most commonly, as described previously, an artery communicates with a vein (arteriovenous [AV] anastomosis), but it may also connect to another artery (arterioarterial [AA] anastomosis) or a vein may communicate with another vein (venovenous [VV] anastomosis). Because the artery determines direction of flow, an AV connection represents unidirectional flow from the artery of one fetus to the vein of the other (Figure 7). The artery of an AV pair sends blood to the cotyledon, and the vein anastomosing with it accepts this blood and transfuses it to the other fetus rather than allowing it to return in its usual circuit back to the originating fetus. Unidirectional flow may occur from and to either fetus, as determined by which fetus the artery originates from.
Most commonly, vascular connections run in both directions (i.e., an AV anastomosis from twin A to B is balanced out by flow from an AV anastomosis that runs from twin B to twin A). Therefore, despite the presence of many such unidirectional communications, in terms of total fluid dynamics, a net balance in blood flow results. In many instances, there may be several unidirectional AV anastomoses that are balanced by the presence of an AA connection. A significant net imbalance in flow is hypothesized to be one of the characteristic causes of TTTS.

In an AA anastomosis, an artery from each fetus meets, and because arteries are relatively high-pressure vessels, a turbulent bidirectional flow results. These connections are end to end and course along the surface of the placenta. They do not penetrate into the placental parenchyma (Figure 8). AAs are often larger than AVs, and whereas several AVs may be present in a given monochorionic placenta, pathologic correlation studies have shown that there is usually only one AA, which is present in 75% of monochorionic placentas. The presence of an AA anastomosis may provide enough balance of flow in an MC twin pregnancy to mitigate the development of true TTTS. This may account for the “near-TTTS” cases with discordant amniotic fluid that are so often referred and followed but never meet definitive criteria for true TTTS and do not seem to carry the same morbidity typically described for TTTS. The presence of AA anastomoses is correlated with selective intrauterine growth restriction (sIUGR) type III.

Unequal placental sharing

A single placenta is meant to sustain a single fetus. When two fetuses share a single placenta, there is no set blueprint for how to achieve this successfully so that each fetus retains an equal share. The umbilical cords may insert anywhere on the placenta (eg, central, marginal, velamentous). A line perpendicular to the midpoint between the two cord insertions may be considered the vascular equator between the circulations. As such, if the cord of one twin inserts centrally and the others twin’s cord inserts anywhere eccentrically (eg. peripheral, marginal, velamentous), the equator would, by definition, result in an unequal placental share for the fetus with the eccentric cord insertion. This may predispose to decreased growth potential and sIUGR of the twin with a smaller share.
(Rand 2009). Most often, the smaller share is sufficient to support growth up to a certain point. It may well be that when the fetus reaches a certain size and its “demand” outstrips the fixed supply of that limited placental share, growth restriction ensues. The small placental share associated with the sIUGR fetus may predispose it to a significantly higher risk for demise. Many MC twins with true TTTS also have underlying unequal placental sharing.

**Fetal complications**

**TRAP**

One percent of all MC twin pregnancies are complicated by a structurally normal twin perfusing an acardiac co-twin, often anencephalic, by means of a unique set of vascular connections. Umbilical artery flow, which normally runs from the fetus toward the placenta, is instead reversed in the acardiac twin, flowing toward it rather than away from it (Figure 9). It receives all its blood volume from the so-called “pump” twin through this reversed arterial connection. Because of the enormous strain of this work, in addition to chronic hypoxia from the double-deoxygenated blood the acardiac twin sends back to the pump, the otherwise normal pump twin has greater than 50% mortality. This is most often manifest as high-output cardiac failure, hydrops, and polyhydramnios in the pump twin. The polyhydramnios serves to complicate matters further by increasing the risk for preterm labor and preterm rupture of the membranes. Overall prognosis depends on the size and vascularity of the acardiac mass.

Intervention in the setting of the TRAP sequence/acardiac twin first began with open hysterotomy and selective delivery of the acardiac mass so as to remove the burden on the otherwise normal pump twin. This evolved to umbilical cord ligation by means of fetoscopy and, ultimately, to less invasive ultrasound-guided methods. Such therapies have included bipolar coagulation, fetoscopic laser, and, most recently, devices causing thermal coagulation by means of radiofrequency ablation (RFA). With RFA, the survival rate of the pump twin is around 85% (Cabassa 2012).
sIUGR

The term ‘selective intrauterine growth restriction’ in monochorionic pregnancies is applicable to cases where the estimated fetal weight (EFW) of the small fetus falls below the 10th percentile. Significant fetal weight discordance is an important element of the clinical picture, which will often accompany this condition, but is not necessary for diagnosis. This is defined by different authors as discordance between the EFW of two fetuses > 25%, and is calculated as the difference between the EFW of the larger twin and the smaller twin divided by the EFW of the larger twin. The clinical significance of cases when both twins’ EFW falls below the 10th percentile without discordance, or cases when discordance exists but the smaller fetus’ EFW is above the 10th percentile, remains to be defined (Valsky 2010).

A definition based on an EFW below the 10th percentile, although not universally established, is widely accepted and tends to be the simplest approach for practical and investigational purposes. However, various diagnostic criteria have been used in the literature, including EFW less than 10th percentile, fetal weight discordance, or fetal abdominal circumference below the 10th percentile, which hamper comparison between studies. The reported prevalence of sIUGR based on an EFW below the 10th centile ranges from 10 to 15%. The reported prevalence of monochorionic pregnancies with an inter-twin birthweight discordance of more than 25% ranges from 11.3% to 19%.

A classification system of sIUGR into three types has been proposed, according to the Doppler patterns in the umbilical artery (UA) in the fetus with IUGR. Accordingly, pregnancies are defined as type I (normal umbilical artery Doppler), type II (persistent absent or reversed end-diastolic flow, AREDF) or type III (intermittent absent/reverse end-diastolic flow, iAREDF). These types not only correlate with distinct clinical forms but also with distinct patterns of placental anastomoses.

The type I Doppler pattern is distinguished by positive diastolic flow in the umbilical artery of the small twin. Placental anastomotic patterns in type I pregnancies are similar to uncomplicated monochorionic pregnancies, resulting in a fair number of anastomosis and bidirectional fetal flow interchange.
Type II pattern is characterized by persistent AREDF in the UA. As in type I, sIUGR type II pregnancies show a distribution of placental anastomoses quite similar to uncomplicated monochorionic twins, but with a more severe placental discordance.

The type III sIUGR is defined by the presence of iAREDF in the UA Doppler of the IUGR twin. The characteristic feature of this Doppler pattern, unique to monochorionic twins, is the alternation of phases of positive with phases of absent/reverse diastolic flow, normally but not always in a cyclical fashion. The observation of this sign indicates the presence of a large placental AA anastomosis, which facilitates transmission of the systolic waveforms of one twin into the umbilical cord of the other one (Valsky 2010).

Type I is associated with a fairly good prognosis. Types II and III are associated with a higher risk of intrauterine demise of the smaller twin, and a high rate of delivery at less than 32 weeks of gestation. Type III is associated with increased risk of neurological injury of the larger twin.

Management in type II and III remains a challenge. Fetal therapy (fetoscopic laser coagulation, cord occlusion) may be an option to improve the prognosis of the larger twin, but the decision must also be weighed against severity of growth restriction, parents’ preferences and technical issues which may include gestational age and placental location (Valsky 2010).

**TAPS**

Monochorionic twins can have a discordant hemoglobin level, which was recently described as twin anemia polycythemia sequence (TAPS). TAPS represents TTTS as it is known by the neonatologist, because it usually presents at the time of birth with a large plethoric twin and usually a small anemic twin. Postnatal diagnosis is based on the presence of chronic anemia with reticulocytosis in the donor and polycythemia in the recipient (Lopriore 2007a).

The reticulocytosis and the absence of hypovolemic shock in the donor allow differentiation from an acute intrafetal transfusion. Absence of fetal cells in the maternal
circulation or red blood cell antibodies excludes feto-maternal hemorrhage and alloimmunization, respectively.

TAPS can occur spontaneously in previously uncomplicated pregnancies (Lopriore 2007a) or after incomplete laser surgery as a treatment for TTTS (Robyr 2006).

Iatrogenic TAPS may occur in up to 13% of ongoing twin pregnancies after laser treatment and is diagnosed by an elevated MCA-PSV > 1.5 multiples of the median (MoM) in one twin, suggesting anemia, and < 0.8 MoM in the other, suggesting polycythemia (Robyr 2006). On the other hand, spontaneous TAPS occurs in about 5% of previously uncomplicated pregnancies, usually after 30 weeks, especially in pairs with late-onset discordant growth. TAPS may also account for some late and previously unexplained intrauterine deaths. However, it remains to be demonstrated if MCA-PSV will identify TAPS in previously uncomplicated pregnancies.

The diagnosis of TAPS can only be made in the absence of TTTS. TAPS and TTTS do occasionally go together, and in < 5% of TTTS cases, the donor will have a MCA-PSV > 1.5 MoM (Kontopoulos 2009).

The placentas of spontaneous TAPS pregnancies show a striking similarity with those of iatrogenic TAPS after incomplete laser surgery. Both have few small unidirectional artery-to-vein anastomoses without compensating artery-to-artery anastomosis, suggesting that TAPS results from a chronic net transfusion across these tiny anastomoses. To all rules there are exceptions, and rare cases with only a small bidirectional anastomosis have been reported. The best management of iatrogenic as well as spontaneous TAPS is currently unknown. Because of its late presentation, the mortality of TAPS is likely to be lower than that of TTTS. Depending on the characteristics of each case, such as gestational age, recurrence after intrauterine transfusion, fetal haemodynamic condition, presence of congenital defects and placental localization, definitive management may consist of elective birth, cord coagulation or laser separation of the anastomoses (Lewi 2010).
**Co-twin demise**

Any complicated MC twin pregnancy has an increased baseline risk for adverse events and may result in demise of one or both twins. When demise of one twin occurs, the well-being and long-term outcome of the surviving co-twin are of significant concern. Demise may occur spontaneously, during expectant management, or after an invasive procedure (i.e., fetoscopic laser coagulation, ultrasound-guided RFA cord coagulation).

The literature on the morbidities of the surviving co-twin is incomplete, complex, and confounded, and it makes counseling challenging. There is up to an estimated 40% risk for adverse neurologic outcome in an MC survivor after co-twin demise. With demise of one twin in a vascularly interconnected pair, the often severe and sudden decrease in blood pressure causes a massive transfusion to the demised twin. This equilibrates within a few minutes; however, depending on the severity and duration of the ischemic period, it may result in end-organ damage. In the management of a complicated monochorionic twin pair, the risk for a potential invasive intervention is constantly weighed against the potential risk incurred by a surviving co-twin if a spontaneous demise is allowed to occur.

Given the potential risk for organ damage, particularly neurologic damage, improving evaluation and assessment of a surviving co-twin are important adjuncts to treatment of monochorionic twins after spontaneous or procedure-related demise. Antenatal ultrasound has been widely used, utilizing fetal neurosonography for signs of ischemic or other pathologic findings. Unfortunately, many such lesions are sonographically occult, because ultrasound is an excellent tool for the diagnosis of hemorrhage and ventriculomegaly but far less so for ischemic white matter injury. Because MRI is the only sensitive imaging modality for the diagnosis of ischemic white matter injury, fetal MRI has recently been added in an attempt to improve antenatal neurologic risk assessment. MRI evidence of injury has been shown as early as 1 day after injury, and all insults were visible by 2 weeks. Moreover, it should be noted that as gestational age increases, image quality on MRI improves vastly (Rand 2009).
**Congenital abnormalities**

Monochorionic twin pregnancies can also be discordant in the presence of congenital abnormalities, which are more common in monochorionic twins, probably due to a teratogenic effect of embryo cleavage, or because of complications of the shared circulation. As such, major congenital defects are found in about 6% of pregnancies and both twin can be affected. Cardiac anomalies are especially prevalent amongst monochorionic twins. Therefore, all monochorionic twins should benefit from detailed sonographic follow-up by experienced sonographers.

In the event of a severe discordant abnormality, selective reduction by fetoscopic or ultrasound-guided cord coagulation has a survival rate of > 80% for the non-affected co-twin. About half of the losses are attributable to intrauterine demise and about half to postnatal losses due to very preterm birth, mostly related to iatrogenic membrane rupture (Lewi 2010).

**TTTS**

Twin to twin transfusion syndrome occurs as a serious complication in 10-15% of monochorionic twin pregnancy, develops typically between 15 and 26 weeks of gestation and is associated with a high perinatal mortality and morbidity (Baschat 2010a).

The *pathophysiology* of TTTS is an unbalanced transfusion of blood across placental vascular anastomoses from one twin (donor) to the other (recipient) (Fisk 2009). In TTTS, the pattern of anastomoses shows a predominance of AV net blood flow from the donor to the recipient fetus. TTTS results in a volume-depleted donor twin with signs of oliguria and oligo-/anhydramnios and a volume overloaded recipient twin with polyuria and polyhydramnios, which may lead to impairment of various organ systems in both affected twins. However, velamentous cord insertion and hormonal factors almost certainly play an important role as well. In the recipient, hypervolemia and atrial distension mediate the release of atrial natriuretic peptide (ANP), resulting in polyuria and subsequent polyhydramnios. While renal renin-angiotensin system (RAS) is suppressed, the recipient has high levels of renin and angiotensin - explained by transfer from the donor and
placental increased production. Elevated vasoactive substances combine with hypervolemia to further increase overload and result in the progressive development of cardiac failure, manifesting as abnormal venous Doppler, cardiac hypertrophy, bivalvular regurgitation, right outflow tract obstruction and eventually hydrops fetalis. In the donor, renal RAS is hyperactivated in association with elevation of other vasoactive proteins, such as endothelin. Renal hypoperfusion leads to oliguria – and consequently oligohydramnios - and eventually to renal tubular dysplasia and atrophy. Donor’s hypovolemia, in combination with placental insufficiency resulting from unequal placental sharing, contribute to the common observation of absent end diastolic velocities in the umbilical artery Doppler in this fetus.

Researchers tried to find markers to predict TTTS in the first trimester. As such, Lewi et al. (2008) have demonstrated that combined first-trimester and 16-week ultrasound examination could predict to some extent cases complicated by TTTS. The difference in crown-rump length (CRL) in the first trimester, abdominal circumference (AC) at 16 weeks, and the presence of discordant amniotic fluid are significant predictors of a complicated fetal outcome. Van Mieghem et al. (2010c) also showed that the best predictor of TTTS was the severity of amniotic fluid discordance corrected for gestational age. Although their algorithms can be implemented in clinical practice for counseling patients and stratification of pregnancy risk, one is unable to predict TTTS in all cases. It is notable that about 30% of MC twin pregnancies with moderate amniotic fluid discordance (not fulfilling the criteria of TTTS), but ultimately progressing to the syndrome, show an increased MPI, and that 40% of MC pregnancies that will progress to TTTS have already abnormal findings in the ductus venosus flow in the first trimester or discordant nuchal translucency measurements. Recently, it was suggested that both increased nuchal translucency and abnormal flow in the ductus venosus in monochorionic twins may suggest early manifestations of haemodynamic imbalance between donor and recipient. Velamentous cord insertion has also been described previously as a risk factor for TTTS. Because the degree of placental sharing cannot be assessed with prenatal ultrasound, a discordant cord insertion may signify unequal sharing. Finally, increased nuchal translucency (NT), membrane folding, and absence of a AA anatomosis have some predictive value for TTTS, but in clinical routine their uptake is challenged by their best accuracy being close to the typical gestational ages at presentation. Unfortunately, these
findings have little sensitivity and specificity and should not be used to predict the disease or to "upstage" it (Martins 2012).

TTTS is usually diagnosed during the recommended fortnightly ultrasound follow-up of asymptomatic monochorionic pregnancies. Although TTTS is not a homogeneous clinical entity, and encompasses a broad spectrum of severity, its diagnosis relies upon strict ultrasound criteria as defined in the Eurofetus trial (Senat 2004) and consist of a confirmed monochorionicity, polyuric polyhydramnios in the recipient twin together, oliguric oligohydramnios in the donor twin, discordant fetal bladder with markedly enlarged bladder in the recipient and very small or non-visible bladder in the donor during the most of the examination (Senat 2004). In Europe polyhydramnios is defined as a deepest vertical pool of amniotic fluid >8 cm before and >10 cm after 20 weeks and oligo/anhydramnios as a deepest pool <2 cm. In contrast, in the United States, the 8 cm cutoff is used more often throughout gestation (Baschat 2011). Both continents agree on the definition of oligohydramnios in the donor’s sac (< 2 cm deepest vertical pocket).

A large number of fetuses that have TTTS may also have a size discrepancy, but this is not required for, or a part of, the diagnosis.

Ultrasound staging of TTTS was introduced in 1999 (Quintero 1999) and provided a reproducible classification. Quintero et al. staged TTTS as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>bladder in the donor twin still visible</td>
</tr>
<tr>
<td>II</td>
<td>bladder in the donor twin no longer visible, no Doppler flow abnormalities</td>
</tr>
<tr>
<td>III</td>
<td>Doppler flow abnormalities (absent/reversed end-diastolic flow in the umbilical artery, absent/reversed a-wave in the ductus venosus)</td>
</tr>
<tr>
<td>IV</td>
<td>hydrops fetalis</td>
</tr>
<tr>
<td>V</td>
<td>demise of one or both twins</td>
</tr>
</tbody>
</table>

Nevertheless, the prognosis is not accurately related to this staging because the natural history of TTTS does not follow an orderly progression through the stages over time. A number of ‘early stage’ cases do not progress and remain at stage 1 or even regress. Progression rates have been reported to vary from 10 to 45.5% (Chalouhi 2011).
This staging system has been shown to correlate with the chance of survival, particularly in cases managed with amnioreduction. In cases treated with laser some studies suggest an association, whereas others do not (Baschat 2011). In a recent meta-analysis a trend was observed for better survival in stages I and II compared with stages III and IV (Rossi 2009).

TTTS entails profound fetal cardiovascular changes which may be present from very early stages in the natural history of the syndrome. Several attempts have been made to develop cardiovascular scores which include cardiac function parameters (Rychik 2007). While the use of such scoring systems is of high interest for research and the understanding of the natural history of the disease, these scores did not prove to be of clinical use for prognostic evaluation of TTTS treated by laser, and did not help significantly in preoperative staging before laser therapy (Baschat 2011).

Left untreated, TTTS is associated with extremely high perinatal mortality and morbidity, which approaches 100% when the onset is in previable gestational age. Perinatal mortality is mainly due to miscarriage or severe preterm birth as a result of the massive polyhydramnios and preterm rupture of the membranes or fetal demise due to severe cardiovascular disturbances (Berghella 2001). Donors and recipients who survive face the risk for morbidity in various organ systems (i.e. brain, cardiac, renal, bowel).

TTTS management has encompassed non-specific, sometimes symptomatic, treatments including amnioreduction, septostomy and even expectant management. To date, the only treatment addressing the pathophysiology of the syndrome is fetoscopic laser photocoagulation (FLP) of placental vessels (Chalouhi 2010). It is a more effective first-line treatment than serial amnioreduction for severe TTTS before 26 weeks. The use of amniodrainage is restricted to late gestational age and following technical failures or limitations of laser. FLP should be considered in the treatment of TTTS to improve perinatal and neonatal outcome. The procedure is usually performed between 15 and 26 weeks’ gestation.

Treatment of stage I disease remains controversial. Conservative management of TTTS stage I is a reasonable option until randomized clinical trials are presented (Rossi 2012).
Complications after FLP include intrauterine fetal death of either fetus (13-30%) and preterm rupture of membranes (10%). Persistence of overt TTTS due to anastomoses missed during surgery (2-14%) and TAPS can occur, but the rate of these complications is critically depending on the surgeon’s experience. If performed correctly, FLP results in a reversal of haemodynamics disturbances associated with TTTS soon after treatment. Reported survival rate for at least one twin is 76-88%. The reported incidence of severe neurodevelopment impairment at 2- to 5-year of age is 13-17%, including a cerebral palsy rate of 6-7% (Baschat 2011, Rossi 2011, Van Klink 2011).
The haemodynamic characteristics of monochorionic (MC) twin pregnancies are enigmatic and remain one of the most challenging problems in contemporary perinatal medicine. The cardiac effect of the underlying hypervolemia or endocrine dysregulation, or both, manifests in the recipient as echocardiographic findings of the syndrome related cardiomyopathy. The Quintero staging system for TTTS has recently been questioned, because more refined measurement of cardiac function may improve evaluation of disease severity and prediction of outcome. Much has been done to increase survival and diminish the cardiac morbidity associated with TTTS (Martins 2012).

Cardiac compromise in twin-to-twin transfusion syndrome and echocardiographic findings

Congenital heart disease (CHD) occurs 12 times more frequently in TTTS than in the general population (Lopriore 2007b). The monozigotic twinning process itself may increase the incidence of CHD, by the unequal division of the inner cell mass, disturbance of laterality and by phenotypic variability of the same genome resulting in discordant cardiovascular anatomy. When considering CHD in TTTS, primary structural cardiac anomalies must be distinguished from acquired cardiac manifestations that result from haemodynamic changes (Silva 2011). Fetal cardiac function has been assessed by ultrasound, using precordial venous Doppler, intracardiac Doppler assessment of transvalvular blood flow, the myocardial performance (or Tei) index (MPI), M-mode assessment of ventricular contractility, the atrioventricular early (E) to late (A) Doppler peak flow velocity index and the speckle tracking-derived strain and strain rate analysis. Doppler assessment of the ductus venosus and the umbilical venous flow is useful in estimating the right atrial pressure curve, and has been integrated in the Quintero staging system with alteration of patterns flow upstaging the disease to stage III (Van Mieghem 2010b). The MPI correlates reasonably with the degree of dysfunction, and enables the assessment of ventricular hypertrophy and outflow tract lesions even in recipients with early stage disease (Fisk 2009). Van Mieghem et al. (2010a) showed that speckle tracking-
derived strain and strain rate analysis can identify fetuses with a failing right ventricle because of TTTS, but may be cumbersome to acquire when polyhydramnios is present and has relatively high interobserver and intraobserver variability (Van Mieghem 2010a).

Recipient twin

When TTTS occurs, 55–100% of recipients present with echocardiographic signs of cardiac compromise (Suetres 2008), including hypertension (Mahieu-Caputo 2003), (bi-)ventricular hypertrophic cardiomyopathy (Karatza 2002), tricuspid regurgitation, ventricular hypokinesia (Stirnemann 2010), abnormal flow patterns in the ductus venosus (Stirnemann 2010), and, most importantly, right ventricular outflow tract obstruction (RVOTO) (Karatza 2002).

Although cardiovascular disorders in recipients may result from increased preload caused by chronic hypervolemia, it is the increased afterload (Lopriore 2007b) resulting from increased arterial resistance and pressure that has been identified by many as a key factor in the pathogenesis of cardiomyopathy (Martins 2012). As such, in about half the cases, the heart is enlarged as a result of hypertrophy rather than ventricular dilatation (Van Mieghem 2010b), and most frequently diastolic dysfunction, rather than systolic dysfunction, is present (Raboisson 2004). The thickened, dysfunctional myocardium causes alterations in ventricular filling. Patterns similar to those seen in restrictive cardiomyopathy occur in 20-30% of cases, and predominantly in the right side (Martins 2012). Failure of the right ventricle, through non-compliance or diastolic dysfunction, can be demonstrated in the ductus venosus by reduced forward blood flow with the atrial contraction. As the dysfunction progresses, the two diastolic waveforms fuse and the Doppler inflow pattern regresses to that typically seen in the first trimester, supporting the notion that RVOTO may be caused by the diminished forward blood flow through the right side of the heart. Also, the ventricular filling time may be shortened, the isovolumetric relaxation prolonged and the MPI increased (Raboisson 2004, Van Mieghem 2010b). Compromise in terms of systolic function may also occur, with a considerable decrease in the shortening fraction in about 30% of the recipients (Karatza 2002), and, again, predominantly at the level of the right ventricle, as demonstrated by the decreased strain in speckle tracking analysis (Van Mieghem 2010b).
Right ventricular hypertrophy may develop progressively, leading to acquired “congenital” pulmonary stenosis or even pulmonary atresia. Also, tricuspid regurgitation occurs in about 30–50% of recipients but is severe in only half of these (Rychik 2007). Mitral regurgitation, on the other hand, is much less frequent (6–14% of cases), yet usually severe (9%) (Rychik 2007). Finally, chronic pressure overload and the increased shear stress associated with TTTS may cause calcification of the aorta and pulmonary artery, with hyperplasia of the intima and media, in the absence of valvular disease (Karatza 2002). Changes also take place in the coronary arteries, which favor supply to the overloaded right ventricle. Ultimately these alterations may lead to fetal hydrops and intrauterine fetal demise (Van Mieghem 2010b).

In most series, abnormal ductus venosus blood flow waveforms are seen in about one in three recipients and a pulsatile umbilical vein in one in ten (Rychik 2007).

It is important to note that, in Quintero stage I, already 45% of cases show signs of ventricular dysfunction in terms of an increased Tei index and that 35% of cases have a fused right ventricular inflow pattern suggestive of diastolic dysfunction. The occurrence of these so-called early findings remains relatively stable over stages I to III (Michelfelder 2007). Nevertheless, other findings such as the left ventricular MPI and mitral and tricuspid regurgitation increase with Quintero stages, suggesting that the Quintero staging system, at least to some degree, reflects progressive fetal cardiovascular compromise. Moreover, as growth of fetal cardiac structures is dependent on the blood flow through them, persistent ventricular dysfunction can lead to secondary anatomic changes (Van Mieghem 2010b, Martins 2012).

Donor twin

In contrast to these changes in recipient twins, acquired cardiac pathology of the donor twin seems to be a much rarer event. Decreased blood volume leading to hypovolemia and reduced placental venous return result in decreased left-sided cardiac output. As the disease progresses, the increase in the feto-placental resistance manifests as absent or reversed end diastolic flow in the umbilical artery (AREDF). Coarctation of the aorta may develop as a result of this subtle imbalance between right and left ventricular outputs, yet insufficient to result in aortic valvular stenosis. Furthermore, the severe placental insufficiency may lead to abnormal Doppler waveforms in the ductus venosus in 5–10% of
donors and 3% present with tricuspid regurgitation or umbilical vein pulsations (Van Mieghem 2009c). A lower MPI has also been documented and, although not significant in most studies, it may be suggestive of hypotension. Finally, ventricular dysfunction may become so severe that hydrops fetalis eventually develops (Martins 2012).

**Staging of TTTS and cardiac profiling**

Although the Quintero staging system estimates the severity of TTTS, it disregards the cardiac involvement of the disease that may be present even at its earlier stages. Therefore, quantifying the magnitude of cardiac derangement through a cardiovascular score might help in early identification of TTTS, more precisely grade the severity of disease, improve decision making for treatment, and help to set a prognosis for possible late cardiovascular sequelae in childhood (Rychik 2007). As such, new staging systems based on the severity of cardiac dysfunction in the recipient fetus have been proposed. The most sensitive one is the Children’s Hospital of Philadelphia scoring system, designed to represent the cardiovascular status of the twins and which correlates with the Quintero staging system (Rychik 2007). The Cincinnati TTTS staging system similarly modifies staging based on severity of recipient’s cardiovascular abnormality as evaluated by fetal echocardiography (Habli 2008). Other attempts have also been made to better classify severity of disease. For example, Murakoshi et al. (2008) subdivided stage III disease on the basis of whether the donor bladder is visible (Stage III atypical) or not (Stage III classical), in the hope that this would differentiate subgroups with respect to fetal prognosis following FLP and help in understanding the pathophysiology of stage III disease. Another approach made by Tan et al. (2004), after showing that AA anastomoses detection predicted better perinatal survival independent of Quintero stage, was subclassifying each stage on the basis of the presence or absence of AA anastomoses (Fisk 2009). Also, Van Mieghem et al. (2009d) have shown that the ejection fraction correlates with MPI. Zilkulnig et al. (1999) demonstrated that abnormal flow in the ductus venosus correlates with tricuspid regurgitation whereas Stirmann et al. (2010) developed cardiac profiling allowing discrimination of cases with significant myocardial dysfunction as well as assessment of the severity of the recipient’s cardiomyopathy. Also, according to Michelfelder et al. (2007), demonstrable, quantifiable changes in both right and left ventricle structure and function occur in recipient twins at even the earliest stages of
TTTS. Most of these methods for the assessment of fetal cardiovascular function are flawed by high interobserver and intraobserver variability, need extensive training, or require hardware that is not easily accessible. Nevertheless, these new cardiac staging systems are useful in the research setting and may play an important part in elucidating the pathophysiology of disease (Van Mieghem 2010b).

**Cardiac function and TTTS management**

In recipients following FLP, there seems to be a progressive improvement of umbilical artery and ductus venosus Doppler flow studies, disappearance of tricuspid regurgitation in 45% of fetuses and improvement in systolic and diastolic function. Acute changes in venous Doppler and ventricular wall thickness likely reflect a decrease in ventricular volume and filling pressures, and the acute improvements in MPI after laser are associated with improved recipient survival (Habli 2008). In fact, in the 48 h following laser, there seems to be normalization of cardiac size, precordial venous Doppler waveforms, valvular regurgitation, and ventricular inflow patterns in half the cases, and the MPI improves in approximately 40% (Sueters 2008). Survival is reduced if this initial improvement is not manifest. Further amelioration in cardiac function continues in the longer term and by about 6 weeks after surgery most have regained normal cardiac function (Van Mieghem 2010b). Because of the capacity of fetal cardiomyocytes to replicate, this recovery seems to proceed faster in utero than after birth (Van Mieghem 2009c). In fact, even severe cardiac dysfunction such as functional pulmonary atresia and hydrops seem to resolve in almost all cases, which is an argument against the use of selective reduction in these fetuses. Nevertheless, a slightly reduced early diastolic ventricular filling may persist as compared to donors (diastolic dysfunction), and recipients remain at an increased risk of occurrence of RVOTO and at a three-fold increased risk (5–8%) of pulmonary stenosis at the time of birth when compared to uncomplicated monochorionic twins (Martins 2012).

In contrast to recipients, the donor twin seems to experience a temporary worsening in cardiac function with increased cardiac size (Sueters 2008), tricuspid regurgitation, ductus venosus alterations, and subcutaneous edema after laser treatment (Van Mieghem 2010b). These findings are probably secondary to a state of relative hypervolemia combined with an abrupt increase in afterload that develops after surgery and disappears in 2–4 weeks. Interestingly, as MPI is also dependent on inherent cardiac muscle
characteristics, these fast venous flow alterations were not mirrored in a significant increase of the MPI at 48 h but only at 2 weeks postoperatively, suggesting that MPI may lag compared with venous Doppler. The donor also experiences an increase in umbilical vein blood volume and flow accompanied by a state of right heart overload, which is in agreement with the notion that FLP reverses blood flow. The increase in the donor’s cardiothoracic ratio after laser treatment is of special interest and can be explained by a state of transient volume overload that initiates a process of cardiovascular remodeling, intrauterine growth restriction that may affect cardiothoracic ratio measurements, cardiac hypertrophy, vasoactive factors that now remain in the donor’s own circulation, residual anastomoses that may initiate reversal of TTTS, and fetal anemia (Sueters 2008). Cardiomegaly as such is not an extremely sensitive index of cardiac overload in comparison with venous Doppler or other Doppler-derived indices. Finally, it has been often suggested that the hostile in-utero environment may cause increased vascular stiffness and raised cardiac afterload in the surviving donor, which has been associated with adult onset of cardiovascular disease such as hypertension and ischemic heart disease (Sueters 2008). Laser treatment can supposedly alter this prenatal vascular programming, with normalization of wall stiffness and cardiac function at the age of 2 years, but this idea has been challenged (Van Mieghem 2010b).

It has recently been investigated whether preoperative fetal cardiac function can predict fetal demise after laser (which occurs in about 18% of recipient fetuses). Shah et al. (2008) demonstrated that the recipient’s cardiovascular profile score can predict outcome to a certain extent. Preoperative abnormal umbilical artery Doppler with AREDF is predictive of loss of the donor following FLP and, when it develops following the procedure, also of the recipient’s demise. In fact, after FLP, the umbilical artery pulsatility index seems to decrease and the ductus venosus pulsatility index to increase in donors, whereas in the recipient the ductus venosus pulsatility index seems to decrease. A significant deviation from these trends seems to have a negative impact on the prognosis for both twins. Whether the impact of FLP is greater in stages I and II or higher remains controversial. According to Baschat et al. (2010b), FLP corrects umbilical venous flow imbalances between TTTS twins through an increase in the umbilical venous return in the donor. A clinical correlate for the successful correction of volume status in these
circumstances seems to be bladder filling whereas peripheral Doppler changes appear to bear no relation to changes in umbilical venous flow (Baschat 2010b). Other preprocedural poor prognostic factor includes discordant growth but after FLP the degree of discordance and the frequency of growth restriction declines (Fisk 2009). Finally, combining functional cardiac ultrasound with amniotic fluid biomarkers may identify recipient fetuses at an increased risk of postoperative demise. It does not seem likely, however, that cardiac function alone will predict outcome, as fetal demise after laser is multifactorial and depends on other factors such as placental sharing or incomplete laser separation (Martins 2012).

Postnatal findings
Few groups have investigated long-term cardiovascular outcome after TTTS. An echocardiographic follow-up study in the University Children’s Hospital in Bonn, Germany, including 89 survivors after TTTS and laser therapy found normal cardiac function at a median age of 21 months. The prevalence of pulmonary stenosis, which was recorded only in recipients, was increased in comparison with the general population (7.8 vs 0.03%) (Herberg 2006). Fesslova et al. (1998), assessed 17 pairs of twin fetuses after TTTS and serial amniocenteses. No specific cardiac involvement was seen in donor twins after birth. In 45% of the recipients there were variable degrees of biventricular hypertrophy and dilatation with tricuspid regurgitation, which became normal in all cases within 40 days to 6 months after birth. Gardiner et al. (2003), examined pulse wave velocity in brachial arteries of twin survivors of TTTS treated with and without laser therapy. The pulse wave velocity discordance seen in the 13 laser treated twin pairs resembled that of dichorionic control subjects (heavier individual with higher pulse wave velocity), whereas the 13 twin pairs after serial amniodrainage showed the opposite inter-twin discordance (increased arterial wall stiffness in the donor) at a median age of 11 months.

In summary, the data provide evidence of normalization of cardiac function after intrauterine severe hemodynamic imbalance once the underlying cause has been removed and illustrate the remarkable adaptability of the developing heart. However, the surviving recipients remain at a small increased risk of pulmonary stenosis. A possible mechanism for this clinically relevant issue may be the chronic right ventricular volume overload, or muscular hypertrophy with severe outflow tract obstruction resulting in
diminished antegrade flow with diminished growth of the right ventricular outflow tract and pulmonary artery. In addition, the release of vasoactive peptides or growth factors may contribute to the development of the cardiac disease (Herberg 2006).

Because of the recipients’ increased risk of right ventricular outflow tract obstruction, intrauterine and postnatal echocardiographic monitoring is warranted.

The cardiovascular system, kidneys and growth seem to recover from the chronic hemodynamic imbalance observed before laser treatment. Intrauterine laser coagulation as a causal therapeutic strategy seems to be the best treatment option for TTTS currently available (Maschke 2011).
### Chapter 5. List of tables

#### Table 1. Most commonly used systolic and diastolic parameters to assess fetal cardiac function.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood volume estimation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>Fraction of blood ejected from the ventricles with each heart beat</td>
<td>2D, M-Mode, 2D speckle tracking</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>Volume of blood being pumped by the ventricle per minute</td>
<td>2D, conventional Doppler, STIC</td>
</tr>
<tr>
<td><strong>Myocardial motion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annular displacement</td>
<td>Distance and velocity of the movement of the atrioventricular valve annulus (MAPSE, TAPSE, SAPSE)</td>
<td>M-Mode, 2D speckle tracking</td>
</tr>
<tr>
<td>Systolic annular peak velocity</td>
<td>Speed of movement of the atrioventricular valve annulus in systole (S')</td>
<td>Spectral or color TDI</td>
</tr>
<tr>
<td><strong>Myocardial deformation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>Amount of deformation (change in length of a myocardial segment from its original length)</td>
<td>Color TDI or speckle tracking imaging</td>
</tr>
<tr>
<td>Strain rate</td>
<td>Speed of deformation (change of strain over time)</td>
<td>Color TDI or speckle tracking imaging</td>
</tr>
<tr>
<td><strong>Times</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection time</td>
<td>Time interval between opening and closure of semilunar valve</td>
<td>Conventional Doppler or spectral/color TDI</td>
</tr>
<tr>
<td>ICT (Isovolumetric contraction time)</td>
<td>Time interval between the start of contraction and the closure of the outflow valve</td>
<td>Conventional Doppler or M-Mode</td>
</tr>
<tr>
<td><strong>Velocity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial acceleration velocity</td>
<td>Myocardial acceleration velocity during isovolumetric contraction</td>
<td>Conventional Doppler or spectral/color TDI</td>
</tr>
<tr>
<td><strong>Diastolic function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precordial vein blood flow patterns (DV and others)</td>
<td>Pattern of blood in precordial veins during atrial contraction that indirectly reflects cardiac compliance</td>
<td>Conventional Doppler</td>
</tr>
<tr>
<td>E/A</td>
<td>Ratio between early (E) and late (A) ventricular filling velocity</td>
<td>Conventional Doppler</td>
</tr>
<tr>
<td>Diastolic annular peak velocities</td>
<td>Speed of movement of the atrioventricular valve annulus in early (E') and late (A') diastole</td>
<td>Spectral or color TDI</td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>Transmitral-to-mitral annular diastolic velocity ratio</td>
<td>Conventional Doppler or spectral TDI</td>
</tr>
<tr>
<td>IRT (Isovolumetric relaxation time)</td>
<td>Time between closure of the aortic valve and opening of the mitral valve</td>
<td>Conventional Doppler or spectral/color TDI</td>
</tr>
<tr>
<td><strong>Global function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPI (myocardial performance index)</td>
<td>Ratio between isovolumetric times (contraction plus relaxation) and ejection time</td>
<td>Conventional Doppler or spectral/color TDI</td>
</tr>
</tbody>
</table>
Table 2. Summary of most important limitations of fetal cardiac function assessment for each technique (Crispi 2012).

<table>
<thead>
<tr>
<th></th>
<th>M-mode</th>
<th>Conventional Doppler</th>
<th>Tissue Doppler</th>
<th>2D speckle tracking</th>
<th>4D STIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal position</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fetal body and respiratory</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>movements</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Changes throughout gestation –</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>normalization</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fetal heart size</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>High fetal heart rate – frame rate</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impossibility of ECG</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Lack of validation in utero</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>
Chapter 6. List of figures

Figure 1. Pathways of the fetal heart and representative oxygen saturation values (in numbers) (Kiserud 2004).

The via sinistra (red) directs well oxygenated blood from the umbilical vein (UV) through the ductus venosus (DV) (or left half of the liver) across the inferior vena cava (IVC), through the foramen ovale (FO), left atrium (LA) and ventricle (LV) and up the ascending aorta (AO) to join the via dextra (blue) in the descending AO. Deoxygenated blood from the superior vena cava (SVC) and IVC forms the via dextra through the right atrium (RA) and ventricle (RV), pulmonary trunk (PA) and ductus arteriosus (DA). The isthmus aortae (arrow) and the section of the left portal vein between the main stem (P) and the DV (striped area) represent watershed areas during hemodynamic compromise. CCA, common carotid arteries; FOV, foramen ovale valve; LHV, left hepatic vein; MHV, medial hepatic vein; PV, pulmonary vein; RHV, right hepatic vein.

Figure 2. Illustration of measuring fetal Tei index (MPI) (Papanna 2011).

(a) Isovolumetric contraction time and (b) isovolumetric relaxation time.
Figure 3. Schematic diagram (a) and M-mode echocardiography (b) illustrating measurement of maximal displacement of the atrioventricular annulus (Matsui 2011).

i.e. distance between end-systole and end-diastole calculated from X-Y coordinates.
Figure 4. TDI of the tricuspid annulus showing velocities in systole and diastole.

Figure 5. Longitudinal and cross-sectional imaging planes demonstrating the aortic isthmus with correct cursor placement for pulsed-wave Doppler imaging (Acharya 2009).
Figure 6. (A) Normal angioarchitechtiture (cotyledon). (B) Superficial view of bidirectional flow into and out of a cotyledon (Rand 2009).

Figure 7. (A) Abnormal intertwin connection: AV anastomosis. (B) Superficial view of unidirectional flow into and out of the cotyledon as a result of the intertwin AV anastomosis (Rand 2009).
Figure 8. (A) AA anastomosis. (B) Superficial view of bidirectional flow in AA anastomosis. (Rand 2009)

Figure 9. Vasculature in TRAP/acardiac twin. a-a, arterioarterial; v-v, venovenous (Rand 2009).
Chapter 1. Rationale of the study

Assessment of MPI, MPI’ and AoI in monochorionic pregnancies

In TTTS, the cardiac function in the recipient fetus is typically compromised because of chronic volume overload as a consequence of the net transfer of blood from the donor to the recipient twin through placental vascular anastomoses, and a subsequent deregulation of the fetal, placental, and maternal renin-angiotensin-aldosterone system. Severe cardiac dysfunction is diagnosed in current clinical practice by abnormal fetal venous Doppler waveforms or, at a later stage, when hydrops appears. This is integrated in the staging system described by Quintero et al (1999) which is almost universally used but has recently been questioned. Fetal survival after therapy depends on stage before laser treatment, but individual outcome is also closely tied to cardiac function. Hence, a direct and more refined measurement of cardiac function may improve evaluation of disease severity and prediction of outcome.

Doppler-based methods to assess the fetal cardiac function, such as the myocardial performance index, have been introduced and validated in fetal medicine. Several groups have demonstrated that alterations in these indices occur in the recipient’s heart at the time of TTTS and that this is not strictly related to Quintero stage at the time of presentation (Martins 2012, Habli 2012). The MPI has been shown to correlate reasonably with the degree of dysfunction even in early disease, being increased in 45% of recipients at Stage I (Michelfelder 2007, Fisk 2009). A lower MPI has been documented in donors and it may be suggestive of hypotension (Van Mieghem 2010). MPI has been used to assess cardiac function after FLP of anastomoses: MPI improves in recipients, but increases in donors as sign of transient impairment of cardiac function postoperatively (Van Mieghem 2009c). Van Mieghem (2009c) constructed a nomogram for RV-MPI and LV-MPI in uncomplicated monochorionic pregnancies, showing that both indices increase during pregnancy – a trend that was previously shown in singletons - reporting a mean
LV-MPI ranging between 0.27-0.33, in contrast to the range between 0.34-0.37 in singletons (Van Mieghem 2009d, Hernandez-Andrade 2007). This confirms findings from Sueters et al (2008b) showing that, even in non-TTTS MCDA twins, cardiac output is significantly higher than in singleton fetuses. Because of a somewhat larger variance of the data, however, the 95% confidence interval and 95th percentile (ranging between 0.39-0.47) was not so different from singletons (between 0.43-0.45). This suggests that, for clinical practice where the 95th percentile is often the only used parameter, singleton charts can be used.

TDI is a robust and reproducible echocardiographic tool that permits a quantitative assessment of motion and timing of myocardial events. Myocardial velocities are a sensitive marker of mildly impaired systolic or diastolic function and therefore useful in the early identification of subtle cardiac dysfunction in preclinical stages. Recently, TDI has been shown to be feasible in fetuses. Tissue Doppler imaging may constitute a more sensitive tool than conventional echocardiography to evaluate cardiac dysfunction (Comas 2010). TDI is used to assess longitudinal and global systolic and diastolic function evaluating annular myocardial velocities and time intervals as well as MPI’. Recently, Divanovic et al (2011) used TDI to demonstrate that concentric hypertrophy is observed in recipient twins affected by TTTS and is associated with impaired ventricular relaxation and shortened filling time.

Doppler echocardiographic assessment of the aortic isthmus blood flow seems to be a promising tool that would help in early identification of fetal circulatory compromise because it provides important information on fetal cardiovascular function, i.e. individual performance of ventricles, relative changes in upper (including brain) and lower (including placenta) body resistances and fetal oxygenation (Acharya 2009). Changes in aortic isthmus blood flow pattern seem to reflect the fetal cardiovascular status accurately and predict the perinatal and long-term neurodevelopmental outcome in IUGR (Del Rio 2008, Figueras 2009, Fouron 2005). Another clinical utility of aortic isthmus (AoI) blood flow measurement could be in the assessment of cardiac function in fetuses at risk of developing heart failure (Acharya 2011). Hence, it may be useful in studying fetal cardiac function in monochorionic twin pregnancies that develop TTTS.
Chapter 2. Aims

We assessed MPI-conventional Doppler, MPI’-TDI and AoI PI in monochorionic twins to understand their normal trend in uncomplicated MC pregnancies, and to study the impact of fetal cardiac function on outcome of MC pregnancies complicated by TTTS in order to improve identification of cases and indications for therapy.

As fetal cardiac function changes during gestation and reference curves for MPI, MPI’ and AoI PI in monochorionic diamniotic twin pregnancies were lacking (Van Mieghem 2009c, Vimpeli 2009, Del Rio 2006), we first constructed nomograms based on a prospective cohort of uncomplicated MCDA twin pregnancies to allow adjustment of data from TTTS pregnancies. This study was also undertaken to determine whether there is any correlation in assessing ventricular function using TDI vs conventional Doppler.

Given the possible role of fetal cardiac function as a prognostic factor for fetal outcome, we aimed to determine the evolution of fetal cardiac function in monochorionic twins complicated by TTTS.
Chapter 3. Materials and methods

We performed a single-center longitudinal study between January 2009 and December 2012 at the University Hospital Spedali Civili of Brescia. The study was approved by the local Ethics Committee and all participants gave written informed consent.

84 women with uncomplicated monochorionic diamniotic twin pregnancies attending the twin pregnancy clinic were invited to undergo an additional obstetric ultrasound examination with the aim of investigating fetal cardiac function (Group I; control). In our centre a transabdominal ultrasound examination is routinely performed at 11–13+6 weeks’ gestation in all multiple pregnancies to define chorionicity, diagnose major fetal defects and for measurement of the CRL and NT thickness of each fetus. Follow up of uncomplicated monochorionic twins includes ultrasound examinations at 16 weeks and every forthnight thereafter until delivery. Frequency of ultrasound scans is increased in case of complications. This population was used to construct nomograms for fetal cardiac function. We excluded monoamniotic pregnancies, cases complicated by congenital cardiac anomaly or arrhythmia, TRAP sequence, mothers younger than 18 years.

Besides this normal population, we also assessed 34 cases of TTTS in monochorionic diamniotic twin pregnancies (Group II; TTTS), if they were both alive at the time of the examinations. TTTS was defined according to the sonographic criteria of oliguric oligohydramnios in the donor twin with a deepest vertical pocket (DVP) ≤ 2 cm and polyuric polyhydramnios in the recipient with a DVP ≥ 10 cm. Staging of the disease was done according to the Quintero criteria (1999). One case was Quintero Stage I, eight were Stage II, twentyfive were Stage III, 0 cases was Stage IV. We used this population, combined with Group I, to validate the MPI (conventional Doppler and TDI) and AoI PI as indicators of cardiac function.

Ten pregnancies were complicated by selective IUGR (Group III; sIUGR) and two pregnancies by intrauterine death without any maternal pathology or fetal anomaly (Group IV; IUD).
All examinations were performed by a single operator with a Philips iU-22 ultrasound system using a C5-1 multifrequency curved array transducer (Philips, Bothell, WA, USA). Fetal heart Doppler studies were performed three times between 18 and 24 weeks of gestation, as this is the most critical period to identify and treat TTTS. Doppler measurements were obtained during a period of fetal quiescence and stable HR, in both twins.

The Tei index was obtained with PW conventional Doppler as described in previous studies (Acharya 2008). Briefly, the isovolumetric contraction and isovolumetric relaxation times were obtained by measuring the time interval between the closure of the AV valve and its subsequent opening in the next cardiac cycle. In addition, the ejection time from the opening to the closing of the semi-lunar valve of the great vessel exiting the corresponding ventricle was also measured. The semi-lunar valve ejection time was then subtracted from the atrioventricular valve time. This value was then divided by the corresponding ejection time.

Left-sided measurements were obtained simultaneously by keeping the Doppler gate at a 3–5 mm width and placing it on the medial leaflet of the mitral valve and left ventricular outflow tract at less than a 20° angle. The Doppler sweep was maximized at 15 cm/s to improve the ability to distinguish the specific valve events. Measurements were obtained by placing the calipers on the center of the valve clicks. If the valve clicks were not easily distinguished, the flow pattern was used to calculate the time interval.

Right-sided measurements are usually obtained separately for the tricuspid and pulmonary valves due to the right-sided valves’ anatomical configuration, in particular after 20 weeks’ gestation. However, we could measure the tricuspid and pulmonary valves flow simultaneously by keeping the Doppler gate at 5mm, thereby removing the inaccuracy involved in measuring the time intervals across different heart beats.

TDI was obtained as described in previous studies (Comas 2010). A clear 4-chamber view was obtained in an apical or basal view. The TDI program was set to the pulsed-wave mode with a sample volume size between 2 and 4 mm. Sample volumes were placed in the basal part of the left ventricular wall (mitral annulus) and right ventricular wall (tricuspid annulus). The insonation ultrasound beam was kept at an angle of <30° to the
orientation of the ventricular wall and no angle correction was applied. The sector widths were minimized to obtain the highest possible frame rates (201–273 frames/s), and the TVI recordings stored as cine loops of at least 5–10 consecutive cardiac cycles. To calculate left and right MPI’ by TDI, the following periods were calculated: ICT’, ET’, and IRT’. Finally, left and right MPI’ were calculated as (ICT’ + IRT’)/ET’. Measurement of all MPI’ components were made from the same cardiac cycle.

Once the aortic isthmus was identified in the longitudinal or cross-sectional view, Doppler velocimetry was performed by placing the Doppler gate (cursor) at the appropriate location (in the longitudinal view, placed just distally to the origin of the left subclavian artery; in the 3-vessel-trachea view, placed in the aortic arch, close to where the aortic arch and the ductus arteriosus converge with the descending aorta), keeping the angle of insonation as low as possible (always less than 30°) (Acharya 2011). Doppler flow velocity waveforms was obtained using color-directed pulsed-wave Doppler interrogation. Pulsed-wave gate size (sample volume) was adjusted according to the size of the aortic isthmus and gestational age to avoid recording signals from the adjacent vessels, and Doppler scale was set to high velocity to reduce aliasing.

Delivery records and pediatric discharge reports of the patients were reviewed after delivery.

**Statistical methods**

The relationship between categorical variables was evaluated in univariate analysis using Chi-square test or the Fisher exact test, as appropriate.

Curves describing the time-trend of myocardial parameters in controls were calculated using random effects mixed models for repeated measures. Linear and quadratic transformation of time were tested and only significant (P<0.05) parameters were used. A logarithmic transformation of myocardial parameters was applied only if the parameter had a non-normal distribution and the random effects mixed model did not converge with the untransformed parameter.

Mean values were compared between two subgroups using the non-parametric Wilcoxon test. Myocardial parameters of TTTS fetuses were compared in time using random effects
mixed models for repeated measures. Mean values were reported in Tables and Figures, with P-values evaluating the significance of changes in time.

Survival of twins affected by TTTS was evaluated through the standard Kaplan-Meier method and the difference in survival between recipients and donors was evaluated through the Log-rank test.

TTTS risk according to cardiac parameters at 18th week was evaluated comparing recipient TTTS twins with controls and donor TTTS twins with controls. The odds ratio of being a TTTS twin was modeled keeping the myocardial parameters as continuous variable and using restricted cubic spline models. Cubic splines are smoothly joined piecewise third-order polynomials (Durrleman 1989). Polynomials are fitted within intervals delimited by knots, and restrictions are placed on the resulting curve to ensure a smooth appearance at the knot points. A three-knots analysis was performed.

All analyses were adjusted for estimated fetal weight and gestational age, when appropriate. All analyses were carried out with the SAS software (SAS Institute, Cary, NC), the R (http://cran.r-project.org/) and Matlab software. All the reported P-values were two sided.

Multilevel regression analysis was performed with MLwiN version 2.26 (Centre for Multilevel Modelling, University of Bristol, United Kingdom) to examine the associations between each parameter and gestational age. In the multilevel analysis, the first level was the variance between measurements obtained from the same fetus, the second was the variance between fetuses within the same pregnancy, and the third was the variance between different pregnancies. Since the variance attributable to the three levels was negligible, further analyses were carried out without multilevel adjustments.

Sensitivity, specificity, positive and negative predictive values for the prediction of TTTS were calculated for cardiac parameters at the 18 and 22 weeks assessment. Confidence intervals were also calculated.
Chapter 4. Results

157 monochorionic pregnancies were enrolled into our study: 93 were uncomplicated, 64 complicated (Figure 1). In the first group, 9 patients were excluded for one or more missing measurements. In the second group, 18 patients were excluded for monoamnioncity, TRAP syndrome or fetal cardiovascular anomalies. 84 uncomplicated pregnancies (Group I; controls) were studied and compared to 46 complicated pregnancies: 34 TTTS (Group II), 10 sIUGR (Group III), 2 IUD not for fetal anomaly or maternal condition (Group IV). Among these complicated pregnancies, 19 with TTTS, 7 with sIUGR and 2 with IUD were prospectively followed-up at our centre since the first trimester. The other ones were referred from other Institutions.

Characteristic of women are described in Table 1: no significant differences in age, parity or ethnicity between controls and complicated pregnancies were found.

Fetal cardiac function was measured as MPI RV and LV, MPI’ RV and LV and AoI PI at a mean gestational age of 18.3 (range 17.1-20.5), 22.3 (21.1-23.5) and 24.3 (22.2-26.2) weeks. A comparison between controls and recipients, donors, sIUGR fetuses was performed.

All the cardiac parameters were significantly different at 18 weeks in recipients versus controls except for MPI’ RV, which was on the contrary the only cardiac parameter significantly different in donors (Tables 2a-2b).

Table 3a describes the longitudinal changes in cardiac parameters from 18 to 24 weeks’ gestation, considering all the pregnancies complicated by TTTS. In recipients twins all parameters changed significantly during this time period. In donors MPI’ RV was the only parameter which changed significantly.

Table 3b describes the same changes as in Table 3a considering only the pregnancies treated by FLP. The following graphs (Figure 2) are a visible demonstration of these changes. In recipients MPI RV, MPI LV, AoI PI and MPI’ LV decreased significantly from 18
to 24 weeks’ gestation. In donors MPI’ RV was confirmed as the only parameter with a significant modification.

Comparing Table 2 to Table 3b to understand the effect of FLP therapy, a significant improvement in recipients’ cardiac function towards mean values in controls was demonstrated.

TTTS risk according to cardiac parameters at 18 weeks measured in recipients is shown in Figure 3a. MPI RV was predictive of TTTS for values ≥ 0.45. For example, with a MPI RV = 0.50, there is a 4 fold risk to become the recipient of a TTTS pregnancy. Also MPI LV, MPI’ LV and AoI PI were useful in prediction of TTTS for values ≥ 0.44, 0.45, 2.45 respectively. If we select only the fetuses diagnosed with TTTS after 18 weeks, MPI RV and LV and AoI PI are confirmed predictive of TTTS (Figure 3b).

Tables 4a-4c and 5a-5c describe sensitivity and specificity of cardiac parameters assessed at 18 or 22 weeks’ gestation in recipients for the development of TTTS. MPI’ LV has the best sensitivity and negative predictive value at 18 weeks confirmed at 22 weeks. Data were not analyzed for the 24 weeks’ examination, since no pregnancy developed TTTS after that examination.

Figures 4a and 4b show that none of the parameters was useful in predicting donors. Only MPI’ RV was different with an inverted trend in all donors.

Figures 5a-5e show the longitudinal change of the cardiac parameters measured in controls.

Figures 6a-6j are a clear illustration of cardiac parameters’ modification in recipients or donors compared to controls.

Figures 7a and 7b show the risk of complications according to the fetal weight discrepancy at 18 weeks: if the discrepancy is ≥ 21 g, there is an increased risk of complications; if it is ≥22.5 g, there is an increased risk of TTTS.

Figure 8 shows the survival in twins affected by TTTS. Recipients had a trend towards a better survival than donors (68 vs 58%) even if the P-value was not significant (0.250).
TTTS fetal therapy and survival are described in Tables 6 and 7. Most of the cases were diagnosed at stage III and underwent FLP. The overall survival was 59%, but at least one fetus survived in 70.8% at stage III.

None of the cardiac parameters was significantly different in sIUGR fetuses at 18 weeks (Table 8) or predictive of the condition (Figure 9). Figure 10 shows the risk of sIUGR according to the fetal weight discrepancy at 18 weeks: if the discrepancy is ≥ 26 g, there is an increased risk of sIUGR. Figure 11 shows the survival in twins affected by sIUGR. sIUGR fetuses had worse survival than the co-twin (68 vs 100%) with a borderline P-value (0.067). The mean gestational age at diagnosis was 20.2 weeks (range 17.2-30).

Table 9 shows the pregnancy outcomes. In the TTTS group, 2 pregnancies underwent pregnancy termination due to recurrent TTTS or pPROM after laser therapy; 1 pregnancy with twins discordant for vermian hypoplasia managed conservatively underwent selective termination at 20 weeks’ gestation. Spontaneous miscarriages complicated 6 pregnancies: 3 before laser therapy, 3 after. Intrauterine death complicated 7 pregnancies (5 donors and 2 recipients), all after laser therapy. In this group the modalities of delivery were vaginal delivery in two cases, Caesarean section in five.

Table 10 shows the neonatal outcomes.
Chapter 5. Discussion

The present study provides additional data on fetal cardiac function in complicated and uncomplicated monochorionic diamniotic twin gestations.

As fetal cardiac function changes during gestation and reference curves for MPI, MPI’ and AoI PI in monochorionic diamniotic twin pregnancies were lacking (Van Mieghem 2009c), we assessed these cardiac parameters in normal monochorionic twins (Group I) and we constructed their normal trend between 18 and 24 weeks’ gestation using a polynomial regression analysis (Figures 5a-5e). All the indices slightly increase during pregnancy – a trend similar to what was previously seen in singletons (Vimpeli 2009, Del Rio 2006, Hernandez-Andrade 2007, Comas 2010, Comas 2011b, Cruz-Martinez 2012).

Given the possible role of fetal cardiac function as a prognostic factor for fetal outcome, we determined the evolution of fetal cardiac function in monochorionic twins complicated by TTTS and treated by FLP therapy. Our findings in monochorionic pregnancies support observations in earlier studies (Rychik 2007, Raboisson 2004, Barrea 2006) showing that fetal systolic and diastolic ventricular function are altered in pregnancies complicated by TTTS, in particular in recipients. Moreover in these twins, we saw a prevalent diastolic dysfunction with earlier modifications in the left ventricle.

Similar to what was presented in previous studies (Barrea 2006, Habli 2008), we showed in a prospective cohort that the cardiac function of the recipient twin improved after FLP therapy. This significant improvement in cardiac indices is a demonstration of FLP’s effectiveness as the increase of amniotic fluid index is a good sign for donors’ wellness. No significant changes were seen in umbilical artery PI, MCA PSV and DV PI, probably due to the small number of twins.

Other groups also suggested that the fetal cardiac function at the time of diagnosis of TTTS and the postoperative evolution of the myocardial performance can predict survival of the recipient (Habli 2008, Shah 2008). We are still unable to conclude this from our data but we had a total survival of 59%.
More importantly, we aimed to study the impact of fetal cardiac function on outcome of MC pregnancies complicated by TTTS in order to improve identification of cases and indications for therapy. All the studies in the Literature are mainly focused on fetal cardiac function in MC pregnancies already complicated by TTTS or in TTTS fetuses undergoing FLP. This is the first study assessing fetal cardiac function at gestations as early as 18 weeks, in order to better predict the development of TTTS. In recipients a global cardiac dysfunction was seen, particularly diastolic and left which was earlier than other modifications. In recipients MPI RV and LV, MPI’ LV and AoI PI have been demonstrated to be predictive test for TTTS. In fact, their negative predictive values are higher than 90% even if their specificity is only around 80% (Tables 4a-4c). The best index is MPI’ LV with a sensitivity of 92% and specificity of 80%. We were unable, nor was it our goal, to improve Quintero’s staging system giving a new score system. However, using these indices (or MPI’ LV alone) we could be reasonably confident to follow up the woman after three or four weeks instead of two when the parameters are below their cut-offs at 18 weeks’ gestation. This could allow a significant reduction in the number of ultrasound scans, and a better allocation of resources, in a setting like ours where, in accordance to data from the literature and international guidelines (Sueters 2006, Vayssière-FCGO 2011, RCOG 2008), monochorionic diamniotic twin pregnancies are scanned at least every two weeks since 16 weeks’ gestation.

A recent study conducted in fetuses with and without congenital heart disease (Acharya 2008) demonstrated that the correlation between Tei indices measured by PD and TD methods is weak. Therefore, it has been suggested not to use the indices interchangeably in the assessment of fetal cardiac function. This study confirmed this suggestion. In fact, if we consider cardiac indices in recipients at 18 weeks’, MPI’ RV is not useful in assessing cardiac function and MPI’ LV shows the best sensitivity and negative predictive value. If we combine MPI LV with MPI’ LV no more informations are given in terms of prediction of complication.

A recent study on AoI PI (Del Rio 2008) demonstrated that this index is >95th centile in 41% IUGR fetuses and found that AoI PI was significantly associated with the risk of adverse perinatal outcome. We found AoI PI be higher in recipients at 18 weeks but not in
donors or sIUGR fetuses. This could be explained by the fact that sIUGR has a different pathophysiology from IUGR in singletons, but also by the number of sIUGR in our study being too small to identify the possible suggested association. It will be interesting to study this issue in a larger number of patients.

As described in the Literature (Crispi 2012), fetal cardiac function assessment had major limitations: several intrinsic particularities of the fetus such as its position, movements, oligohydramnios and small size required expertise to acquire adequate images and might sometimes hamper complete evaluation; even acquisition and interpretation of techniques were critical; we didn’t have ECG co-registration, critical to identify time events, nevertheless we always analyzed a complete cardiac cycle; cardiac time periods were not displayed individually, but as MPI and MPI’ composite parameters; we couldn’t follow up all patients due to some referrals; we decided to scan only three times the patients during the pregnancies to improve patients’ compliance but we chose the gestational age at higher risk for TTTS. We also excluded the 20 weeks’ scan since it was coincident with the anomaly scan, and would have made the appointment too long to maintain the woman’s confort.

We described novel parameters of fetal cardiac function in uncomplicated monochorionic diamniotic pregnancies. We showed that in TTTS the donor’s cardiac function is not grossly abnormal but in recipients it is abnormal at time of TTTS with normalization after FLP. We identified cardiac indices predictive of the subsequent development of TTTS, and suggest a possible role of these indices in the planning of monochorionic diamniotic pregnancy follow-up.

Given the role of LV function in maintaining fetal brain perfusion, and its alteration observed in recipients, it would be interesting to correlate in utero cardiac function with long-term neurologic outcomes.
## Table 1. Characteristics of women

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<th>IUGR No. 10</th>
<th>P-value</th>
<th>IUD No. 2</th>
<th>P-value</th>
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<th>In vitro fertilization</th>
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Table 2a. Cardiac function and fetal weight in TTTS twins versus controls at 18th week

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<tr>
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<th>Controls (n=168)</th>
<th>Donors (n=28)</th>
<th>Recipients (n=27*)</th>
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<td>Mean (SD)</td>
<td>P-value</td>
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<tr>
<td></td>
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<td>P-value</td>
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</table>

*One recipient difficult to measure.

Table 2b. Cardiac function in TTTS twins diagnosed after the 18th week (n=12) versus controls at 18th week

<table>
<thead>
<tr>
<th></th>
<th>Control (n=168)</th>
<th>Donors (n=12)</th>
<th>Recipients (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>MPI RV</strong></td>
<td>0.40 (0.08)</td>
<td>0.42 (0.08)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>MPI LV</strong></td>
<td>0.36 (0.08)</td>
<td>0.42 (0.10)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>AoI PI</strong></td>
<td>2.34 (0.16)</td>
<td>2.39 (0.15)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>MPI' RV</strong></td>
<td>0.49 (0.08)</td>
<td>0.41 (0.05)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>MPI' LV</strong></td>
<td>0.43 (0.07)</td>
<td>0.45 (0.08)</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>UA PI</strong></td>
<td>1.42 (0.17)</td>
<td>1.54 (0.42)</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>MCA PSV</strong></td>
<td>23.6 (4.8)</td>
<td>25.6 (4.3)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Weight g</strong></td>
<td>171 (45)</td>
<td>167 (36)</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Table 3a. Cardiac function in TTTS twins at 18\textsuperscript{th}, 22\textsuperscript{nd} and 24\textsuperscript{th} weeks

<table>
<thead>
<tr>
<th></th>
<th>Donors</th>
<th></th>
<th></th>
<th></th>
<th>Recipients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>18\textsuperscript{th}</td>
<td>22\textsuperscript{nd}</td>
<td>24\textsuperscript{th}</td>
<td>P-value</td>
<td>No.</td>
<td>18\textsuperscript{th}</td>
</tr>
<tr>
<td>MPI RV</td>
<td>27</td>
<td>0.41</td>
<td>0.42</td>
<td>0.43</td>
<td>0.39</td>
<td>26</td>
<td>0.53</td>
</tr>
<tr>
<td>MPI LV</td>
<td>27</td>
<td>0.40</td>
<td>0.41</td>
<td>0.42</td>
<td>0.84</td>
<td>26</td>
<td>0.53</td>
</tr>
<tr>
<td>AoI PI</td>
<td>27</td>
<td>2.40</td>
<td>2.42</td>
<td>2.47</td>
<td>0.09</td>
<td>26</td>
<td>2.58</td>
</tr>
<tr>
<td>MPI' RV</td>
<td>27</td>
<td>0.43</td>
<td>0.44</td>
<td>0.46</td>
<td>&lt;0.01</td>
<td>26</td>
<td>0.51</td>
</tr>
<tr>
<td>MPI' LV</td>
<td>27</td>
<td>0.44</td>
<td>0.43</td>
<td>0.46</td>
<td>0.24</td>
<td>26</td>
<td>0.63</td>
</tr>
<tr>
<td>UA PI</td>
<td>28</td>
<td>1.54</td>
<td>1.53</td>
<td>1.30</td>
<td>0.13</td>
<td>27</td>
<td>1.64</td>
</tr>
<tr>
<td>MCA PSV</td>
<td>28</td>
<td>24.8</td>
<td>29.9</td>
<td>28.0</td>
<td>0.06</td>
<td>27</td>
<td>26.2</td>
</tr>
<tr>
<td>DV PI</td>
<td>20</td>
<td>0.92</td>
<td>0.73</td>
<td>0.62</td>
<td>&lt;0.01</td>
<td>16</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Note: No. is the number of assessed twins at 18\textsuperscript{th}, 22\textsuperscript{nd}, 24\textsuperscript{th} week.
Table 3b. Cardiac function in TTTS twins at 18\textsuperscript{th}, 22\textsuperscript{nd} and 24\textsuperscript{th} weeks in women undergoing laser therapy

<table>
<thead>
<tr>
<th></th>
<th>Donors</th>
<th></th>
<th></th>
<th>P-value</th>
<th>Recipients</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>18\textsuperscript{th}</td>
<td>22\textsuperscript{nd}</td>
<td></td>
<td>24\textsuperscript{th}</td>
<td>P-value</td>
<td>No.</td>
<td>18\textsuperscript{th}</td>
</tr>
<tr>
<td>MPI RV</td>
<td>21</td>
<td>0.39</td>
<td>0.41</td>
<td>0.42</td>
<td>0.35</td>
<td></td>
<td>20</td>
<td>0.54</td>
</tr>
<tr>
<td>MPI LV</td>
<td>21</td>
<td>0.38</td>
<td>0.40</td>
<td>0.41</td>
<td>0.39</td>
<td></td>
<td>20</td>
<td>0.55</td>
</tr>
<tr>
<td>AoI PI</td>
<td>21</td>
<td>2.38</td>
<td>2.44</td>
<td>2.49</td>
<td>&lt;0.01</td>
<td></td>
<td>20</td>
<td>2.59</td>
</tr>
<tr>
<td>MPI' RV</td>
<td>21</td>
<td>0.43</td>
<td>0.43</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td></td>
<td>20</td>
<td>0.51</td>
</tr>
<tr>
<td>MPI' LV</td>
<td>20</td>
<td>0.44</td>
<td>0.43</td>
<td>0.46</td>
<td>0.67</td>
<td></td>
<td>20</td>
<td>0.64</td>
</tr>
<tr>
<td>UA PI</td>
<td>22</td>
<td>1.57</td>
<td>1.60</td>
<td>1.29</td>
<td>0.27</td>
<td></td>
<td>21</td>
<td>1.67</td>
</tr>
<tr>
<td>MCA PSV</td>
<td>22</td>
<td>24.9</td>
<td>29.7</td>
<td>27.9</td>
<td>0.24</td>
<td></td>
<td>21</td>
<td>25.7</td>
</tr>
<tr>
<td>DV PI</td>
<td>17</td>
<td>0.96</td>
<td>0.76</td>
<td>0.62</td>
<td>0.20</td>
<td></td>
<td>13</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Note: No. is the number of twins at 18\textsuperscript{th}, 22\textsuperscript{nd}, 24\textsuperscript{th} week.
Table 4a. Sensitivity (SE) and Specificity (SP) of cardiac function parameters assessed at 18 weeks in Recipients gestation for the development of TTTS. (Prevalence = 13.4%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI RV cut off 0.45</td>
<td>0.615</td>
<td>0.425-0.776</td>
<td>0.798</td>
<td>0.731-0.851</td>
<td>0.320</td>
<td>0.930</td>
</tr>
<tr>
<td>MPI LV cut off 0.44</td>
<td>0.692</td>
<td>0.500-0.835</td>
<td>0.869</td>
<td>0.810-0.912</td>
<td>0.451</td>
<td>0.948</td>
</tr>
<tr>
<td>MPI' LV cut off 0.45</td>
<td>0.923</td>
<td>0.759-0.979</td>
<td>0.804</td>
<td>0.737-0.857</td>
<td>0.421</td>
<td>0.985</td>
</tr>
<tr>
<td>AoI PI cut off 2.46</td>
<td>0.731</td>
<td>0.539-0.863</td>
<td>0.816</td>
<td>0.750-0.867</td>
<td>0.380</td>
<td>0.951</td>
</tr>
</tbody>
</table>

Table 4b. Sensitivity (SE) and Specificity (SP) combining cardiac function parameters (Combining Multiple Tests) assessed at 18 weeks in Recipients for the development of TTTS. (Prevalence = 13.4%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI LV + MPI' LV</td>
<td>0.692</td>
<td>0.500-0.835</td>
<td>0.958</td>
<td>0.917-0.980</td>
<td>0.720</td>
<td>0.950</td>
</tr>
<tr>
<td>AoI PI + MPI' LV</td>
<td>0.731</td>
<td>0.539-0.863</td>
<td>0.982</td>
<td>0.949-0.994</td>
<td>0.864</td>
<td>0.959</td>
</tr>
</tbody>
</table>

Table 4c. Sensitivity (SE) and Specificity (SP) of cardiac function parameters assessed at 18 weeks in Recipients diagnosed with TTTS after 18 weeks’ gestation for the development of TTTS (n.12). (Prevalence = 6.7%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI RV cut off 0.45</td>
<td>0.500</td>
<td>0.254-0.746</td>
<td>0.798</td>
<td>0.730-0.851</td>
<td>0.150</td>
<td>0.957</td>
</tr>
<tr>
<td>MPI LV cut off 0.44</td>
<td>0.667</td>
<td>0.391-0.862</td>
<td>0.869</td>
<td>0.810-0.912</td>
<td>0.266</td>
<td>0.973</td>
</tr>
<tr>
<td>MPI' LV cut off 0.45</td>
<td>1.000</td>
<td>0.758-1</td>
<td>0.804</td>
<td>0.737-0.857</td>
<td>0.267</td>
<td>1.000</td>
</tr>
<tr>
<td>AoI PI cut off 2.46</td>
<td>0.667</td>
<td>0.391-0.862</td>
<td>0.815</td>
<td>0.750-0.867</td>
<td>0.205</td>
<td>0.972</td>
</tr>
</tbody>
</table>
Table 5a. Sensitivity (SE) and Specificity (SP) of cardiac function parameters assessed at 22 weeks in Recipients gestation for the development of TTTS. (Prevalence = 9.7%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI RV cut off 0.52</td>
<td>0.33</td>
<td>0.120-0.550</td>
<td>0.93</td>
<td>0.900-0.970</td>
<td>0.35</td>
<td>0.96</td>
</tr>
<tr>
<td>MPI LV cut off 0.48</td>
<td>0.44</td>
<td>0.210-0.670</td>
<td>0.90</td>
<td>0.860-0.950</td>
<td>0.33</td>
<td>0.95</td>
</tr>
<tr>
<td>MPI' RV cut off 0.48</td>
<td>0.83</td>
<td>0.660-1</td>
<td>0.41</td>
<td>0.340-0.480</td>
<td>0.13</td>
<td>0.82</td>
</tr>
<tr>
<td>MPI' LV cut off 0.53</td>
<td>0.78</td>
<td>0.590-0.97</td>
<td>0.91</td>
<td>0.870-0.950</td>
<td>0.48</td>
<td>0.92</td>
</tr>
<tr>
<td>AoI PI cut off 2.51</td>
<td>0.56</td>
<td>0.330-0.780</td>
<td>0.73</td>
<td>0.670-0.800</td>
<td>0.18</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table 5b. Sensitivity (SE) and Specificity (SP) combining cardiac function parameters (Combining Multiple Tests) assessed at 22 weeks in Recipients for the development of TTTS. (Prev = 9.7%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI LV + MPI' LV</td>
<td>0.44</td>
<td>0.250-0.630</td>
<td>0.97</td>
<td>0.940-0.999</td>
<td>0.62</td>
<td>0.94</td>
</tr>
<tr>
<td>AoI PI + MPI' LV</td>
<td>0.55</td>
<td>0.320-0.780</td>
<td>0.99</td>
<td>0.970-1</td>
<td>0.83</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Table 5c. Sensitivity (SE) and Specificity (SP) of cardiac function parameters assessed at 22 weeks in Recipients diagnosed with TTTS after 22 weeks’ gestation for the development of TTTS (n=4). (Prevalence = 2.3%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SE</th>
<th>CI (SE)</th>
<th>SP</th>
<th>CI (SP)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPI RV cut off 0.52</td>
<td>0.50</td>
<td>0.010-0.990</td>
<td>0.93</td>
<td>0.890-0.970</td>
<td>0.15</td>
<td>0.99</td>
</tr>
<tr>
<td>MPI LV cut off 0.48</td>
<td>0.50</td>
<td>0.010-0.990</td>
<td>0.91</td>
<td>0.860-0.950</td>
<td>0.11</td>
<td>0.98</td>
</tr>
<tr>
<td>MPI' RV cut off 0.48</td>
<td>0.00</td>
<td>0.000-1</td>
<td>0.41</td>
<td>0.340-0.480</td>
<td>0.04</td>
<td>0.94</td>
</tr>
<tr>
<td>MPI' LV cut off 0.53</td>
<td>0.75</td>
<td>0.330-1</td>
<td>0.91</td>
<td>0.870-0.950</td>
<td>0.17</td>
<td>0.98</td>
</tr>
<tr>
<td>AoI PI cut off 2.51</td>
<td>0.50</td>
<td>0.010-0.99</td>
<td>0.74</td>
<td>0.680-0.810</td>
<td>0.40</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Table 6. TTTS therapy

<table>
<thead>
<tr>
<th>Category</th>
<th>Pregnancies No. 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTTS stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td>III</td>
<td>25</td>
</tr>
<tr>
<td>IV-V</td>
<td>0</td>
</tr>
<tr>
<td>I Fetal therapy</td>
<td></td>
</tr>
<tr>
<td>laser</td>
<td>28</td>
</tr>
<tr>
<td>Conservative management</td>
<td>3</td>
</tr>
<tr>
<td>Spontaneous miscarriage before laser</td>
<td>2</td>
</tr>
<tr>
<td>Delivery</td>
<td>1</td>
</tr>
<tr>
<td>II Fetal therapy after laser</td>
<td></td>
</tr>
<tr>
<td>Voluntary termination of pregnancy/cord occlusion</td>
<td>3*</td>
</tr>
<tr>
<td>laser</td>
<td>3°</td>
</tr>
<tr>
<td>GA at diagnosis (mean (range))</td>
<td>18,5 (16-24,3)</td>
</tr>
<tr>
<td>GA at I laser (mean (range))</td>
<td>19,3 (17,2-24,1)</td>
</tr>
</tbody>
</table>

*1 termination of pregnancy at 18,3w for pPROM after laser, 1 termination of pregnancy at 20,3 w for recidive TTTS III, 1 selective termination for brain anomaly

°2 laser for recidive, 1 for TAPS
### Table 7. TTTS fetal survival

<table>
<thead>
<tr>
<th>Category</th>
<th>Pregnancies No. 33*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td></td>
</tr>
<tr>
<td>0 twin</td>
<td>9/33 (27.3%)</td>
</tr>
<tr>
<td>1 twin</td>
<td>9/33 (27.3%)</td>
</tr>
<tr>
<td>2 twins</td>
<td>15/33 (45.5%)</td>
</tr>
<tr>
<td>≥ 1 twin</td>
<td>24/33 (72.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>39/66 (59%)</td>
</tr>
<tr>
<td>Survival ≥1 fetus</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>100%</td>
</tr>
<tr>
<td>Stage II</td>
<td>75% *</td>
</tr>
<tr>
<td>Stage III</td>
<td>70.8%</td>
</tr>
</tbody>
</table>

*1 pregnancy lost to follow up: IUD of the donor after laser but II twin lost to follow up
° 68.8% if we consider 1 NND at 25 weeks

### Table 8. Cardiac function and fetal weight in sIUGR twins versus controls at 18\textsuperscript{th} week

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=168)</th>
<th>Normally grown (F1) (n=10)</th>
<th>sIUGR (F2) (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>MPI RV</td>
<td>0.40 (0.08)</td>
<td>0.36 (0.05)</td>
<td>0.41 (0.08)</td>
</tr>
<tr>
<td>MPI LV</td>
<td>0.36 (0.08)</td>
<td>0.34 (0.04)</td>
<td>0.41 (0.11)</td>
</tr>
<tr>
<td>AoI PI</td>
<td>2.34 (0.16)</td>
<td>2.33 (0.16)</td>
<td>2.19 (0.74)</td>
</tr>
<tr>
<td>MPI’ RV</td>
<td>0.49 (0.08)</td>
<td>0.43 (0.08)</td>
<td>0.44 (0.08)</td>
</tr>
<tr>
<td>MPI’ LV</td>
<td>0.43 (0.07)</td>
<td>0.41 (0.06)</td>
<td>0.42 (0.07)</td>
</tr>
<tr>
<td>UA PI</td>
<td>1.42 (0.17)</td>
<td>1.37 (0.20)</td>
<td>1.58 (0.39)</td>
</tr>
<tr>
<td>MCA PSV</td>
<td>23.6 (4.8)</td>
<td>26.4 (4.6)</td>
<td>29 (6.6)</td>
</tr>
<tr>
<td>Weight g</td>
<td>171 (45)</td>
<td>196 (61)</td>
<td>144 (37)</td>
</tr>
</tbody>
</table>

P-values for comparisons:
Table 9. Pregnancy outcomes: comparison between uncomplicated pregnancies vs TTTS or sIUGR or IUD

<table>
<thead>
<tr>
<th>Category</th>
<th>No complication</th>
<th>TTTS No. 33°</th>
<th>p-value</th>
<th>sIUGR No. 10</th>
<th>p-value</th>
<th>IUD No. 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery modalities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>83</td>
<td>23</td>
<td>0.0001</td>
<td>9*</td>
<td>0.20</td>
<td>1</td>
<td>0.046</td>
</tr>
<tr>
<td>VD</td>
<td>1</td>
<td>2</td>
<td>0.19</td>
<td>1**</td>
<td>0.023</td>
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<td>0.078</td>
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<td>IUD ≥ 1 fetus</td>
<td>0</td>
<td>8**</td>
<td>0.0001</td>
<td>0</td>
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<td>GA at birth</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≥ 34</td>
<td>70</td>
<td>17</td>
<td>0.0008</td>
<td>5</td>
<td>0.26</td>
<td>1</td>
<td>0.086</td>
</tr>
<tr>
<td>&lt; 34</td>
<td>14</td>
<td>8</td>
<td>0.43</td>
<td>5</td>
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<td>1</td>
<td>0.32</td>
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<td>Mean GA at birth</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35,3 (26,1-38)</td>
<td>34,3 (25-40)</td>
<td>0.69</td>
<td>34,3 (29,6-40,4)</td>
<td>0.98</td>
<td>31,2 (23,4-39)</td>
<td>0.71</td>
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<td>Maternal complications</td>
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<tr>
<td>pPROM</td>
<td>8</td>
<td>5</td>
<td>0.513</td>
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<tr>
<td>PTD</td>
<td>14*</td>
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<td>0.01</td>
<td>0</td>
<td>0.35</td>
<td>1’</td>
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</tr>
<tr>
<td>other</td>
<td>2**</td>
<td>1***</td>
<td>1.00</td>
<td>0</td>
<td>1.00</td>
<td>0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*2 cervical cerclage, 12 spontaneous labour before CS
**1 TAPS at birth, 1 placenta praevia
* 1 pregnancy lost to follow up: IUD of the donor after laser but II twin lost to follow up
** 2 Vaginal delivery, 5 caesarean section, 1 twin IUD
*** vaginal bleeding
^ 2 selective termination of pregnancy
` 1 selective termination of pregnancy
’ HELLP syndrome
Table 10. Neonatal outcomes: comparison between uncomplicated pregnancies vs TTTS or sIUGR or IUD

<table>
<thead>
<tr>
<th>Category</th>
<th>No complication</th>
<th>TTTS No. 42*</th>
<th>P-value</th>
<th>sIUGR No. 7</th>
<th>P-value</th>
<th>IUD No. 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Birth Weight g (range)</td>
<td>2159 (789-2900)</td>
<td>1913 (530-3500)</td>
<td>0.037</td>
<td>1291 (980-1980)</td>
<td>0.00073</td>
<td>2100 (700-3500)</td>
<td>0.97</td>
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<tr>
<td>NICU admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>21</td>
<td>10</td>
<td>0.048</td>
<td>3</td>
<td>0.055</td>
<td>0</td>
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<tr>
<td>No</td>
<td>147</td>
<td>29</td>
<td></td>
<td>4</td>
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<td>Neonatal complications</td>
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<td>RDS</td>
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<td>8</td>
<td>0.034</td>
<td>3</td>
<td>0.015</td>
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<td>IVH</td>
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<td>0</td>
<td>1.00</td>
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<tr>
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<td>0.49</td>
<td>0</td>
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<td>0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*3 neonatal death: one at 25 weeks’ after selective termination, two at 22.1 weeks’ due to spontaneous miscarriage.
Chapter 7. List of figures

Figure 1. Monochorionic pregnancies enrolled into the study

- 157 monochorionic pregnancies: longitudinal follow-up
  - 93 uncomplicated
    - 84 controls
    - 9 missing measurements
  - 64 complicated
    - 10 monochorionic
    - 2 TRAP
    - 6 Fetal cardiovascular anomalies
    - 19 TTS + 15 TTS
    - 7 sIUGR + 3 sIUGR
    - 2 IUD

- 18 monochorionic pregnancies: from referral hospitals
Figure 2. Cardiac function in TTTS twins at 18th, 22nd and 24th weeks in women undergoing laser therapy

<table>
<thead>
<tr>
<th></th>
<th>week 18</th>
<th>week 22</th>
<th>week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>0.39</td>
<td>0.41</td>
<td>0.42</td>
</tr>
<tr>
<td>Recipients</td>
<td>0.54</td>
<td>0.48</td>
<td>0.44</td>
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</table>

MPI RV changes in time
MPI LV changes in time

<table>
<thead>
<tr>
<th>week 18</th>
<th>week 22</th>
<th>week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>0.38</td>
<td>0.40</td>
</tr>
<tr>
<td>Recipients</td>
<td>0.55</td>
<td>0.48</td>
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</table>
### AoI PI changes in time

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<th>week 18</th>
<th>week 22</th>
<th>week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>2.38</td>
<td>2.44</td>
<td>2.49</td>
</tr>
<tr>
<td>Recipients</td>
<td>2.59</td>
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</table>

### MPI’ RV changes in time

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<th>week 22</th>
<th>week 24</th>
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</thead>
<tbody>
<tr>
<td>Donors</td>
<td>0.43</td>
<td>0.43</td>
<td>0.47</td>
</tr>
<tr>
<td>Recipients</td>
<td>0.51</td>
<td>0.51</td>
<td>0.53</td>
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</table>
**MPI’ LV changes in time**

<table>
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<th>week 18</th>
<th>week 22</th>
<th>week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>0.44</td>
<td>0.43</td>
<td>0.46</td>
</tr>
<tr>
<td>Recipients</td>
<td>0.64</td>
<td>0.61</td>
<td>0.56</td>
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</table>

**UA PI changes in time**

<table>
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<th></th>
<th>week 18</th>
<th>week 22</th>
<th>week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>1.57</td>
<td>1.60</td>
<td>1.29</td>
</tr>
<tr>
<td>Recipients</td>
<td>1.67</td>
<td>1.52</td>
<td>1.22</td>
</tr>
</tbody>
</table>
Figure 3a. TTTS risk according to cardiac parameters at 18th week. Recipient twins and control twins were selected.
Figure 3a. Continue

Aoi PI at 18th week

UA PI at 18th week

MCA PSV at 18th week
Figure 3b. TTTS risk according to cardiac parameters at 18th week. Recipient twins diagnosed with TTTS after 18 weeks and control twins were selected.
Figure 3b. Continue

![Graphs showing odds ratio for AoI PI at 18th week, UA PI at 18th week, and MCA PSV at 18th week.](image-url)
Figure 4a. TTTS risk according to cardiac parameters at 18th week. Donor twins and control twins were selected.
Figure 4a. Continue

AoI PI at 18th week

UA PI at 18th week

MCA PSV at 18th week
Figure 4b. TTTS risk according to cardiac parameters at 18\textsuperscript{th} week. Donor twins diagnosed with TTTS after 18 weeks and control twins were selected.

- **MPI RV at 18th week**

- **MPI LV at 18th week**

- **MPI' RV at 18th week**

- **MPI' LV at 18th week**
Figure 4b. Continue

Aol PI at 18th week

UA PI at 18th week

MCA PSV at 18th week
Figure 5a. Longitudinal change of MPI RV in controls

Figure 5b. Longitudinal change of MPI LV in controls
Figure 5c. Longitudinal change of AoI PI in controls

$$AoI\ PI = 2.0933 \text{ week} + 0.01348$$

Figure 5d. Longitudinal change of MPI' RV in controls

$$MPI' RV = 0.3631 + 0.006219 \text{ week} + 0.000945 (\text{week}^3)$$
Figure 5e. Longitudinal change of MPI' LV in controls

MPI' LV = 0.3631 + 0.006219 week + 0.000945 \text{ (week)}^2
Figure 6a. MPI RV measurements in Controls (white dots) and Donors (black dots)

Figure 6b. MPI LV measurements in Controls (white dots) and Donors (black dots)
Figure 6c. AoI PI measurements in Controls (white dots) and Donors (black dots)

Figure 6d. MPI' RV measurements in Controls (white dots) and Donors (black dots)
Figure 6e. MPI’LV measurements in Controls (white dots) and Donors (black dots)

Figure 6f. MPI RV measurements in Controls (white dots) and Recipients (black dots)
Figure 6g. MPI LV measurements in Controls (white dots) and Recipients (black dots)

Figure 6h. AoI PI measurements in Controls (white dots) and Recipients (black dots)
Figure 6i. MPI’ RV measurements in Controls (white dots) and Recipients (black dots)

Figure 6j. MPI’LV measurements in Controls (white dots) and Recipients (black dots)
Figure 7a. Fetal weight discrepancy (g) detected at 18th week and risk of any complication
Figure 7b. Fetal weight discrepancy (g) detected at 18th week and risk of TTTS. TTTS and control twins were selected.
Figure 8. Survival of twins affected by TTTS

Tick marks on the curves represent deliveries
*1 pregnancy lost to follow up.
Figure 9. sIUGR risk according to cardiac parameters at 18th week. sIUGR twins (F2) and control twins were selected.
Figure 9. Continue

**AoI PI at 18th week**

**UA PI at 18th week**

**MCA PSV at 18th week**
Figure 10. Fetal weight discrepancy (g) detected at 18th week and risk of sIUGR. sIUGR and control twins were selected.
Figure 11. Survival of twins affected by sIUGR

F1 (10 deliveries out of 10)

F2 (7 deliveries out of 10)

P-value: 0.067

Tick marks on the curves represent deliveries
REFERENCES


Crispi F, Gratacos E. Fetal cardiac function: technical considerations and potential research and clinical applications. Fetal Diagn Ther 2012a;32:47–64.


Maschke C, Diemert A, Hecher K, Bartmann P. Long-term outcome after intrauterine laser treatment for

Matsui H, Germanakis I, Kulinskaya E, Gardiner HM. Temporal and spatial performance of vector velocity
Michelfelder E, Gottliebson W, Border W, Kinsel M, Polzin W, Livingston J, et al. Early manifestations and
spectrum of recipient twin cardiomyopathy in twin–twin transfusion syndrome: relation to Quintero stage.

Mori A, Uchida N, Ishiguro Y, Atsuko T, Kanako M, Mikio M. Evaluation of cardiac function of the fetus by
inferior vena cava diameter pulse waveform. Am Heart J 207;154:789-794.

Murakoshi T, Ishii K, Nakata M, Sago H, Hayashi S, Takahashi Y, et al. Validation of Quintero stage III sub-
classification for twin–twin transfusion syndrome based on visibility of donor bladder: characteristic

O’Donoghue K, Cartwright E, Galea P, Fisk NM. Stage I twin–twin transfusion syndrome: rates of progression

Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006; 367:
356–367.

Papalla R, Mann LK, Molina S, Johnson A, Moise KJ. Changes in the recipient fetal Tei index in the peri-
operative period after laser photocoagulation of placental anastomoses for twin-transfusion syndrome.
Prenatal Diagnosis 2011;31:176-180.

19:480–484.


late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in

Rossi C, D’Addario V. The efficacy of quintero staging system to assess severity of twin-twin transfusion
syndrome treated with laser therapy: a systematic metaanalysis and review of literature. Am J Perinatol

Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin

Rossi C, D’addario V. Survival Outcomes of Twin–Twin Transfusion Syndrome Stage I: A Systematic Review

abnormality and development of a cardiovascular score to assess severity of disease. Am J Obstet Gynecol

Shah AD, Border WL, Crombleholme TM, Michelfelder EC. Initial fetal cardiovascular profile score predicts


Valsky DV, Eixarch E, Martinez JM, Crisp F, Gratacós E. Selective intrauterine growth restriction in monochorionic twins: pathophysiology, diagnostic approach and management dilemmas. Seminars in Fetal & Neonatal Medicine, 2010;342e348.


Objectives: To systematically review the predictive accuracy of first trimester nuchal translucency (NT) for twin-to-twin transfusion syndrome (TTTS).

Methods: Medline and Web of Science were searched for articles published in any language using the keywords ‘feto fetal transfusion’ and ‘nuchal translucency’. Two reviewers extracted clinical and methodological study characteristics and test accuracy data. Accurate data were used to form 2 × 2 data tables comparing NT >95th centile in one of both fetuses, intertwin NT discordance >20% and the occurrence of TTTS.

Results: Of 28 citations identified, 9 met the criteria for the systematic review. For a NT >95th centile, the sensitivity for the subsequent development of TTTS ranged from 0% to 75%, with a specificity ranging from 83% to 100%. For an intertwin NT discordance >20%, the sensitivity ranged from 3% to 64%, with aspecificity ranging from 61% to 100%. Summary receiver operating characteristic curves are shown in the Figure.

Conclusions: NT >95th centile in one of both fetuses, and intertwin NT discordance >20% are not clinically useful predictors of the subsequent development of TTTS. Therefore, strict ultrasound follow up is recommended for the timely diagnosis of TTTS in monochorionic twin pregnancies.


Objectives: Our aim was to evaluate the initial results of selective fetoscopic laser coagulation of placental equator for twin – to twin transfusion syndrome (TTTS).

Methods: This was a prospective cohort study performed in a tertiary referral centre. The sonoendoscopic approach was used to identify the placental vascular equator and to photocoagulate crossing vessels.

Results: Between April 2008 and March 2010, a total of 35 monochorionic diamniotic pregnancies, complicated by severe twin–twin transfusion syndrome before 26 weeks of gestation, underwent fetoscopic laser coagulation of placental blood vessels by3 operators. Median gestational age was 20+1 weeks (interquartile range (IQR) 18+1–22+6 weeks) at fetoscopy and 34+4 weeks (IQR32+4–36+0 weeks) at birth. There was at least one survivor in 66% (23/35) of pregnancies, and the overall survival rate was 54%(38/70). On average, seven vessels were ablated during each of the procedures, with a median operative time of 40 minutes (IQR30–50 minutes). Recurrence of TTTS complicated 11% (4/35) of cases. Intraamniotic bleeding occurred in 2/35 patients. One of these cases was further complicated by maternal hemoperitoneum requiring blood transfusion and surgery.

Conclusions: Our results of fetoscopic laser treatment for twin–twin transfusion syndrome are comparable to those reported in the literature.