



Fetal ventricular strain in uncomplicated and selective growth-restricted monochorionic diamniotic twin pregnancies and cardiovascular response in pre-twin–twin transfusion syndrome

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KEYWORDS: cardiovascular; monochorionic; selective intrauterine growth restriction; sIUGR; TTTS; twin–twin transfusion syndrome; ventricular strain

CONTRIBUTION

What are the novel findings of this work?

Monochorionic diamniotic (MCDA) twin pregnancies that go on to develop twin–twin transfusion syndrome (TTTS) present with intertwin discordance in global longitudinal ventricular strain and ductus venosus early filling time as a percentage of the cardiac cycle (DVeT%), as well as impaired myocardial velocities in some recipients, before Quintero staging criteria are met. We describe the gestational-age response and variability of strain in uncomplicated MCDA twin pregnancies. Unlike in singleton pregnancy with intrauterine growth restriction, in selective intrauterine growth restriction (sIUGR), there is augmented left ventricular (LV) strain.

What are the clinical implications of this work?

Despite the pressing need for an early predictor of TTTS in routine screening of MCDA twin pregnancy due to its sudden onset, the variability of ventricular strain due to biological responses and technical limitations and the difficulty of offline analysis limit its assessment to a research tool. Study of the cardiovascular response in cases of suspected TTTS may help to predict its development. Introduction of intertwin discordance measurement via DVeT% appears more practical and useful in routine screening. The higher LV strain in MCDA twin pregnancy with isolated sIUGR warrants further study to elucidate its mechanism.

ABSTRACT

Objectives Our primary aim was to confirm whether intertwin discordance in ventricular strain and ductus venosus (DV) time intervals predicts twin–twin transfusion syndrome (TTTS). Secondary aims were to create gestational-age ranges for ventricular strain in uncomplicated monochorionic diamniotic (MCDA) twin pregnancies without selective intrauterine growth restriction (sIUGR) and to characterize the relationship of ventricular strain with gestational age in MCDA twin pregnancies with sIUGR that did not develop TTTS.

Methods In the period 2015–2018, we enrolled 150 MCDA twin pregnancies consecutively into this prospective, blinded study of global longitudinal left and right ventricular strain. With the observer blinded to twin pairing and pregnancy outcome, videoclips of the four-chamber view, which had been recorded during ultrasound surveillance in the usual window for development of TTTS (16–26 completed gestational weeks), underwent offline measurement of strain. Uncomplicated MCDA twin pregnancies, without sIUGR, were used to test the association between strain, gestational age and estimated fetal weight using mixed-effects multilevel regression. Inter-rater reliability was tested in 208 strain measurements in 31 fetuses from pregnancies which did not develop TTTS and within-fetus variation was assessed in 16 such fetuses, in which multiple four-chamber views were taken on the same day. The effect of sIUGR on

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strain in otherwise uncomplicated MCDA twin pregnancy was analyzed. MCDA twin pregnancies were defined as 'pre-TTTS' when, having been referred for TTTS evaluation, they did not satisfy Quintero staging criteria, but subsequently developed TTTS requiring laser treatment. MCDA pregnancies which did not develop TTTS comprised the 'non-TTTS' group. Cardiovascular parameters measured in these cases included tissue Doppler parameters and DV early filling time as a percentage of the cardiac cycle (DVeT%). Intertwin strain and DVeT% discordance was compared between non-TTTS and pre-TTTS cases, matched for gestational age.

Results Paired strain data were available for intertwin comparison in 127/150 MCDA twin pregnancies, comprising 14 pre-TTTS and 113 non-TTTS pregnancies, after exclusions. Scans were collected at a median frame rate of 97 (range, 28–220) Hz. Laser therapy was performed at a median gestational age of 20.6 (range, 17.2–26.6) weeks. There were no group differences in right (RV) or left (LV) ventricular strain discordance between 68/113 non-TTTS and 13/14 pre-TTTS MCDA twin pregnancies < 20 completed gestational weeks (RV, $P = 0.338$; LV, $P = 0.932$). DVeT% discordance > 3.6% was found in eight of 13 pre-TTTS pregnancies. In non-TTTS pregnancies, the estimated variability in ventricular strain within each twin during the day was high (RV, 19.7; LV, 12.9). However, within each pair (intertwin variation), variability was low (RV, 5.5; LV, 2.9). Interclass correlation reflecting the proportion of total variability represented by the variability between twin pairs was low (RV, 0.22; LV, 0.18). Both RV ($P < 0.001$) and LV ($P = 0.025$) strain showed a negative association with gestational age. Among non-TTTS MCDA twin pregnancies, LV strain was, on average, higher by 1.83 in sIUGR compared with normally grown fetuses ($P = 0.023$), with no statistically significant difference in RV strain ($P = 0.271$).

Conclusions Although ventricular strain has been reported previously as a possible predictor of developing TTTS, in this blinded, prospective study, we found no significant intergroup differences in ventricular strain in pre-TTTS compared with age-matched non-TTTS MCDA twin pregnancies. We recommend using DVeT% discordance as a more practical screening tool in MCDA twin pregnancies. This study also provides new information on the changes with gestational age, and the biological and technical variation, of global longitudinal ventricular strain in uncomplicated MCDA twin pregnancies and those with isolated sIUGR. © 2019 The Authors. *Ultrasound in Obstetrics & Gynecology* published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.

INTRODUCTION

The initiating events of twin-to-twin transfusion syndrome (TTTS) remain under-explored, in part because uncomplicated monochorionic diamniotic (MCDA) twin pregnancies undergo ultrasound surveillance in a variety

of medical facilities and only those manifesting signs of TTTS are referred to fetal medicine centers for evaluation and, if confirmed, laser therapy¹. Hence, the pathophysiological triggers involved in the transition from balanced to unbalanced intertwin transfusion, resulting in TTTS, remain largely unknown^{2–5}. In addition, selective intrauterine growth restriction (sIUGR) is common in MCDA twin pregnancies and is thought to be due to unequal early cytoplasmic division of the egg further compounded by unequal placental sharing⁶. However, the influence of sIUGR on cardiovascular function in monochorionic pregnancies and on the development of TTTS has not been characterized.

We have reported previously on early cardiovascular changes that may be useful in the prediction of progression to TTTS in MCDA twin pregnancies. Firstly, in a study comparing ventricular strain in MCDA twin pregnancies without and those with TTTS, we observed intertwin discordance, with impaired left ventricular (LV) recipient strain, in MCDA twin pregnancies with Stage-I or Stage-II TTTS and in two control MCDA twin pregnancies which had no abnormal Doppler parameters, but which subsequently developed TTTS. Right ventricular (RV) strain was impaired significantly only in Stage-III and Stage-IV TTTS⁷. Impaired cardiac strain in Stages I and II was not accompanied by fetal echocardiographic findings such as atrioventricular valve regurgitation, and we hypothesized that impaired LV strain may be an early marker of cardiac response in TTTS; the most plausible explanation was that this was secondary to increased afterload in the recipient twin associated with placental production of vasoactive substances, a possible initiating process of TTTS. Secondly, we and others have described shorter filling times in the waveform of the ductus venosus (DV) in recipient twins^{8–10}. DV early filling times were found to be discordant before Quintero staging criteria were satisfied in MCDA twin pregnancies that developed TTTS. Receiver-operating-characteristics-curve analysis confirmed that an intertwin difference in DV early filling times as a percentage of the cardiac cycle (DVeT%) > 3.6% was predictive of the development of TTTS, with 83% sensitivity and 80% specificity⁸. Discordance in DV filling times was not observed in MCDA twin pregnancies complicated only by sIUGR, and this may assist in their differentiation from TTTS¹⁰.

The primary aim of this prospective, blinded study was to confirm whether intertwin discordance in ventricular strain and in DV time intervals predicts TTTS. Secondary aims, using MCDA twin pregnancies which did not develop TTTS ('non-TTTS'), were to create gestational-age ranges for ventricular strain in those without sIUGR and to characterize the relationship of ventricular strain with gestational age in those with sIUGR.

METHODS

This was a prospective cohort study performed in MCDA twin pregnancies undergoing biweekly surveillance at one of seven maternal-fetal medicine satellite clinics within

the Memorial Hermann Hospital System. The obstetric sonographers were experienced in Doppler ultrasound examination of twin pregnancies and, in addition to the anatomy scan at about 20 gestational weeks, obtained monthly biometry and biweekly four-chamber cardiac clips, amniotic fluid indices and umbilical-cord Doppler measurements from presentation onwards throughout pregnancy.

Following institutional review board approval (HSC-MS-14-0632), 150 women with MCDA twin pregnancy within the usual gestational-age range for development of TTTS (between 16 and 26 completed weeks' gestation) were counseled and enrolled consecutively during the period 2015 to 2018. We excluded MCDA twin pregnancies with established TTTS, twin anemia-polycythemia sequence (TAPS) or structural congenital heart defects diagnosed at the first ultrasound examination.

TTTS was defined according to Quintero staging criteria (Table 1)¹. TAPS was diagnosed when there was discordance in the middle cerebral artery peak systolic velocity (MCA-PSV), based on the suggested Leiden protocol, using multiples of the median (MoM), i.e. if the recipient had MCA-PSV < 1 MoM and the donor had MCA-PSV > 1.5 MoM¹¹. Atypical Stage-III TTTS comprised cases in which the amniotic fluid volume met the criteria for TTTS and abnormal Doppler indices satisfied Quintero stage III, but a fetal bladder was still visible in the donor fetus. For the purposes of this study, we defined a MCDA twin pregnancy as 'pre-TTTS' if it was referred for TTTS evaluation, at which it did not satisfy the Quintero staging criteria, but subsequently developed TTTS requiring laser treatment. These pregnancies also underwent fetal echocardiography (echo) and functional measurements, including standard cardiac-valve measurements and Doppler evaluation to assess the combined cardiac output indexed for estimated fetal weight (EFW), tissue Doppler velocities and DV waveform time intervals.

The study endpoint was laser intervention for TTTS. In our center, we offer laser for Quintero stage II or greater. In cases of Stage-I TTTS, we would offer laser for maternal discomfort due to polyhydramnios (usually, maximum vertical pocket > 14 cm) or cervical length < 1.5 cm on transvaginal ultrasound.

To determine the change in RV and LV strain values according to gestational age, from 16 to

26 completed weeks' gestation, we used MCDA pregnancies that did not develop TTTS and were not affected by sIUGR. Non-TTTS MCDA twin pregnancies with isolated sIUGR were analyzed as a separate cohort. We defined sIUGR as: one twin < 10th percentile for EFW, > 25% discordance in EFW, and type I, II or III according to the Barcelona protocol^{12,13}.

Before the study commenced, all sonographers were trained to optimize four-chamber cardiac clips in order to achieve images with good contrast and frame rates as high as possible (aiming for 100 Hz). Most scans were performed using GE Voluson E8 (GE Healthcare, Zipf, Austria) ultrasound machines, equipped with RAB6-D or M6-C probes, and 19 of the 150 pregnancies were examined using a Siemens S2000 (Siemens, Erlangen, Germany) machine, equipped with a 6C2 probe. All data were stored on Viewpoint (GE Healthcare) in DICOM. All DICOM clips were anonymized by a single observer (I.E.A.), with twin pairs separated, and imported into speckle-tracking software (Cardiac Performance Analysis, TomTec, Unterschleissheim, Germany) for offline analysis. Batch measurement of global longitudinal LV and RV strain was performed by two investigators (A.A., H.M.G.), who were blinded to twin pairing and pregnancy outcome. Global peak systolic strain is defined as the relative shortening (as a percentage) of the ventricle during systole and was reported by the software as a negative percentage, but, according to convention, we express this herein as a positive percentage value¹⁴. We use the terms 'impaired' for a less negative result and 'augmented' for a more negative result.

Statistical analysis

Sample size was based on a power calculation from our previous study⁷, to test the hypothesis with 90% power at a significance level of 5%.

Intertwin discordance was calculated as RV or LV strain in heavier/recipient twin minus that in lighter/donor twin. Intertwin discordance in strain was compared between cases that later developed TTTS and those that did not using multilevel modeling with repeated measures within each pregnancy. All models included adjustment for gestational age by main effects or interaction with case/control group when indicated by the hypothesis. Within-gestational-age differences between strain discordance were tested by Wilcoxon's rank sum test. A multivariable, multilevel model (using repeated measures within each pregnancy and repeated measures within each fetus) was fit to determine the association between strain and gestational age. A separate model was fit to determine the association between strain and EFW and the effect of sIUGR on ventricular strain (mixed model with intertwin RV difference as outcome). Non-linear associations with gestational age and EFW were tested for optimal model fit by Akaike information criterion minimization.

Inter-rater reliability was tested in 208 strain measurements (104 LV and 104 RV), made in the first 31 non-TTTS pregnancies by a single investigator (C.W.)

Table 1 Quintero staging criteria¹

Quintero stage	Findings
Stage I	Deepest vertical pocket > 8 cm in one amniotic sac and < 2 cm in other amniotic sac
Stage II	Non-visualization of fetal urinary bladder in donor
Stage III	Doppler abnormalities in donor (IIID) or recipient (IIIR)
Stage IV	Hydrops in one or both fetuses
Stage V	Demise of one or both fetuses

IIID, Stage-III donor; IIIR, Stage-III recipient.

and repeated by a second one 1 year later (A.A.). The second observer was blinded to the initial results and both were blinded to the twin pairing as well as to the pregnancy outcome. In addition, 16 fetuses from non-TTTS pregnancies had more than one four-chamber clip recorded on the same day and these 39 observations were used to estimate within-fetus variation. Intertwin and inter-rater reliability in RV and LV strain measures were calculated by mixed-effects models accounting for repeated measures within each pregnancy and within each twin. The R packages, 'lme4' and 'rmcorr', were used to estimate intertwin and inter-rater correlation. Analyses were carried out using Stata SE 15 (<https://www.stata.com/>) and R version 3.5.2 (<https://cran.r-project.org/>).

RESULTS

Among the 150 MCDA twin pregnancies enrolled consecutively, there were 15 which were pre-TTTS and 135 which did not develop TTTS. We excluded 22 of the latter pregnancies, due to failure to measure strain in both fetuses (seven pregnancies) or failure to measure it in one fetus of a pair (15 pregnancies), at a median gestational age of 19.6 (range, 17–26) weeks' gestation. Thus, paired offline strain measurements were available for intertwin comparison in 128 pregnancies, including 15 which were pre-TTTS and 113 which were non-TTTS (of which 98 were uncomplicated and 15 were affected by isolated sIUGR). One of the TTTS cases was excluded post enrolment (it was referred at TTTS Stage II and enrolled

erroneously, then regressed, but donor strain could not be measured before TTTS re-occurred), leaving 14 pre-TTTS cases.

Table 2 shows the maternal and cohort characteristics of the entire cohort of 127 MCDA pregnancies and the subgroup of the 14 pre-TTTS cases, which included two with secondary TAPS. Of these, 13 had a fetal echo within 2 weeks prior to laser therapy (one of the two TAPS cases did not have echo data); Tables 3 and 4 present their cardiovascular measurements and intertwin differences. There was DVeT% discordance > 3.6% in eight of the 13 cases. The group of 98 non-TTTS, non-sIUGR MCDA twin pregnancies with 196 normally grown fetuses included a total of 292 strain measurements between 16 and 26 completed week's gestation.

Overall, sIUGR was present in 15/113 (13%) non-TTTS and 6/14 (43%) pre-TTTS MCDA twin pregnancies. Intertwin weight discordance in these sIUGR cases was similar for non-TTTS and pre-TTTS MCDA twin pregnancies (median, 30% (range, 21–45%) *vs* median, 35% (range, 31–43%)). Furthermore, two non-TTTS pregnancies had both twins affected by IUGR (EFW < 10th centile) and this was accounted for by multilevel modeling.

There was no difference in the frame rates captured in the four-chamber views between the 83 pre-TTTS clips and the 371 non-TTTS clips (median frame rate, 102 Hz (range, 46–133 Hz) *vs* 96 Hz (range, 28–220 Hz)).

Table 2 Maternal and cohort characteristics for 127 monochorionic diamniotic (MCDA) twin pregnancies, overall and according to gestational age at fetal echo

Characteristic	All	16–26 weeks		16–20 weeks	
		Pre-TTTS	Non-TTTS (without sIUGR)	Pre-TTTS	GA-matched non-TTTS
Pregnancies	127	14	98	11	68
Paired observations*	196	23	146	13	81
sIUGR	21 (17)	6 (43)	0	5 (45)	6 (9)
Maternal age (years)	30 (18–44)	30 (25–42)	30 (18–44)	28 (25–42)	30 (18–44)
Maternal ethnicity					
Asian	7 (6)	0	6 (6)	0	4 (6)
African-American	16 (13)	1 (7)	13 (13)	1 (9)	8 (12)
Hispanic	40 (31)	3 (21)	34 (35)	3 (27)	23 (34)
Caucasian	45 (35)	9 (64)	30 (31)	6 (55)	24 (35)
Other or N/A	19 (15)	1 (7)	15 (15)	1 (9)	9 (13)
Gravidity					
1	39 (31)	5 (36)	29 (30)	5 (45)	23 (34)
2	43 (34)	5 (36)	36 (37)	4 (36)	22 (32)
3	22 (17)	1 (7)	19 (19)	1 (9)	13 (19)
≥ 4	20 (16)	3 (21)	12 (12)	1 (9)	8 (12)
N/A	3 (2)	0	2 (2)	0	2 (3)
Parity					
0	53 (42)	5 (36)	42 (43)	5 (45)	33 (49)
1	44 (35)	6 (43)	35 (36)	5 (45)	22 (32)
2	19 (15)	2 (14)	16 (16)	0	8 (12)
≥ 3	11 (9)	1 (7)	5 (5)	1 (9)	5 (7)

Data are presented as *n*, *n* (%) or median (range). *For both right and left ventricles. GA, gestational age; N/A, not available; pre-TTTS, MCDA twin pregnancies not fulfilling Quintero staging criteria, but which subsequently developed twin–twin transfusion syndrome (TTTS) (including twin anemia–polycythemia sequence) requiring laser treatment; sIUGR, selective intrauterine growth restriction.

Table 3 Cardiovascular parameters at fetal echo (FE) within 2 weeks before laser treatment, in 13 monochorionic diamniotic twin pregnancies referred for evaluation for twin–twin transfusion syndrome (TTTS), which did not satisfy Quintero staging criteria, but subsequently developed TTTS

Case	FE–laser interval (days)	GA (weeks)	Intertwin weight discordance (%)	Twin type	Strain			Z-score						CCOi (mL/kg/min)
					LV	RV	DVeT%	RV e'	RV a'	RV s'	LV e'	LV a'	LV s'	
2	1	21+6	5	Recipient	19.2	18.2	21.0	-2.48*	-0.11	-1.60	-2.13*	-1.49	-3.52*	259
				Donor	19.3	19.6	19.7	-2.48*	-0.11	-1.60	-2.13*	-1.49	-3.52*	259
13	1	23+1	13	Recipient	13.4	12.1	18.6	-0.51	0.27	-0.03	0.52	6.84*	1.24	322
				Donor	17.9	13.8	25.0	-3.25*	0.03	-2.25*	1.19	1.66	0.02	415
5	3	20+1	35	Recipient	24.1	20.5	20.0	-3.64*	-0.37	-2.86*	0.77	1.27	-2.61*	304
				Donor (sIUGR)	19.5	22.6	28.9	-2.69*	-0.03	-1.38		-1.40	-1.85	273
9	5	17+6	34	Recipient	24.8	11.0	22.5	-3.60*	-0.37	-1.31	2.48*	1.69	0.48	307
				Donor (sIUGR)	23.3	25.7	25.6		-0.43	-1.64		-1.93	-2.54*	245
7	6	25+5	43	Recipient	15.5	21.8	21.1	0.15	-0.17	-1.99	1.13	4.58*	1.71	252
				Donor (sIUGR)	12.5	15.6	23.7	-1.94	0.04	0.59	0.55	0.62	-0.47	503
8	6	20+6	7	Recipient	27.8	21.0	22.8	0.75	0.44	3.37*	0.86	4.38*	2.21*	754*
				Donor	28.3	23.2	20.4	3.49*	0.43	4.54*	-0.57	4.38*	0.92	805*
4	5	16+5	13	Recipient	16.0	15.9	20.5	-1.78	-0.07	-1.03	-3.49*	-1.36	-2.63*	243
				Donor	27.2	29.8	28.6	-2.46*	-0.30	-2.87*	-3.49*	-1.36	-2.63*	243
6	7	16+3	37	Recipient	21.6	24.9	17.4	-0.99	-0.10	-2.46*	-2.74*	-0.46	-2.33*	731*
				Donor (sIUGR)	13.1	13.7	24.2	-4.35*	-0.45	-3.22*	-3.16*	0.72	-3.01*	285
11	7	16+2	15	Recipient	25.4	23.2	22.5	-2.17*	-0.07	-1.99	-0.90	-1.96	-3.98*	731*
				Donor	25.1	22.5	29.6	-3.15*	0.08	-1.00	-3.16*	-0.91	-2.84*	389
12	7	17+1	34	Recipient	26.5	18.4	18.0	-2.84*	-0.18	-1.55	-0.27	-1.56	-3.57*	489
				Donor (sIUGR)	17.3	26.8	19.9	-3.05*	-0.25	-2.32*	-2.78*	-1.61	-3.46*	448
14	9	21+6	31	Recipient	18.7	15.5	23.7	-0.94	0.18	-1.48	-3.32*	-0.09	-2.11*	372
				Donor (sIUGR)	10.4	23.1	27.6	-0.18	0.01	-1.42	-1.82	0.07	-0.89	434
1	14	20+5	25	Recipient	11.9	23.7	19.2	-1.25	0.31	-1.03	-1.14	1.87	-1.81	436
				Donor	21.8	26.4	24.0	-2.17*	0.16	-1.59	-0.63	1.52	-1.81	441
10	14	18+2	17	Recipient	17.6	16.1	10.2		0.15	-1.55		-1.36	-6.08*	242
				Donor	17.7	20.7	21.5	-2.28*	-0.11	-2.67*	-4.39*	-3.05*	-4.24*	214*

*Abnormal value (Z-score < -2 or > +2). a', late diastolic velocity; CCOi, combined cardiac output indexed for estimated fetal weight; DVeT%, ductus venosus early filling time (as percentage of cardiac cycle); e', early diastolic velocity; GA, gestational age at FE; LV, left ventricular; RV, right ventricular; s', systolic velocity; sIUGR, selective intrauterine growth restriction.

Table 4 Intertwin discordance and cardiovascular parameters at fetal echo (FE) within 2 weeks before laser treatment, in 13 monochorionic diamniotic twin pregnancies referred for evaluation for twin–twin transfusion syndrome (TTTS), which did not satisfy Quintero staging criteria, but subsequently developed TTTS

Case	GA (weeks)	TTTS stage at laser	FE–laser interval (days)	Intertwin weight discordance (%)	Donor sIUGR	DVeT% discordance*	Strain discordance*	
							LV	RV
2	21+6	Atypical IIID	1	5	No	1.3	0.0	-1.4
13	23+2	III+TAPS	1	13	No	-6.4	-4.5	-1.6
5	20+4	Atypical IIID	3	35	Yes	-8.9	4.6	-2.1
9	18+4	IIID	5	34	Yes	-3.1	1.5	-14.7
7	26+4	II	6	43	Yes	-2.6	3.0	6.3
8	21+5	Atypical IIID	6	7	No	2.4	-0.5	-2.2
4	17+3	III	5	13	No	-8.1	-11.2	-14.0
6	17+3	III	7	37	Yes	-6.8	8.6	11.2
11	17+2	IIID	7	15	No	-7.1	0.3	0.7
12	18+1	IIID	7	34	Yes	-1.9	9.3	-8.4
14	23+1	I	9	31	Yes	-3.9	8.3	-7.6
1	22+5	II	14	25	No	-4.8	-9.9	-2.7
10	20+2	I	14	17	No	-11.3	-0.2	-4.6

*Discordance calculated as: Recipient – Donor. IIID, Stage-III donor; DVeT%, ductus venosus early filling time (as percentage of cardiac cycle); GA, gestational age at laser treatment; LV, left ventricular; RV, right ventricular; sIUGR, selective intrauterine growth restriction.

Intertwin strain discordance

The 81 paired strain measurements in gestational-age-matched non-TTTS MCDA twin pregnancies at 16–20 weeks showed no significant intertwin discordance in mean strain: RV, -0.4 (95% CI, -2.1 to 1.4) and LV, 0.23 (95% CI, -1.1 to 1.5), pre-TTTS cases showed greater, but not significant, variability in mean strain discordance: RV, -2.32 (95% CI, -5.9 to 1.3) and LV, -0.39 (95% CI, -3.1 to 3.9). There was no significant difference in RV or LV strain discordance between pre-TTTS and non-TTTS groups ($P = 0.338$ and $P = 0.932$).

Figure 1 shows intertwin discordance in strain from 13 paired measurements (11 pre-TTTS pregnancies) before 20 completed gestational weeks, at a median of 7 (range, 2–14) days before diagnosis of TTTS, for RV and LV. No statistical analysis was performed because of the small sample size, but strain in the recipient was impaired compared to that of the corresponding donor twin in about half of these cases: 9/13 RV (7/11 pregnancies) and 6/13 LV (6/11 pregnancies).

Figure 2 compares intertwin discordance in strain before 20 completed gestational weeks between the 11

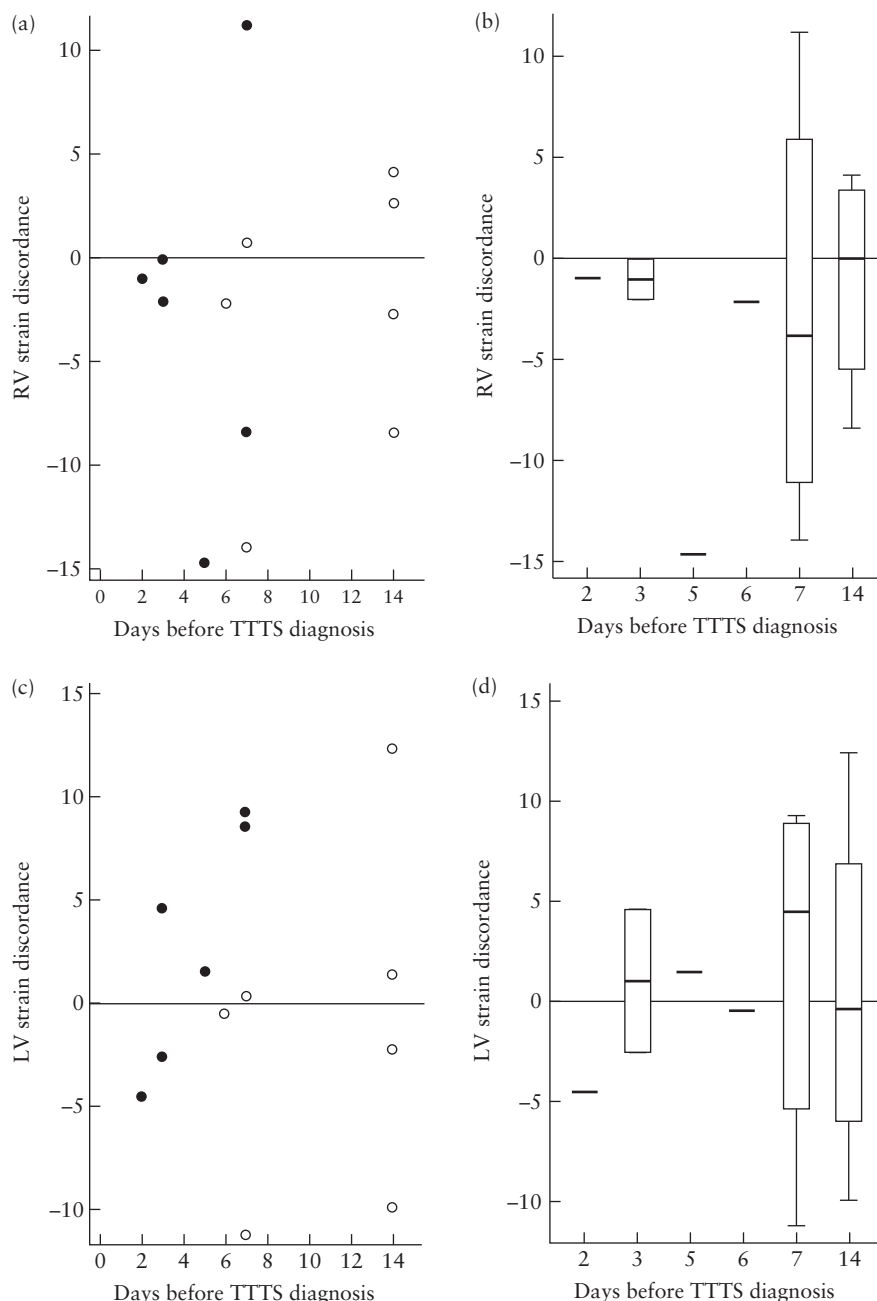


Figure 1 Intertwin right (RV) (a,b) and left (LV) (c,d) ventricular strain discordance before 20 completed gestational weeks, in 11 mono-chorionic diamniotic pregnancies which later developed twin–twin transfusion syndrome (TTTS) (13 observations), according to time interval between measurement and diagnosis of TTTS. (a,c) Scatterplots, showing those with (●) and those without (○) selective intrauterine growth restriction. (b,d) Box-and-whiskers plots, with boxes showing median and interquartile range (IQR), and whiskers showing IQR plus 1.5 × IQR.

pre-TTTS and 68 non-TTTS MCDA twin pregnancies without sIUGR (median gestational age of entire cohort at time of measurement, 19.7 (range 16–20) weeks). No intertwin RV or LV strain discordance was observed at any gestational age among non-TTTS pregnancies, but there were significant differences at some gestational weeks in the pre-TTTS group. Impaired RV strain was observed in the recipient twin at 17 weeks: mean strain difference, -15.32 (95% CI, -22.4 to -8.3) and at 20 weeks: mean strain difference, -2.15 (95% CI, -2.7 to -1.6), with augmented RV strain in the recipient twin at 19 weeks: mean strain difference, 3.36 (95% CI, 2.2 to 4.5). Impaired LV strain was observed in the recipient twin at 18 weeks: mean strain difference, -2.42 (95% CI, -2.7 to -2.1). However, this analysis contained only between two and four pre-TTTS pregnancies at each gestational week and cannot account for the variability and timing of onset of TTTS.

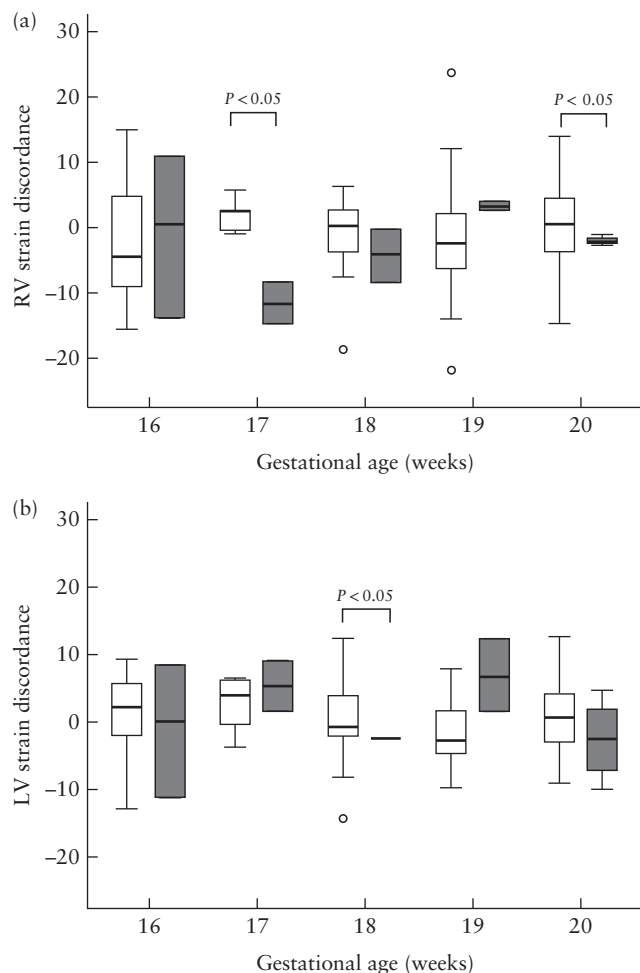


Figure 2 Box-and-whiskers plots, showing intertwin right (RV) (a) and left (LV) (b) ventricular strain discordance before 20 completed gestational weeks, in 11 monochorionic diamniotic (MCDA) twin pregnancies which later developed twin–twin transfusion syndrome (13 observations) (■), compared with 68 that did not (□), according to gestational age. Boxes show median and interquartile range (IQR), and whiskers are IQR plus $1.5 \times$ IQR. Circles are outliers.

Association between strain and gestational age in non-TTTS MCDA twin pregnancy

In the non-TTTS, non-sIUGR group, between 16 and 26 weeks, RV strain showed a strong negative association with gestational age. The association was linear, with an average reduction in RV strain of 0.38 with each additional week of gestation ($P < 0.001$), the decrease being strongest after 19 gestational weeks (Figure 3a). LV strain showed a similar significant but non-linear relationship with gestational age ($P = 0.025$), increasing slightly between gestational ages 16 and 19 weeks but then decreasing after 19 weeks with an average decrease of 0.37 per week of gestation (Figure 3b). The association between strain and EFW is provided in Appendix S1.

sIUGR and strain measurements in non-TTTS MCDA twin pregnancy

Overall, sIUGR was present in 15/113 non-TTTS MCDA twin pregnancies (42 measurements) and we compared these with the 98 that were not affected by sIUGR. LV strain was, on average, higher by 1.83 (augmented) in non-TTTS sIUGR ($P = 0.023$), while RV strain showed

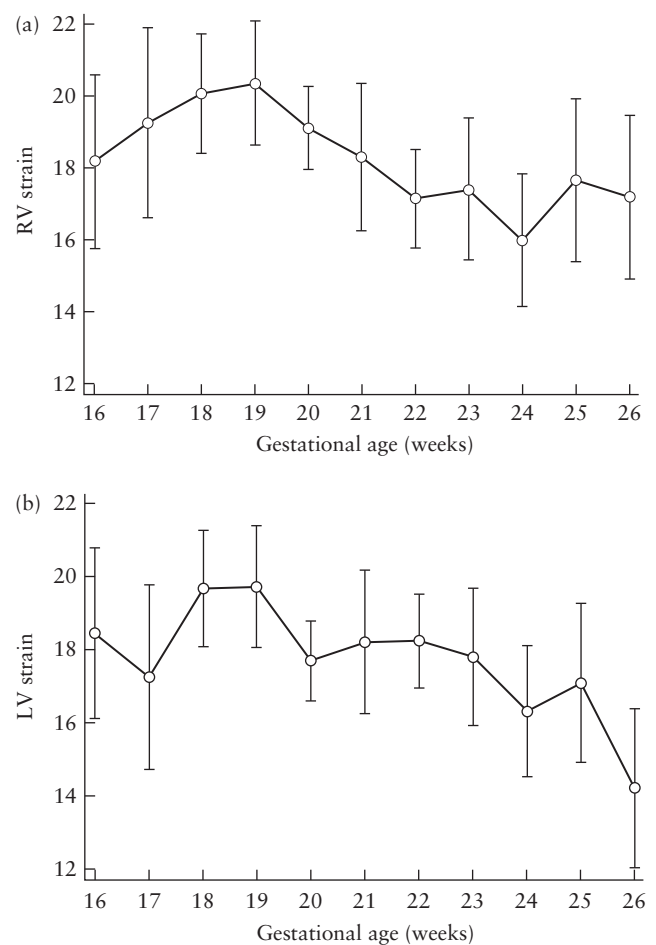


Figure 3 Right (RV) (a) and left (LV) (b) ventricular strain according to gestational age in 98 non-TTTS, non-sIUGR monochorionic diamniotic twin pregnancies, between 16 and 26 weeks' gestation ($n = 292$ observations). Mean and 95% CI are shown.

no statistically significant difference between the groups ($P = 0.271$) (Figure 4). There was no significant intertwin discordance in RV or LV strain in sIUGR non-TTTS pregnancies.

Variability of strain measurements in non-TTTS MCDA twin pregnancy

In the MCDA twin pregnancy non-TTTS group, 16 fetuses from 11 pregnancies had multiple four-chamber clips recorded on the same day, creating a total of 39 paired LV and RV strain observations. The estimated variability in ventricular strain within each twin during the day was high (LV, 12.9 and RV, 19.7). However, within each pair (intertwin variation), variability was low (LV, 2.9 and RV, 5.5). Interclass correlation, reflecting the proportion of total variability represented by the variability between twin pairs, was low (intraclass correlation coefficients: LV, 0.18 and RV, 0.22).

Inter-rater reliability of strain measurements

Overall, both LV and RV strain showed moderate to high inter-rater correlation. LV strain ($r = 0.53$) showed

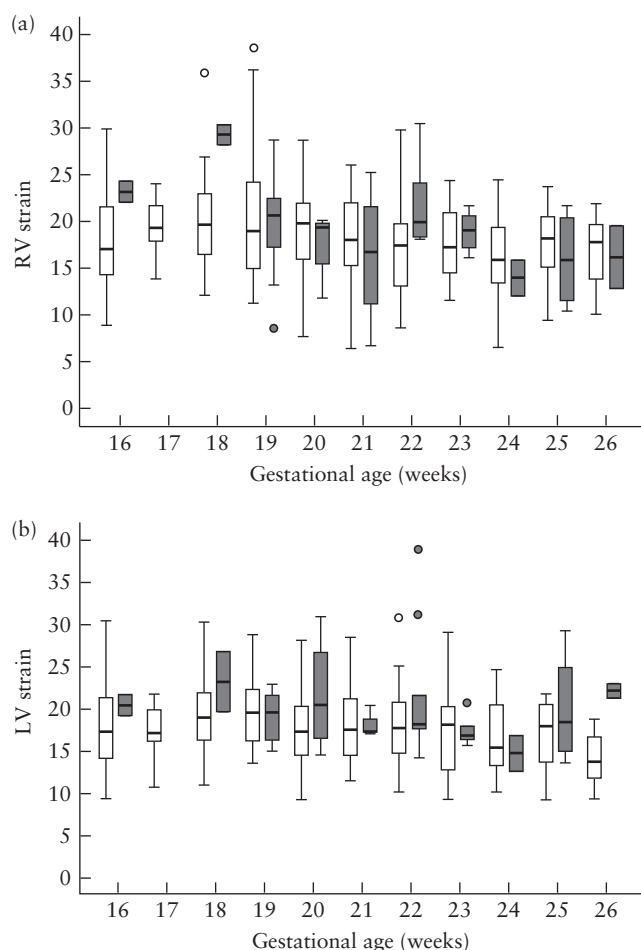


Figure 4 Right (RV) (a) and left (LV) (b) ventricular strain according to gestational age in non-TTTS monozygotic twin pregnancies with (●) and those without (○) selective intrauterine growth restriction. Boxes are median and interquartile range (IQR), and whiskers are IQR plus $1.5 \times$ IQR. Circles are outliers.

higher inter-rater correlation than did RV strain ($r = 0.30$) (Figure 5). Correlation was stronger, particularly for LV strain, after 20 gestational weeks ($r = 0.67$) (Figure 5).

sIUGR and cardiovascular measurements in pre-TTTS pregnancies

Cardiovascular measurements made during fetal echo in The Fetal Center up to 2 weeks prior to laser therapy for 13/14 pre-TTTS pregnancies are given in Table 3, with myocardial velocity Z-scores lying outside the normal range indicated. Intertwin differences are given in Table 4.

The prevalence of sIUGR in pre-TTTS was 43% (6/14 pregnancies). There was absent or reversed end-diastolic flow (AREDF) in the umbilical artery in three of six sIUGR pre-donor fetuses (Cases 5, 6 and 9) and in the DV of two (Cases 4 and 8). No pre-donor satisfied the criteria for Stage-I TTTS at the time that these abnormal Doppler findings were identified. No pre-recipient had hydrops, but there were pulsations in the umbilical artery in Cases 10 and 13, resulting in AREDF in the DV in Case 10. In Case 5, the recipient had mild tricuspid regurgitation and, in Case 6, the recipient had mild mitral regurgitation. Monophasic tricuspid- or mitral-valve inflow Doppler resulted in elevated measurements of late-diastolic myocardial velocities in four fetuses. Intertwin discordance in DVEt% > 3.6% up to 2 weeks prior to laser therapy was evident in eight of 13 pre-TTTS cases (Cases 1, 4, 5, 6, 10, 11, 13 and 14), indicating unbalanced intertwin transfusion (Table 4).

DISCUSSION

Monozygosity in twin pregnancy is characterized by placental anastomoses connecting the circulations of the twins. Twins from uncomplicated MCDA pregnancies, with no apparent functional abnormality, have shown altered vascular responses in childhood, and this has been attributed to fetal vascular programming in response to hemodynamic stressors and placental vasoactive products^{2-5,7-10,15-21}. In contrast, few cardiac sequelae have been reported in childhood survivors of TTTS, despite severe fetal-echo findings, indicating rapid cardiac remodeling once TTTS has been eliminated by laser (or delivery after serial amnioreduction)²²⁻²⁴.

Currently, we know little of the effects of intertwin transfusion and placental influences on cardiac function in normal MCDA twin pregnancies and, before they satisfy the Quintero staging criteria, in those which will go on to develop TTTS. This study characterizes global longitudinal ventricular strain, measured from four-chamber clips collected prospectively during ultrasound surveillance in the usual window for development of TTTS (16–26 completed gestational weeks), to determine if altered strain is an early feature in the development of TTTS.

We observed a gestational age-associated reduction in both RV and LV strain, as is seen in singleton pregnancy²⁵, with no significant intertwin differences

in uncomplicated MCDA twin pregnancies or in those developing sIUGR but not TTTS. The 13–20% variation in strain according to gestational age in MCDA twins is similar to that reported in singletons, and apparently due largely to biological variation, as the inter-rater variability was within 5 units in two-thirds of measurements. Physiological variation in strain in uncomplicated MCDA twin pregnancy was characterized by analysis of 39 four-chamber clips in 16 fetuses recorded on the same day. Strain showed high individual variability, but between pairs there was low variability, suggesting that pairs of twins respond similarly to the intrauterine environment. In contrast, we found an increased, but varying, pattern of intertwin strain discordance in pregnancies that went on to develop TTTS. This could imply that development of unbalanced intertwin transfusion may be associated with early cardiovascular responses and adds to our knowledge of ‘pre-TTTS’ pathophysiology. The impaired ventricular strain observed in our recipient twins supports our previous study reporting impaired cardiovascular strain in recipients and their recovery following laser treatment⁷.

In this study, we have attempted to address the limitations of previous reports, which have had low technical success due to the presence of TTTS, as well as issues such as observers not being blinded to twin pairing and outcome, very low frame-rate clips and the use of singleton pregnancies as controls^{26–28}.

There are certain technical considerations in the measurement of strain that may explain differences between the current and our previous work⁷. It is recognized that absolute values for cardiac function differ between ultrasound machines and software^{29,30}, but not necessarily probes³¹. Furthermore, manual tracing and adjustment of the endocardial border may lead to reader bias in strain measurement. We reduced reader bias by blinding the observer to twin pairing and outcome. However, the offline measurement of strain and lack of automation makes it unwieldy in clinical practice; thus, it remains a research tool and is unlikely to prove useful during TTTS screening.

A fetal echo is performed in all MCDA twin pregnancies referred to The Fetal Center with suspected TTTS.

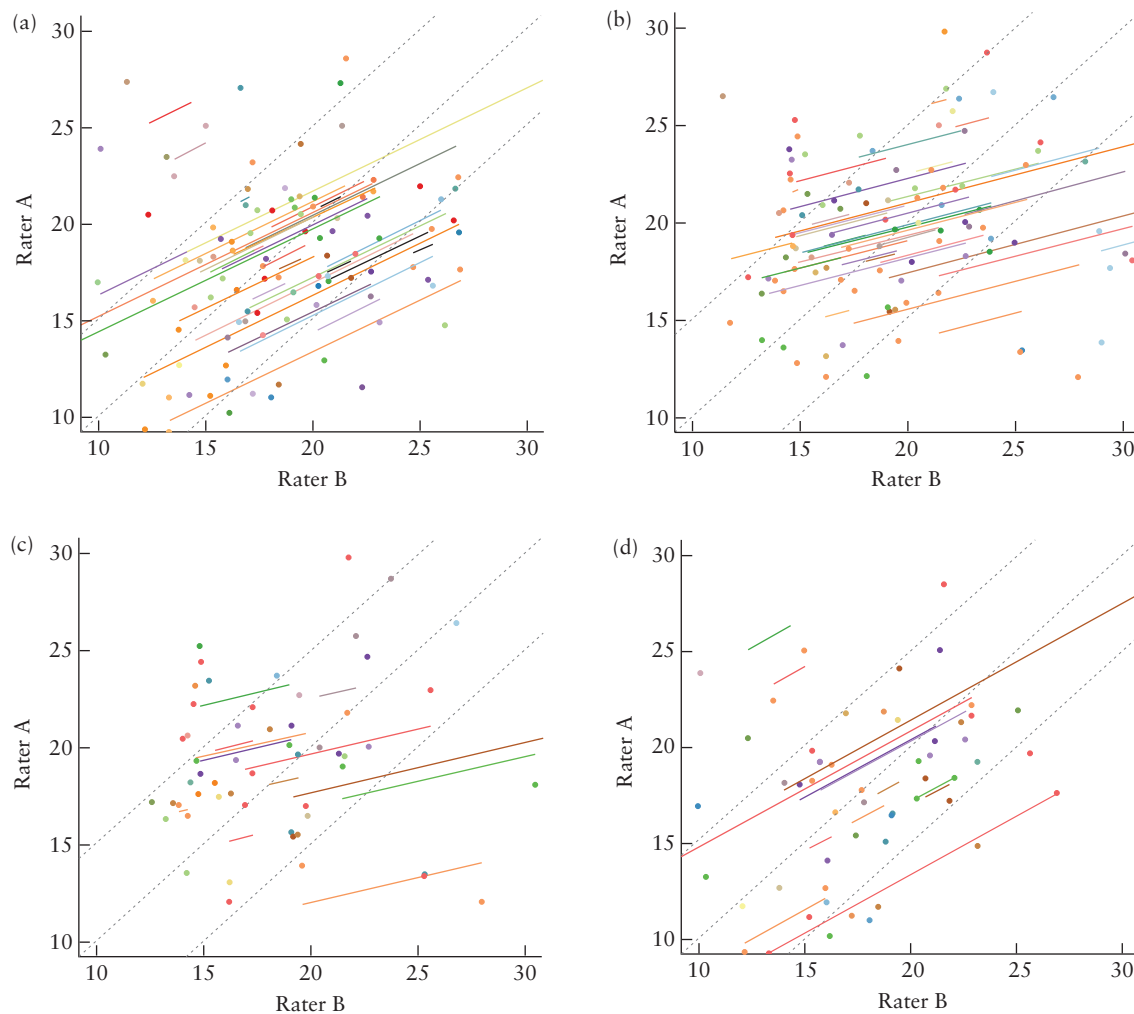


Figure 5 Inter-rater correlation of ventricular strain measurement in monochorionic diamniotic twin pregnancies: (a) left ventricular (LV) strain overall ($r = 0.53$ (range, 0.28 to 0.72)); (b) right ventricular (RV) strain overall ($r = 0.30$ (range, 0 to 0.55)); (c) RV strain after 20 weeks' gestation ($r = 0.35$ (range, -0.28 to 0.77)); (d) LV strain after 20 weeks' gestation ($r = 0.67$ (range, 0.16 to 0.90)). Diagonal lines indicate ± 5 units difference between measures; 68% of all RV strain measures and 67% of all LV strain measures agreed within 5 units.

Our protocol includes DV waveform analysis, tissue Doppler and measures of longitudinal function. These showed reduced early diastolic and systolic myocardial velocities in those pregnancies which went on to develop TTTS. We also confirmed intertwin discordance in DVEt% of > 3.6% in eight of 13 pregnancies which went on to develop TTTS, in agreement with our previous observation that an intertwin discordance in DVEt% of > 3.6% reflects impaired ventricular filling^{4,8}. In contrast, cardiac Doppler waveforms were normal in almost all cases and AREFD in the umbilical artery was seen only in fetuses that became donors and had sIUGR. Abnormality of myocardial velocities and DVEt% support the conclusion that altered intertwin strain values in pregnancies that are 'pre-TTTS' represent true pathophysiology.

In this study we found that LV strain results were significantly augmented in sIUGR non-TTTS fetuses. This intriguing finding is likely linked to reduced placental share and in contrast to the impaired strain reported in singleton IUGR fetuses, which is due to placental disease and maternal vasculopathy^{32,33}.

The potential bias associated with manual assessment of ventricular strain is well recognized. We attempted to minimize this by our study design. It was prospective, and the ultrasound clips were downloaded and anonymized by an administrative member of the team, who assigned a unique identification number so that the investigators measuring strain were blinded to twin pairing and study group. Pregnancy outcome data were not revealed until the study was ready for statistical analysis.

Sonographer training prior to commencement of the study, and the use of similar ultrasound equipment for assessment of most pregnancies, resulted in studies of similar quality from all our clinical centers, and in successful strain analysis similar to that reported in singleton pregnancies and our previous study over a similar gestational-age range²⁵. However, the early gestational age at which TTTS typically develops makes measurement of strain challenging. The improved inter-rater correlation seen after 20 weeks' gestation suggests that measurement of strain may be a less useful predictor of TTTS than is measurement of DVEt%.

We included strain results measured 1 day before laser therapy in one case of TAPS. While the effects of anemia and polycythemia on strain are not known, the intertwin strain differences did not fall outside the confidence intervals of the rest of the cohort and are unlikely to have altered significantly the results presented here. Furthermore, our preliminary data do not show a difference in perinatal outcome between TTTS and TTTS-TAPS cases following laser therapy³⁴.

We do not have additional mechanistic data to help explain the augmented LV strain measured in the non-TTTS fetuses with sIUGR. It is possible that this represents early changes before established brain sparing, although MCA Doppler measurements were normal in all cases. Additionally, color Doppler flow in the aortic isthmus was prograde, but we did not record pulsed Doppler indices³⁵.

Conclusions

While in this blinded, prospective study, we report intertwin discordance in strain in MCDA pregnancies which would go on to develop TTTS, in contrast to the lack of discordance in age-matched uncomplicated MCDA twin pregnancies, the technical constraints of strain measurement cast doubt on the clinical utility of this method in the early diagnosis and management of TTTS at this time. While there is much we do not yet understand because of the limited ultrasound protocols during biweekly monitoring in many countries, this study suggests that DVEt% discordance may be a more practical screening tool in the surveillance of MCDA twin pregnancy.

This study also provides new information on the variation with gestational age, and the biological and technical variation of global longitudinal ventricular strain in uncomplicated MCDA twin pregnancy and those with isolated sIUGR.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:



Appendix S1 Association between strain and estimated fetal weight