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Changes in ductus venosus flow profile in twin–twin transfusion syndrome: role in risk stratification

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KEYWORDS: diamniotic; ductus venosus; monochorionic; twin pregnancy; twin–twin transfusion syndrome

ABSTRACT

Objectives *To evaluate changes in ductus venosus (DV) waveforms and the timing of these changes in twin–twin transfusion syndrome (TTTS), to relate these to disease severity and to assess the clinical applicability of the suggested measurements in the prediction of TTTS.*

Methods *DV time intervals and velocity-time integrals (VTI) normalized to cardiac cycle and total VTI, respectively, as well as velocity ratios were analyzed in 149 monochorionic diamniotic (MCDA) twin pairs. Pregnancies were assigned to the following groups: uncomplicated MCDA (*n = *29); TTTS Stages I+II (*n = *50); TTTS Stages III+IV (*n = *49); and pre-TTTS (*n = *21), of which 14 remained stable and seven progressed to TTTS. Intertwin differences were calculated as larger/recipient minus smaller/donor and related to disease severity. Receiver–operating characteristics curve analysis was used to distinguish TTTS* vs *uncomplicated MCDA and pre-TTTS progressing to TTTS* vs *non-progressing pre-TTTS. Intra- and interobserver reliability of measurement of DV parameters were evaluated using intraclass correlation coefficients (ICCs).*

Results *No intertwin differences in DV parameters were found in uncomplicated MCDA pregnancies. Diastolic VTIs and filling times were significantly shorter in recipient twins in TTTS cases and in larger pre-TTTS twins in comparison with their cotwins. Time intervals, VTIs and velocity ratios correlated significantly with Quintero stages. An intertwin difference in early filling time (eT) normalized to cardiac cycle, eT (%) ≤ −3.6%, could differentiate TTTS from uncomplicated MCDA pregnancies (82.8% sensitivity; 79.8% specificity) and eT (%) ≤ −2.8% predicted progression to TTTS (73.1% sensitivity; 67.4% specificity).*

Conclusions *DV flow profiles and timing of waveform events are already altered in pre-TTTS and early-stage disease, reflecting abnormal ventricular filling and circulatory imbalance. Intertwin comparison of filling times and VTI may allow prediction of evolving TTTS in MCDA pregnancies.*

INTRODUCTION

The ductus venosus (DV) is a unique vessel in the fetal circulation connecting the intra-abdominal umbilical vein to the inferior vena cava at its inlet to the heart[1]. Under physiologic conditions it allows shunting of 20–30% of the oxygen- and nutrient-rich umbilical blood directly into the right atrium, bypassing the hepatic circulation[2,3]. The trumpet-shaped geometry and narrow configuration of the DV cause acceleration of this blood flow, facilitating preferential streaming from the right into the left atrium[1,4–7].

Unlike the umbilical vein, the flow in the DV is phasic and provides information on atrial pressure-volume changes during the cardiac cycle[4]. During atrial relaxation, rapid filling of the atria occurs, which is augmented by the descent of the atrioventricular ring during ventricular systole[8–10] and reflected by an increase in the DV velocity that peaks at the S-wave. Ventricular relaxation results in a decrease in DV velocity during late systole (v-wave). After the opening of the atrioventricular valves, ventricular filling occurs, with blood draining from the atria into the ventricles causing a pressure drop in the atria and resulting in acceleration of blood flow in the DV (D-wave). Finally, atrial contraction augments ventricular filling and the pulse wave is propagated into the DV in a retrograde manner (a-wave)[11]. Assessment of pulsatile flow in the DV using the pulsatility index for veins (PIV) or qualitatively grading flow during atrial contraction (forward/absent/reversed) has become the standard of clinical practice to predict adverse perinatal outcome and to establish the risk of chromosomal anomalies in

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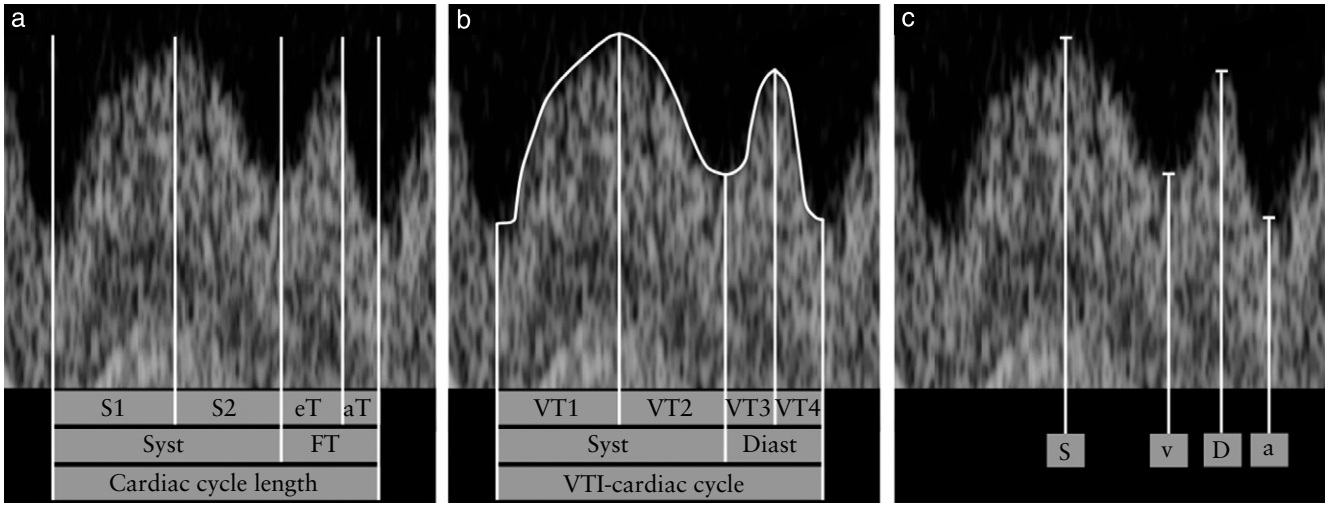

Figure 1 Annotated ductus venosus Doppler waveforms showing acquisition of time intervals, velocity-time integrals (VTIs) and peak velocities. (a) Ductus venosus time intervals. S1, acceleration time during ventricular systole, measured from nadir of a-wave to peak of systole; S2, deceleration time during end-systolic ventricular relaxation, from peak of systole to nadir between systole and diastole; Syst, sum of S1 and S2; eT, early diastolic filling time, from onset of diastolic filling to beginning of fast deceleration caused by atrial contraction; FT, total diastolic filling time, from onset of diastolic filling to nadir during atrial contraction; aT, late diastolic filling time, obtained by subtracting eT from FT. S1 (%) = S1 (ms)/cardiac cycle length (ms), S2 (%) = S2 (ms)/cardiac cycle length (ms), eT (%) = eT (ms)/cardiac cycle length (ms), aT (%) = aT (ms)/cardiac cycle length (ms), FT (%) = FT (ms)/cardiac cycle length (ms), syst (%) = syst (ms)/cardiac cycle length (ms). (b) Ductus venosus VTIs. VT1, acceleration during ventricular systole, measured from nadir of a-wave to peak of systole; VT2, deceleration during systole, measured from peak of systole to nadir; Syst, sum of VT1 and VT2; VT3, acceleration during ventricular diastole, measured from nadir of v-wave to peak of diastole; VT4, deceleration during diastole, measured from peak of diastole to nadir of a-wave; Diast, sum of VT3 and VT4. VT1 (%) = VT1/VTI-cardiac cycle, VT2 (%) = VT2/VTI-cardiac cycle, VT3 (%) = VT3/VTI-cardiac cycle, VT4 (%) = VT4/VTI-cardiac cycle, VT1-syst (%) = syst/VTI-cardiac cycle, VT1-diast (%) = diast/VTI-cardiac cycle. (c) Ductus venosus peak velocities of S-, v-, D- and a-waves.

Table 1 Fetal characteristics of monochorionic diamniotic (MCDA) pregnancies included in the study, according to twin–twin transfusion syndrome (TTTS) status

Characteristic	*Uncomplicated MCDA (n = 29)*	*Pre-TTTS (n = 21)*	*TTTS Stages I + II (n = 50)*	*TTTS Stages III + IV (n = 49)*
Gestational age (weeks)	20.8 ± 1.5	21.8 ± 2.9	21.0 ± 2.7	19.8 ± 2.0
EFW larger twin/recipient (g)	391 ± 114	516 ± 261	441 ± 209	357 ± 151
EFW smaller twin/donor (g)	359 ± 103	424 ± 240	352 ± 172	275 ± 141
EFW discordance*(%)	8.0 ± 6.1	19.4 ± 10.6	21.5 ± 12.5	25.0 ± 12.7

Values are given as mean ± SD. *Discordance calculated as [(larger − smaller)/larger] × 100. EFW, estimated fetal weight.

first-trimester screening[12–15]. However, these indices do not directly reflect mechanical events or their timing during the cardiac cycle[16,17].

Previous studies have shown shortened filling times in the recipients' DV in twin–twin transfusion syndrome (TTTS). Attempts to classify DV velocity patterns have been undertaken in fetuses with altered PIV, including twins complicated by TTTS, but have not shown a specific velocity pattern[18–20].

The aims of this study were to quantify the changes in DV waveforms and timing of events that occur in TTTS, to relate these to disease severity and to assess the clinical applicability of these measurements in prediction of TTTS.

METHODS

This was part of a prospective cohort study enrolling women with monochorionic diamniotic (MCDA) pregnancy referred to The Fetal Center at Children's Memorial Hermann Hospital (UTHealth School of Medicine, Houston, TX, USA) between 1 July 2013 and 31 August 2015.

All women were assessed for complications in MCDA pregnancies, including TTTS, twin anemia–polycythemia sequence (TAPS) and selective intrauterine growth restriction (sIUGR; defined as intertwin estimated fetal weight (EFW) discordance of > 25%) by two-dimensional ultrasound and Doppler evaluation of the fetal cardiovascular system. TTTS was categorized into Stages I–V according to Quintero *et al.*[21]. For further analysis, pregnancies were assigned to one of four groups: uncomplicated MCDA; TTTS Stages I + II; TTTS Stages III + IV; and pre-TTTS, defined as amniotic fluid discordance not explained by anomalies detected on anatomic survey with either oligohydramnios in the smaller twin (maximal vertical pocket (MVP) < 2 cm) or polyhydramnios in the larger twin (MVP > 8 cm), but not meeting the criteria for TTTS

Table 2 Ductus venosus (DV) Doppler parameters of monochorionic diamniotic (MCDA) pregnancies included in the study, according to twin–twin transfusion syndrome (TTTS) status

	Uncomplicated MCDA (n = 29)				*Pre-TTTS (n = 21)*				*TTTS Stages I + II (n = 50)*				*TTTS Stages III + IV (n = 49)*			
Variable	*Larger twin*	*Smaller twin*	Δ	P	*Larger twin*	*Smaller twin*	Δ	P	*Recipient twin*	*Donor twin*	Δ	P	*Recipient twin*	*Donor twin*	Δ	P
Time intervals (%)																
S1	31.2 ± 5.1	31.0 ± 5.1	0.1 ± 6.9	1.0	31.6 ± 6.2	31.1 ± 4.8	0.5 ± 6.9	1.0	30.2 ± 5.6	29.6 ± 4.8	0.6 ± 6.3	1.0	26.5 ± 4.7	29.4 ± 4.3	−2.8 ± 6.7	0.401
S2	34.2 ± 4.5	35.0 ± 4.5	−0.8 ± 5.5	1.0	38.2 ± 5.9	33.7 ± 3.0	4.5 ± 5.6	0.051	40.8 ± 6.0	34.1 ± 4.1	6.7 ± 6.6	< 0.001	44.7 ± 6.6	32.0 ± 4.2	12.7 ± 7.3	< 0.001
Syst	65.4 ± 4.9	66.1 ± 4.1	−0.7 ± 5.9	1.0	69.8 ± 3.7	64.8 ± 5.4	5.0 ± 6.6	0.114	71.0 ± 4.2	63.7 ± 4.9	7.3 ± 5.3	< 0.001	71.3 ± 5.3	61.4 ± 4.9	9.9 ± 6.3	< 0.001
FT	34.6 ± 4.9	33.9 ± 4.1	0.7 ± 5.9	1.0	30.2 ± 3.7	35.2 ± 5.4	−5.0 ± 6.6	0.111	29.0 ± 4.2	36.3 ± 4.9	−7.3 ± 5.3	< 0.001	28.7 ± 5.3	38.6 ± 4.9	−9.9 ± 6.3	< 0.001
eT	25.4 ± 3.9	24.2 ± 3.2	1.2 ± 4.3	1.0	19.5 ± 3.2	26.3 ± 6.2	−6.9 ± 7.3	0.006	18.5 ± 4.5	24.2 ± 3.7	−5.8 ± 4.6	< 0.001	16.8 ± 5.1	27.1 ± 5.2	−10.3 ± 7.5	< 0.001
aT	9.2 ± 2.6	9.7 ± 3.0	−0.5 ± 3.4	1.0	10.8 ± 2.7	8.9 ± 7.0	1.9 ± 7.4	1.0	10.5 ± 3.0	12.1 ± 3.8	−1.6 ± 4.1	0.519	11.9 ± 5.2	11.5 ± 6.6	0.4 ± 8.4	1.0
VTIs (%)																
VT1	29.2 ± 5.3	29.7 ± 5.0	−0.5 ± 7.1	1.0	32.4 ± 6.3	29.9 ± 5.6	2.5 ± 8.8	1.0	29.5 ± 4.3	29.5 ± 5.1	0.0 ± 6.0	1.0	24.9 ± 5.0	29.0 ± 4.7	−4.2 ± 7.5	0.037
VT2	38.5 ± 5.1	38.8 ± 4.4	−0.4 ± 6.0	1.0	40.4 ± 7.0	36.0 ± 3.7	4.3 ± 7.6	1.0	45.5 ± 6.7	37.1 ± 4.4	8.4 ± 7.4	< 0.001	52.1 ± 8.8	34.5 ± 5.3	17.6 ± 9.9	< 0.001
VT3	15.6 ± 2.9	15.1 ± 3.1	0.5 ± 3.6	1.0	13.1 ± 2.4	16.9 ± 3.4	−3.8 ± 4.3	0.040	12.8 ± 3.5	16.0 ± 3.1	−3.2 ± 4.6	0.001	11.5 ± 4.5	17.2 ± 2.9	−5.7 ± 5.1	< 0.001
VT4	16.7 ± 3.3	16.3 ± 3.0	0.4 ± 4.0	1.0	14.1 ± 2.9	17.1 ± 3.6	−3.0 ± 3.8	0.166	12.3 ± 2.7	17.4 ± 3.8	−5.1 ± 4.1	< 0.001	11.6 ± 3.2	19.3 ± 3.7	−7.7 ± 4.0	< 0.001
Syst	67.7 ± 5.3	68.6 ± 4.1	−0.9 ± 5.6	1.0	72.8 ± 4.0	66.0 ± 6.2	6.8 ± 6.8	0.019	75.0 ± 5.1	66.6 ± 5.4	8.3 ± 6.2	< 0.001	76.9 ± 6.3	63.5 ± 4.9	13.5 ± 6.4	< 0.001
Diast	32.3 ± 5.3	31.4 ± 4.1	0.9 ± 5.6	1.0	27.2 ± 4.0	34.0 ± 6.2	−6.8 ± 6.8	0.019	25.0 ± 5.1	33.4 ± 5.4	−8.3 ± 6.2	< 0.001	23.1 ± 6.3	36.5 ± 4.9	−13.5 ± 6.4	< 0.001
Velocity ratios																
S/v	1.42 ± 0.23	1.63 ± 0.77	−0.22 ± 0.74	1.0	1.55 ± 0.30	1.62 ± 0.35	−0.06 ± 0.44	1.0	1.52 ± 0.20	1.56 ± 0.38	−0.04 ± 0.42	1.0	1.88 ± 0.94	1.63 ± 0.52	0.25 ± 0.08	0.399
v/D	0.80 ± 0.08	0.73 ± 0.12	0.06 ± 0.13	0.529	0.75 ± 0.12	0.70 ± 0.14	0.05 ± 0.18	1.0	0.79 ± 0.09	0.74 ± 0.15	0.05 ± 0.18	0.904	0.76 ± 0.17	0.73 ± 0.15	0.03 ± 0.22	1.0
D/a	2.59 ± 1.33	2.76 ± 1.43	−0.17 ± 2.01	1.0	4.74 ± 9.84	5.86 ± 8.62	−1.11 ± 13.47	1.0	3.40 ± 2.00	5.25 ± 8.15	−1.85 ± 8.06	1.0	27.82 ± 20.84	8.72 ± 14.59	19.1 ± 27.33	0.004
S/D	1.11 ± 0.08	1.13 ± 0.14	−0.01 ± 0.14	1.0	1.13 ± 0.11	1.09 ± 0.12	0.04 ± 0.14	1.0	1.19 ± 0.12	1.11 ± 0.10	0.08 ± 0.14	0.040	1.31 ± 0.22	1.11 ± 0.08	0.20 ± 0.21	< 0.001
S/a	2.92 ± 1.67	3.21 ± 2.10	−0.29 ± 2.67	0.991	5.57 ± 11.62	6.07 ± 7.97	−0.50 ± 14.37	1.0	4.11 ± 2.65	5.83 ± 8.97	−1.73 ± 8.91	1.0	34.39 ± 25.89	9.73 ± 16.02	24.66 ± 32.40	0.001
v/a	1.98 ± 0.72	1.93 ± 0.79	0.05 ± 1.14	1.0	3.55 ± 7.56	3.61 ± 4.61	−0.06 ± 8.89	1.0	2.68 ± 1.56	3.42 ± 4.56	−0.75 ± 4.60	1.0	23.10 ± 18.26	5.46 ± 8.40	17.64 ± 21.32	< 0.001

Data presented as mean ± SD. DV time intervals are normalized to cardiac cycle length, and velocity-time integrals (VTIs) are expressed as percentage of total VTI of one cardiac cycle. Holm–Bonferroni correction was applied for multiple comparisons, with $P < 0.05$ considered statistically significant. Definitions of each parameter are provided in Figure 1. Δ, intertwin difference; Diast, diastolic; Syst, systolic.

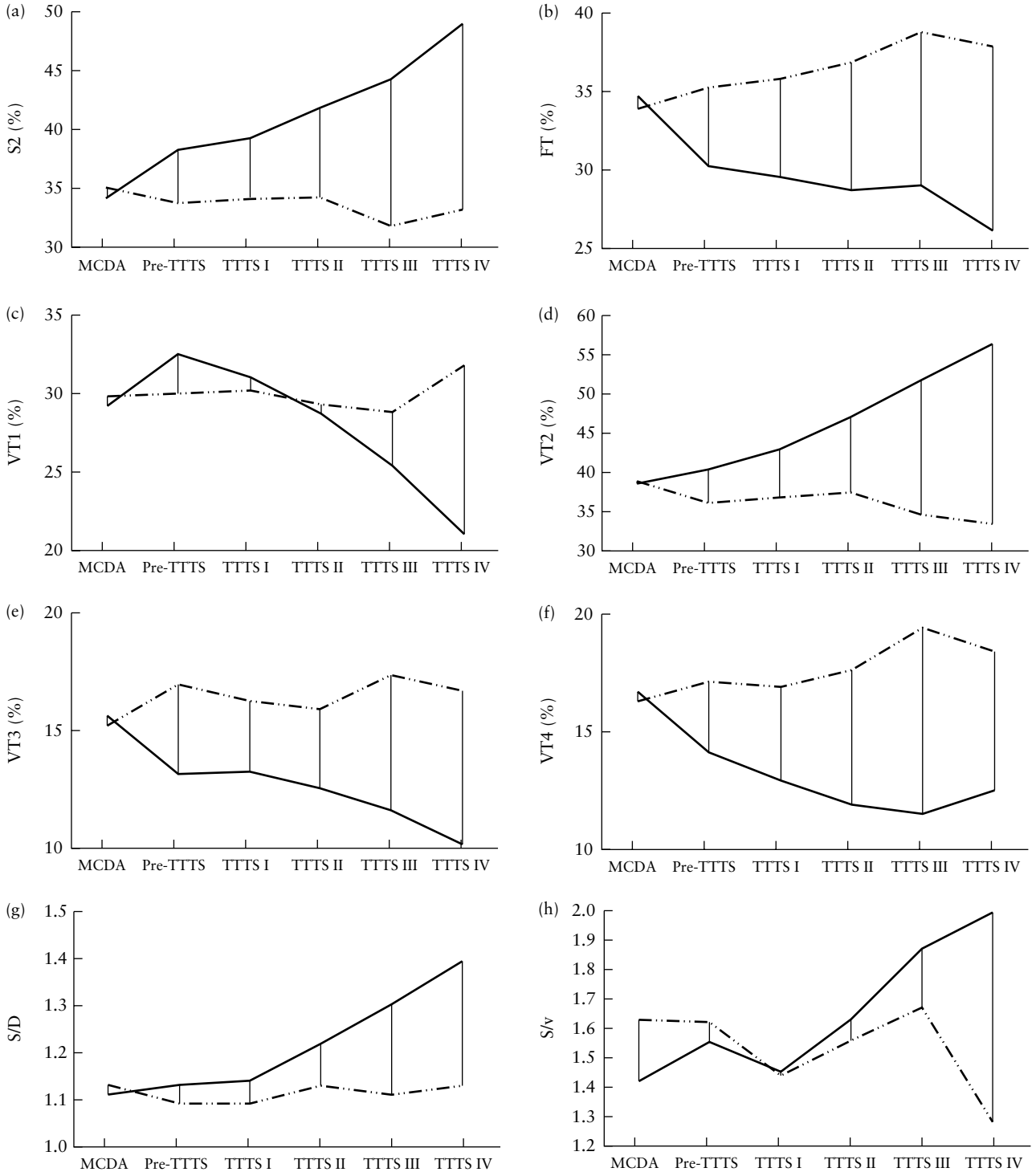

Figure 2 Graphs displaying association of intertwin differences in ductus venosus (DV) Doppler parameters with Quintero staging. (a) Deceleration time during end-systolic ventricular relaxation (S2); (b) total diastolic filling time (FT); (c) velocity-time integral (VTI) of acceleration during ventricular systole (VT1); (d) VTI of deceleration during systole (VT2); (e) VTI of acceleration during ventricular diastole (VT3); (f) VTI of deceleration during diastole (VT4); (g) S/D-wave peak velocity ratio; (h) S/v-wave peak velocity ratio. Mean ductus venosus parameters in recipient/larger (——) and donor/smaller (– · · – · ·) twins and average intertwin difference (vertical lines) in uncomplicated monochorionic diamniotic (MCDA) pregnancies and those complicated by twin–twin transfusion syndrome (TTTS).

Stage I or sIUGR. In women with serial ultrasound examinations only the first was used, but outcomes were known for all and those with pre-TTTS were subsequently assigned to one of two groups: pre-TTTS with progression; or pre-TTTS remaining stable. Only patients with adequate images from both fetuses were included in the analysis. Fetuses with sIUGR were excluded; the karyotype was usually not known. There were no maternal exclusion criteria and all TTTS patients included were studied before selective fetoscopic laser

Table 3 Inter- and intraobserver reliability of ductus venosus (DV) Doppler parameters as evaluated in 20 fetuses (10 twin pairs)

Parameter	*Interobserver ICC*	*Intraobserver ICC*
Time intervals		
S1 (%)	0.689 (0.194–0.878)	0.707 (0.281–0.883)
S2 (%)	0.820 (0.544–0.929)	0.836 (0.341–0.945)
Systolic (%)	0.917 (0.791–0.967)	0.889 (0.725–0.956)
FT (%)	0.917 (0.791–0.967)	0.889 (0.725–0.956)
eT (%)	0.901 (0.731–0.962)	0.956 (0.876–0.983)
VTIs		
VT1 (%)	0.686 (0.188–0.877)	0.639 (0.135–0.854)
VT2 (%)	0.867 (0.666–0.947)	0.878 (0.443–0.961)
VT3 (%)	0.685 (0.225–0.874)	0.801 (0.489–0.922)
VT4 (%)	0.672 (0.206–0.868)	0.667 (0.182–0.867)
Systolic (%)	0.914 (0.784–0.966)	0.874 (0.688–0.950)
Diastolic (%)	0.914 (0.784–0.966)	0.874 (0.688–0.950)
Velocity ratios		
S/v	0.876 (0.678–0.951)	0.797 (0.495–0.919)
v/D	0.882 (0.679–0.955)	0.744 (0.365–0.898)
D/a	0.995 (0.988–0.998)	0.973 (0.932–0.989)
S/D	0.700 (0.244–0.881)	0.774 (0.418–0.911)
S/a	0.995 (0.987–0.998)	0.979 (0.947–0.991)
v/a	0.997 (0.993–0.999)	0.990 (0.975–0.996)

Inter- and intraobserver reliability of DV time intervals normalized to cardiac cycle length, and velocity-time integrals (VTIs) expressed as percent of total VTI. Definitions of each parameter are provided in Figure 1. ICC, intraclass correlation coefficient.

photocoagulation. The study protocol was approved by the Institutional Review Board of UTHealth School of Medicine (HSC-MS-15-0328).

Images were obtained prospectively during routine fetal echocardiography of all MCDA pregnancies using a GE Voluson E8 ultrasound machine (GE Healthcare, Zipf, Austria) with a RAB6-D or an M6-C probe. The DV was assessed in a mid-sagittal plane, optimized for a high signal-to-noise ratio and sweep speed set to display four to six uniform consecutive waveforms. DV Doppler tracings were exported to a cardiac image-analysis software package (Image Arena; TomTec, Unterschleissheim, Germany) and analyzed offline. These measurements included: DV time intervals during systole (S1, S2), the diastolic filling time (FT), early diastolic filling time (eT) and late diastolic filling time (aT); velocity-time integrals (VTI) during systole (VT1, VT2) and diastole (VT3, VT4); and peak velocities during ventricular systole (S-wave), ventricular end-systole (v-wave), ventricular diastole (D-wave) and atrial systole (a-wave) (Figure 1). To account for intertwin heart rate differences, time intervals and VTI were normalized to cardiac cycle length and total VTI. The S/v, v/D, D/a, S/D, S/a and v/a ratios were calculated from the four measured absolute peak velocities. In fetuses with reversed flow during atrial contraction (negative a-wave), the absolute value of the negative velocity of the a-wave was added to the actual value obtained for the S-, v- and D-waves, and divided by 1, as described previously[20]. Intertwin-pair differences were calculated as larger or recipient twin minus the smaller or donor twin.

Table 4 Area under receiver–operating characteristics curve for intertwin differences of ductus venosus (DV) time intervals, velocity-time integrals (VTIs) and S/D velocity ratio in predicting twin–twin transfusion syndrome (TTTS) status of monochorionic diamniotic (MCDA) twin pregnancies

Parameter	*TTTS* vs *uncomplicated MCDA*	*Pre-TTTS progression + TTTS Stage I* vs *uncomplicated MCDA + pre-TTTS stable*
Time intervals		
S2 (%)	0.866	0.674
Systolic (%)	0.867	0.729
FT (%)	0.867	0.729
eT (%)	0.893	0.730
VTIs		
VT2 (%)	0.877	0.694
VT3 (%)	0.795	0.695
VT4 (%)	0.870	0.699
Diastolic (%)	0.911	0.760
Velocity ratio		
S/D	0.739	0.605

Intertwin differences of time intervals normalized to cardiac cycle length, and velocity-time integrals (VTIs) expressed as percentage of total VTI. Definitions of each parameter are provided in Figure 1.

Inter- and intraobserver variability was tested in 20 additional fetuses (10 twin pairs) including uncomplicated MCDA pregnancies and TTTS cases. Three separate DV recordings were acquired during the same ultrasound examination; analysis was carried out twice by one investigator (interobserver variability) and once by a second investigator (intraobserver variability) using the same methodology as described above.

Statistical analysis

Analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA). To minimize possible confounders, each twin-pair functioned as its own control. Intertwin differences were assessed using the Wilcoxon signed rank test, and differences between two independent groups were analyzed using the Mann–Whitney *U*-test. The Kruskal–Wallis test was used for comparisons of more than two groups. Correlations were quantified using Spearman's correlation coefficient (ρ). Receiver–operating characteristics (ROC) curves were analyzed to determine optimal cut-off values. Inter- and intraobserver reproducibility were evaluated using intraclass correlation coefficients (ICCs). A value of $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Pregnancy demographics

A total of 193 sequential MCDA pregnancies presented to The Fetal Center during the study period, of which 149 (77.2%) were included in the analysis. Twenty-seven (14.0%) were excluded because they had no pre-laser echocardiogram, three (1.6%) were excluded because DV

waveforms were of poor quality in one or both twins and 14 (7.3%) cases were excluded because the pregnancy was complicated by sIUGR.

One-hundred and forty-nine paired observations of the DV were analyzed in 298 fetuses: 29 uncomplicated MCDA pregnancies acted as controls and 21 pregnancies were classified as pre-TTTS (14 twin pairs remained stable and seven progressed to TTTS; the time interval from the measurement to development of TTTS was 12 ± 6 days). Ninety-nine pregnancies had a diagnosis of TTTS. The disease severity according to Quintero stages was distributed as follows: TTTS Stage I, $n = 19$; TTTS Stage II, $n = 31$; TTTS Stage III, $n = 44$; TTTS Stage IV, $n = 5$. Fetal characteristics are shown in Table 1. The mean gestational age at echocardiography was 20.6 ± 2.5 weeks and was lower in the TTTS Stage III + IV group compared with the pre-TTTS group ($P = 0.030$). The mean EFW discordance was $21.2 \pm 13.4\%$ and it was significantly higher in TTTS and pre-TTTS pregnancies compared with uncomplicated MCDA pregnancies ($P < 0.01$).

Ductus venosus alterations

Reversal of flow in the DV (negative a-wave) was present in 26 (59.1%) of 44 recipients and in eight (18.2%) of 44 donors with TTTS Stage III and in three (60.0%) of five recipients with TTTS Stage IV. DV time intervals (%), VTIs (%) and peak velocity ratios for the larger/recipient twin and the smaller/donor twins and the corresponding intertwin difference in each group are shown in Table 2. There was a significant correlation between Quintero stages and the intertwin difference in S2 ($\rho = 0.444$, $P < 0.001$), FT ($\rho = -0.221$, $P = 0.028$), eT ($\rho = -0.386$, $P < 0.001$), VT2 ($\rho = 0.521$, $P < 0.001$), VT3 ($\rho = -0.258$, $P = 0.010$), VT4 ($\rho = -0.321$, $P = 0.001$) and diastolic VTI (VT-diast, $\rho = -0.358$, $P < 0.001$). Similarly, recipient S/D ratio ($\rho = 0.321$, $P = 0.001$) and S/v ratio ($\rho = 0.214$, $P = 0.034$) correlated with Quintero staging. The mean values and intertwin differences for S2, FT, VT1, VT2, VT3, VT4, S/D and S/v were associated with disease severity, as shown in Figure 2. Estimated fetal weight did not correlate with DV time intervals (%) and VTIs (%) in the study cohort ($n = 298$ fetuses) or in the uncomplicated MCDA fetuses alone ($n = 58$). A negative association between gestational age and a-wave dependent DV ratios was observed: D/a ($\rho = -0.324$, $P < 0.001$); S/a ($\rho = -0.316$, $P < 0.001$); v/a ($\rho = -0.372$, $P < 0.006$).

Reproducibility

Intraclass correlation coefficients for inter- and intraobserver variability for the parameters obtained are shown in Table 3.

Clinical applicability

ROC curve analysis was conducted to determine the clinical applicability of altered DV physiology (Table 4). Normalization of DV time intervals as a percentage of the cardiac cycle universally improved the area under the ROC curve (AUC) and was superior to absolute time intervals in milliseconds.

The best AUC to differentiate between TTTS and uncomplicated MCDA twin pregnancies was the VTI (%) during diastole at 0.911. Cut-off values for this variable, and for the early (eT (%)) and complete (FT (%)) filling times normalized to the cardiac cycle were calculated to differentiate between groups of pregnancies. In Table 5 we report cut-off values with their sensitivity and specificity for TTTS cases *vs* uncomplicated MCDA pregnancies, and for pre-TTTS cases that progressed grouped with TTTS Stage I cases ($n = 26$) *vs* uncomplicated MCDA twins combined with pre-TTTS cases that did not progress ($n = 43$).

Table 5 Performance of best predictors for differentiation of twin–twin transfusion syndrome (TTTS) status of monochorionic diamniotic (MCDA) pregnancies

Parameter	*Cut-off (%)*	*Sensitivity (%)*	*Specificity (%)*
TTTS *vs* uncomplicated MCDA			
VTI-diastolic (%)	≤ −3.7	79.3	85.9
eT (%)	≤ −3.6	82.8	79.8
FT (%)	≤ −4.6	79.3	77.8
Progression *vs* non-progression*			
VTI-diastolic (%)	≤ −3.3	73.1	60.5
eT (%)	≤ −2.8	73.1	67.4
FT (%)	≤ −3.5	69.2	60.5

Cut-off values are calculated as larger/recipient twin minus smaller/donor twin. Time intervals are normalized to cardiac cycle length, and velocity-time integrals (VTIs) are expressed as percentage of total VTI. *Pre-TTTS progression + TTTS Stage I *vs* uncomplicated MCDA + pre-TTTS stable. eT (%), early diastolic filling time; FT (%), total diastolic filling time; VT-diast (%), diastolic velocity-time integral.

DISCUSSION

In TTTS, placental vascular anastomoses allow a net transfer of fluid from the donor to the recipient[22]. This has an important effect on the venous compartment and alters loading of the heart. In the present study we measured time intervals, absolute velocity ratios and VTIs, which account for both alterations in timing and relative velocities, to characterize changes in DV physiology. We found that even in early TTTS there is an alteration in time intervals and VTIs of the DV flow profile, thus providing new insight into the disease pathophysiology and allowing risk stratification in emerging TTTS.

We have shown that with increasing TTTS severity, there is a relative prolongation of the systolic component of the DV. This can be attributed to the prolongation observed in late systole, expressed as higher values of S2 (%) and VT2 (%). The diastolic phase of the DV is reduced, resulting in a relative decrease in early and total filling times (eT (%), FT (%)) and in the VTI during this period (VT3 (%), VT4 (%)). During

fetal life, the ventricles operate near the limit of the Frank–Starling function curve under physiologic conditions and thus have a limited capability to increase stroke volume with increasing filling pressures[1]. It is likely that the prolongation of the systolic component in the recipients' DV is caused by increased ventricular end-systolic pressure and impaired ventricular relaxation in TTTS. These findings are in agreement with previous observations reporting shortening of diastolic filling times[18,19] and prolongation of late systole[19]. We add to these previous observations by first demonstrating the relationship with disease severity and second showing that early signs of evolving congestive heart failure are apparent in the recipients' DV flow profile in pre- and early TTTS. Absolute velocity ratios were only altered in advanced TTTS. There was a relative decrease in forward flow during early diastole and ventricular relaxation before the opening of the atrioventricular valves compared with ventricular systole (higher S/D and S/v ratios); findings that are also most likely to reflect increased ventricular end-systolic pressure impairing ventricular filling[20].

Our ROC curve analyses confirm that intertwin pair differences in DV measurements can distinguish uncomplicated MCDA from cases of TTTS. However, we have also demonstrated that significant alterations in DV pathophysiology already exist in pregnancies with prestage disease presenting with essential features of TTTS, but not yet meeting criteria for Quintero Stage I[21]. When we compared uncomplicated MCDA pregnancies and pre-TTTS cases that did not progress to TTTS with prestage cases that ultimately developed TTTS and Stage I cases, the best differentiating parameter was the early diastolic filling time (eT (%)). The VTI accounts for alterations in both timing and relative velocities at discrete phases during the cardiac cycle and showed a good correlation with Quintero stages. However, as relative velocities were only altered later in disease, VTIs have better AUC, sensitivity and specificity in differentiating uncomplicated MCDA pregnancies from TTTS cases, but did not improve the sensitivity and specificity in risk stratification of pre-TTTS cases. Both time intervals and VTIs can be obtained easily using routine obstetric ultrasound equipment and demonstrated good inter- and intraobserver reproducibility.

A limitation of this study is that there is no uniform definition of 'pre-TTTS'. It is accepted that the pathogenesis of TTTS is based on placental vascular anastomoses that allow net transfer of blood from the donor to the recipient[23]. However, these anastomoses are present in almost all MCDA placentae and allow balanced intertwin transfusion[22]. They change in size with advancing gestation and spontaneous resolution of early-stage TTTS has been reported[24,25]. Therefore, early prediction of disease progression will remain challenging.

We studied sequential cases presenting to The Fetal Center during this study period, provided that they had a pre-laser echocardiogram, the DV waveforms were of good quality in both twins and the pregnancy was not complicated by sIUGR. We have documented the reasons for excluding 44 (22.8%) pregnancies and believe that the final study cohort does not introduce important selection bias.

In order to allow calculation of cut-off values with an appropriate sample size we grouped prestage cases with uncomplicated MCDA pregnancies and prestage cases that progressed with TTTS Stage 1. This may limit the application of our findings to pre-TTTS cases.

TTTS is characterized by transfer of important vasoactive substances and volume shifts from the donor to the recipient. Early alteration of ventricular filling causes distinct changes in the DV with relative shortening of filling times in the recipient compared with its donor cotwin. These alterations occur early in the pathogenesis of TTTS and may allow prediction of evolving TTTS in MCDA pregnancies. We recommend the inclusion of DV time intervals in the routine surveillance of MCDA pregnancies.

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