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Placental pathology findings and birth weight discordance

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Summary

Purpose: To assess the fetal, perinatal, and maternal outcomes in twin pregnancy according to chorionicity. *Materials and Methods:* This was a retrospective cohort study of 1,571 twin pregnancies with placental pathological examination collected from 2000-2010. Fetal, neonatal, and maternal outcomes of twins were compared via multivariate analysis. *Results:* Placenta anastomosis, unequal placenta sharing, cord size, and cord insertion type were found to be the key elements that impacted growth discordance in twin gestations. Higher rates of severe growth discordance were negatively associated with higher frequencies of anastomosis. Placentas in monochorionic twins were more likely to have shared arteries/veins. *Discussion:* Monochorionic placentas compensate for lack of nutritional flow by penetrating to other placenta surfaces. Compensation for lack of vascular sufficiency would mean a fused placenta or sharing more portions of the placenta. Higher rates of unequal placenta sharing among growth discordant twins is reported irrespective of chorionicity. *Conclusion:* Attention to placenta pathology is important in growth discordant twins.

Key words: Twin; Placenta; Chorionicity; Growth discordance.

Introduction

Independent of gestational age at delivery, twins with significant growth discordance have poorer perinatal outcomes [1-3]. The pathophysiology of birth weight discordance (BWD) has been studied broadly. In monochorionic twins (MC) twins, significant statistical differences are attributed to hemodynamic factors such as twin to twin transfusion syndrome (TTTs) [4], unequal placenta sharing [5], and placenta cord insertion [6]. In dichorionic twins (DC) twins a pathological entity or variation in genetic makeup of twins is assumed [7]. Placenta examinations, gross and microscopic, are useful to uncover the nature of intrauterine impairment of twins' growth. The authors aimed to assess the pathological characteristics of placenta and cord in relation to BWD in twin gestations.

Materials and Methods

Data was collected from pathological records, retrospectively. Here, the authors briefly describe how the records were obtained. Placentas were collected and labeled as A (1 cord clamp) or B (2 cord clamps) according to birth order. Placentas were placed in plastic bags after delivery and kept at 4°C until processed, usually within 24 hours of delivery. Placenta examination was carried out in the pathology department of the Children and Women (C&W) hospital in British Columbia, Canada. Pathologists who examined the placentas had access to the clinical information. The placentas were placed on a clean surface, adherent clots were removed, and the membranes and umbilical cords were excised before they were weighed. There was a systematic approach to attribute placental mass to each twin so that the total placental weight was recorded for each placenta. In DC placentas with fused placentas, the proportion of placenta belonging to each twin was determined by measuring the length, width, and thickness in each of the two placental disks. Measurement of placental thickness was carried out in three areas of the placental disk, and the mean thickness was then recorded. Cord length and its distances from the placenta margin, from the membrane and from the other cord were also measured. Umbilical cord insertions into the disc of the placenta and more than one cm away from the marginal border were defined as (para) central, cord insertions within one cm of the disc edge were defined as marginal and cord insertions directly into the membranes were defined as velamentous. The number of vessels in the cord was recorded. A composite variable was created from cord properties inclusive of cord prolapse, number of cord vessels less than three, and existence of cords knots or entanglements.

The evaluation of placental chorionicity was performed by examination of the inter-twin membrane. Separated twin placentas were examined in the same way as those of singletons. Fused placentas can be MC or DC. The dividing membrane was examined to identify chorionicity. The dividing membrane in a MC pregnancy is thin and translucent without any chorionic layer, while that of a DC placenta is thicker as it contains two chorionic layers between the amniotic sacs. The dividing membrane was then sampled as a membrane roll or in "T section" form. Identification of T form was considered confirmation of chorionicity.

Equal placental sharing was defined as 40% to 60% of the placenta attributed to each twin. The authors chose this range because preliminary data revealed that twins with 40/60 sharing and 50/50 sharing had similar gestational ages at delivery and degree of BWD [8]. Unequal placental sharing was defined as one twin receiving blood from more than 60% of the placenta.

In histological examination of the placenta, vascular-thrombotic lesions (infarction, chorangioma, subchorial fibrin deposition, and retro placental hematoma) were recorded. Arteries were identified as vessels that are situated superficial in relation to the veins. A composite score was created for all of the pathological lesions

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for the purpose of analysis.

Anastomosis between fetal vessels was recorded. Anastomosis was identified by the presence of an impaired vessel from one twin feeding an area drained by the co-twin. Injection studies were performed in fresh specimen to identify unidirectional arteriovenous shunt(s) between donor and recipient. Placentas were also assessed for maternal or fetal inflammatory response corresponding to chorionitis, chorioamnionitis, and chorionic villi inflammation. A composite variable was created using these items plus cases with inflammation or infection of the maternal or fetal side of the chorionic membrane.

Diagnosis of TTTs was made by the referring obstetrician and was designated on the pathology requisition. Placenta abruptio and invasive of trophoblast such as placenta accreta was also recorded.

Completed pathology reports for twins were printed from online pdf records or from hard copies of pathology records stored in hospital charts. Data were then abstracted from these records into an Excel database. Twin pregnancies were included in the data if mothers and babies were linked, babies had a calculated estimated gestational age at birth ≥20 weeks, and the mother did not have a termination procedure. The pathology data were then linked to delivery outcome data such as gestational age and birth weight by Perinatal Services British Columbia (2012): PSBC. POPDATABC (2015). Data Extract. PSBC (2014). The link was possible using the personal health number, maternal and baby identification number, and date of birth. The final linked records were stored on Secure Research Environment on a Virtual Private Network and contained 1,571 pairs (n=3,142 individual twins) of twin pregnancy data.

Inter-twin BWD was defined as a difference in birth weight of twins and was calculated using the following formula: (birth weight larger twin - birth weight smaller twin)/birth weight larger twin) 100%. To identify the trend of change in growth discordance, twins were divided into four groups: 10% or less, 11-20%, 21-30%, and more than 30%. Twins were then divided by three cut off points (10%, 20%, and 30%) and the results were compared using 20% and 30% cut off points versus 10% (reference category).

Fetal growth restriction was calculated using gestational age and BWD. Small for gestational age (SGA) was defined as birth weight less than the 10th percentile, whereas appropriate for gestational age (AGA) was defined as birth weight between the 10th and 90th percentile, according to a twin-adjusted gestational age nomogram [9].

The authors excluded pathology reports of twins born with congenital anomalies, missing birth weight data, and those born at less than 20 weeks of gestation. Some other known confounders from the literature were excluded. These are cases with TTTs [10], and twins with one single stillbirth [11]. In addition twins with smaller than 500 gram weights and those who underwent reduction procedures in utero were excluded as the growth pattern might be different in these cases [12]. No exclusions were made because of pregnancy complications (e.g. hypertension, diabetes), and therefore selection bias is not expected to have occurred.

The frequency of placental and cord characteristics was compared between MC and DC twin pregnancies. The placenta and cord characteristics were then assessed as an important factor in prediction of BWD and growth restriction. Frequency of occurrence of placenta/cord outcomes were compared between moderate (>20%) or severe (>30%) growth discordant twins and concordant twins (>10%). Comparisons were made between lighter twins of BWD pairs and the larger co-twins and concordant controls. The relative frequencies of adverse events were also analyzed between twins with SGA compared with AGA/LGA twins. Analyses were then stratified by chorionicity.

Results

After exclusion of cases noted in the methods section above, there were 1,466 pairs of twins (n=2,932 twins) in the analytical data. Of these, 702 twins (23.9%) were MC and 2230 twins (76.1%) were DC. Table 1 illustrates the clinical characteristics of the cohorts.

Monochorionic twins were delivered at an earlier mean gestational age and were on average 236 grams lighter than their DC counterparts. No significant difference was found between the MC and DC groups in terms of twins' sex.

Relationship between BWD and chorionicity

Investigating the trend of change in BWD amongst MC and DC cohorts, twins were compared in four groups: 10% or less, 11-20%, 21-30%, and more than 30% growth discordance. Frequency of BWD ≥ 30% was about two times higher in MC twins compared with DC twins (9.5% versus 5.0%, $p = 0.01$). Similarly, BWD of 21-30% had a higher frequency in MC gestations compared with DC ones. The authors then stratified BWD three times to ≥ 10%, ≥ 20%, and ≥ 30%. BWD of ≥ 10% was found to be similar between MC and DC cohorts. Overall, 45.3% of the cohort were growth concordant (≥ 10% BWD), while 16.9% were moderately discordant (≥ 20% BWD). Severe discordance was found in 6.6% of twins (BWD ≥ 30%). Analysis of stratified data for chorionicity showed that both moderate and severe discordant twin groups were statistically significantly different in terms of frequency in MC and DC cases. Higher frequencies of BWD twins (≥ 20% or ≥ 30%) were found in MC cases ($p = 0.01$, Table 1).

Chorionicity and pathology results

Table 2 shows characteristics of placenta and cord, overall and stratified by chorionicity. Compared to DC placentas, placenta anastomosis and unequal placenta sharing were significantly more common in MC placentas ($p = 0.01$ for both comparisons). Average values for placenta weight, length, width, and thickness were also higher in MC placentas compared to DC placentas. MC placentas had shorter cords than DC placentas ($p = 0.01$). Cord distances from the margin and from the other cord were longer in DC placentas ($p = 0.01$, $p = 0.05$, respectively). Insertion of cord was found to be an insignificant variable in this class of comparisons.

Relationship between BWD and pathology results

The relationship between BWD and pathology results was then analyzed (Table 3). Two thresholds of > 20% and > 30% were selected for further analysis as these levels were found to be statistically significantly different in the comparison between MC and DC (see Table 2).

Higher frequencies of unequal placenta sharing were found in twins with moderate growth discordance (BWD

Table 1. — *Comparing clinical characteristics of monochorionic and dichorionic twins in the cohorts born in C&W hospital (1,466 pairs, n=2932).*

		Overall	Monochorionic	Dichorionic	*p* value
Birth weight, g, mean ± SD		2,356.58 ± 596.41	2,120 ± 690.17	2,356.89 ± 626.73	0.01
Gestational age, Wk		34.56 ± 3.36	33.85 ± 3.69	34.78±3.22	0.01
BWD	10% or less	1,602 (54.6%)	380 (54.1%)	1,222 (54.8%)	0.01
	11-20%	834 (28.4%)	164 (23.4%)	670 (30.0%)	
	21-30%	317 (10.8%)	91 (13.0%)	226 (10.1%)	
	> 30%	179 (6.1%)	67 (9.5%)	112 (5.0%)	
BWD > 10%		1,330 (45.3%)	322 (45.9%)	1,008 (45.2%)	0.76
BWD > 20%		496 (16.9%)	158 (22.5%)	338 (15.2%)	0.01
BWD > 30%		194 (6.6%)	75(10.7%)	119 (5.3%)	0.01
Sex	Male	1,475 (50.3%)	358 (51.0%)	1,118 (50.1%)	0.36
	Female	1,456 (49.6%)	344 (49.0%)	1,112 (49.9%)	
Growth	AGA	2,133 (72.7%)	521 (74.2%)	1,612 (72.3%)	0.01
	LGA	629 (21.5%)	120 (17.1%)	509 (22.8%)	
	SGA	170 (5.8%)	61 (8.7%)	109 (4.9%)	

g: gram; Wk: Week; SD: standard deviation; BWD: birth weight discordance; AGA: appropriate for gestational age; LGA: large for gestational age; SGA: small for gestational age.

Table 2. — *Comparing characteristics of placenta and cord by chorionicity for twins born in C&W hospital (1,466 pairs, n=2932).*

	Overall	Monochorionic	Dichorionic	*p*
Placenta				
Chorionic villi inflammation	264 (9.0%)	54 (7.7%)	210 (9.4%)	0.09
Chorionitis	213 (7.3%)	54 (7.7%)	159 (7.1%)	0.34
Anastomosis	263 (9.0%)	229 (32.6%)	34 (1.5%)	0.01
Unequal placenta sharing	138 (30.4%)	65 (40.0%)	73 (24.9%)	0.01
Composite of inflammation	388 (13.2%)	82 (11.7%)	306 (13.7%)	0.09
Composite placenta lesions	54 (13.2%)	18 (2.6%)	36 (1.6%)	0.07
Cord composite	8 (0.3%)	<5 (0.7%)	<5 (0.2%)	0.29
Placenta other	36 (1.2%)	7 (1.0%)	29 (1.3%)	0.34
Placenta weight, g, mean ± SD	535.05 ± 228.99	686.18 ± 216.77	500.46 ± 217.46	0.01
Placenta length, cm, mean ± SD	22.07 ± 5.17	23.53 ± 4.24	21.73 ± 5.31	0.01
Placenta width, cm, mean ± SD	17.01 ± 3.98	19.08 ± 3.85	16.52 ± 3.85	0.01
Placenta thickness, cm, mean ± SD	2.13 ± 0.46	2.24 ± 0.54	2.09 ± 0.44	0.01
Cord				
Cord length, cm, mean±SD	27.44 ± 12.67	25.77 ± 12.42	27.99 ± 12.71	0.01
Cord distance from margin, cm, mean ± SD	4.61 ± 2.88	4.40 ± 2.36	4.68 ± 2.26	0.01
Cord distance from membrane, cm, mean ± SD	6.62 ± 3.99	6.91 ± 4.24	4.67 ± 2.26	0.21
Cord distance from other cord, cm, mean ± SD	14.15 ± 8.32	13.28 ± 5.30	14.50 ± 9.24	0.05
Cord insertion type				
Marginal	2024 (69.0%)	472 (76.4%)	1552 (77.1%)	0.84
Circle	481 (16.4%)	114 (18.4%)	367 (18.2%)	
Velamentous	125 (4.3%)	32 (5.2%)	93 (4.6%)	

SD: Standard deviation.

≥ 20%) as well as severe growth discordance (BWD ≥ 30%). Twins with severe discordance had a higher frequency of velamentous cord insertion (VCI) compared with both central (32.7% *vs.* 19.5%, $p = 0.02$) and marginal (12.1% *vs.* 5.4%, $p = 0.01$) insertions.

Twins with growth discordance (at both levels of moderate and severe growth discordance) had shorter cords ($p = 0.01$) for both moderate and severe growth discordant twins). Lower frequencies of chorionitis and composite inflammation were found in growth discordance of ≥ 30% compared to growth concordant twins.

Table 3. — *Comparisons of placenta and cord characteristics in groups with and without ≥20% BWD or ≥ 30% growth discordance for twin gestations registered at C&W hospital (1466 pairs, n=2932).*

	BWD					
	less than 20%	20% and more	*p* value	less than 30%	30% and more	*p*
Placenta						
Chorionic villi inflammation	221 (9.1%)	43 (8.7%)	0.43	251 (9.2%)	13 (6.7%)	0.15
Chorionitis	179 (7.3%)	34 (6.9%)	0.39	206 (7.5%)	7 (3.6%)	0.02
Anastomosis	209 (8.6%)	54 (10.9%)	0.06	245 (8.9%)	18 (9.3%)	0.48
Unequal placenta sharing	74 (22.2%)	64 (12.9%)	0.01	114 (4.1%)	24 (12.4%)	0.02
Composite of inflammation	325 (13.3%)	63(12.7%)	0.38	372 (13.6%)	16 (8.2%)	0.04
Composite placenta lesions	49 (2.0%)	<5 (1.0%)	0.08	53 (1.9%)	<5 (2.6%)	0.11
Cord composite	7 (0.3%)	<5 (1.0%)	0.59	7 (0.3%)	<5 (2.6%)	0.42
Placentation	33 (1.4%)	<5 (1.0%)	0.12	34 (1.2%)	<5 (2.6%)	0.57
Average placenta weight, g	534.37±228.00	538.62±234.54	0.77	534.13±230.36	550.07±205.61	0.49
Average placenta length, cm	22.02 ± 5.14	22.35 ± 5.29	0.32	22.04 ± 5.16	22.61 ± 5.23	0.27
Average placenta width, cm	16.98 ± 3.96	17.16 ± 4.05	0.48	17.00 ± 3.99	17.14 ± 3.84	0.73
Average placenta thickness, cm	2.13 ± 0.47	2.12 ± 0.43	0.74	2.12 ± 0.46	2.15 ± 0.46	0.64
Cord						
Cord length, cm	28.14 ± 12.73	24.19 ± 11.89	0.01	27.76 ± 12.61	23.07 ± 12.68	0.01
Cord distance from margin, cm	4.61 ± 2.24	4.63 ± 2.45	0.54	4.63 ± 2.29	4.39 ± 2.31	0.09
Cord distance from membrane, cm	6.54 ± 3.84	6.90 ± 4.62	0.81	6.63 ± 3.93	6.25 ± 4.69	0.22
Cord distance from the other cord, cm	14.23 ± 8.85	13.81 ± 5.60	0.30	14.29 ± 8.53	12.54 ± 5.10	0.46
Cord insertion type						
Marginal	1,949 (80.0%)	377 (76.0%)	0.21	2,176 (79.7%)	142 (73.1%)	0.01
Central	388 (15.9%)	93 (19.3%)		446 (16.3%)	35 (18.0%)	
Velamentous	99 (4.1%)	26 (5.0%)		108 (3.9%)	17 (8.7%)	
Velamentous *vs.* marginal	99 (4.1%)	26 (5.2%)	0.39	108 (4.0%)	17 (8.8%)	0.01
Velamentous *vs.* central	99 (4.1%)	26 (5.2%)	0.14	108 (4.0%)	17 (8.8%)	0.01
Marginal *vs.* central	1,688 (69.2%)	336 (67.7%)	0.08	1,901 (69.6%)	123 (63.4%)	0.19

Table 4. — *Unadjusted and adjusted odds of anastomosis and unequal placenta sharing in relation to BWD >20% and >30%.*

		BWD ≥ 20%		BWD ≥ 30%	
		Unadjusted	Adjusted*	Unadjusted	Adjusted*
Placenta	Anastomosis	1.30 (0.95-1.79)	0.92 (0.64-1.31)	0.96 (0.58-1.59)	0.57 (0.33-0.98)*
	Unequal placenta sharing	4.26 (2.70-6.70)	4.56 (2.70-6.70)*	2.01 (1.08-3.72)	2.00 (1.07-3.76)*

*Adjusted for chronicity.

Unadjusted and adjusted relationship between BWD and pathology findings

The variables of interest for regression analysis were anastomosis, unequal placenta sharing, cord length, and cord insertion types. Twins with ≥ 30% BWD were less likely to develop anastomosis (0.57, 95% CI 0.33 to 0.98) compared with those with < 30% BWD, after adjustment for chorionicity. Odds of unequal sharing of placentas were significantly higher in moderate and severe growth discordant twins (Table 4).

Linear regression analysis suggested that compared to the reference category (BWD below 30%), cord length was three cm shorter in twins with severe BWD (≥ 30%) while adjusted for chorionicity, sex discordance, and gestational age (Table 5). The impact of chorionicity, however, was of great interest as the same model suggested that adjusted for BWD, sex discordance, and gestational age, DC twins had shorter cords compared to MC by about three cm.

Adjusted for chorionicity and gestational age, ordinal regression analysis of cord insertion type suggested that BWD ≥ 30% was related to type of cord insertion for two comparisons: "velamentous *vs.* central" and "velamentous *vs.* marginal" insertion. If the twin pair had severe growth discordance (≥ 30%), his/her ordered log-odds of having a VCI compared with marginal cord increased by 2.23 cm. Also, if a twin pair had severe growth discordance, his/her ordered log-odds of having VCI decreased by 0.86 times of that of a twin with central cord insertion (Table 5). The above analysis was repeated for BWD ≥ 20% with no significant results (data are not shown).

Lighter discordant versus heavier concordant twins

The results for the lighter discordant twins (n=236) were compared to the combined group of heavier co-twins and twins with concordant birth weight (n=1214). Unequal placenta sharing was more frequent among lighter discordant

Table 5. — *Regression analysis of cord length and type of cord insertion, beta, significance of each term in the model, and 95% confidence interval.*

Dependent variable	Independent variable	Beta (SE)	*p* value	95% CI
Cord length	BWD ≥ 30%*	-3.13 ± 0.84	0.060	-0.06 to -2.02
	Chorionicity	0.98 ± 0.53	0.001	0.89 to 3.12
	Sex discordance	-1.20 ± 0.46	0.010	-2.10 to -0.29
	Gestational age	0.684 ± 0.07	0.001	-0.52 to 9.06
Marginal *vs.* central	BWD ≥ 30%*	-0.05 ± 0.25	0.830	-5.40 to 0.432
Velamentous *vs.* central		0.86 ± 0.17	0.001	0.537 to 1.185
Velamentous *vs.* marginal		2.23 ± 0.26	0.001	1.73 to 2.73

*Adjusted for chorionicity (Ref=MC), sex discordance, and gestational age.

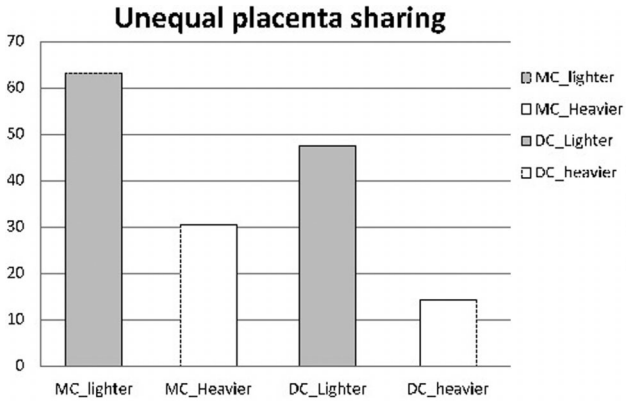

Figure 1. — Relative frequency of unequal placenta sharing in birth weight discordant twin pairs registred in C&W hospital (1,466 pairs, n=2932).

twins compared with the comparison group (52.6% *vs.* 19.9%, $p = 0.01$). Lighter discordant twins had heavier placentas (584.44 ± 227.61 grams *vs.* 511.41 ± 229.29 grams, $p = 0.01$), longer placenta length (23.18 ± 5.01 cm *vs.* 21.68 ± 5.03 cm, $p = 0.01$), wider placenta width (17.82 ± 4.16 cm *vs.* 16.61 ± 3.89 cm, $p = 0.01$) and shorter cords (27.48 ± 12.47 cm *vs.* 25.25 ± 12.03 cm, $p = 0.01$) compared to the combined heavier twins and twins with concordant birth weight.

These results were then stratified by chorionicity to determine if these relationships were present in both MC and DC twins. Unequal placenta sharing was more frequent in lighter discordant twins of DC compared to the heavier DC cohort (Figure 1). Similarly, lighter discordant twins in the MC cohort had significantly higher frequencies of unequal placenta sharing when compared with their heavier DC counterparts.

In the DC cohort, a significant association was found between placenta weight and BWD. Compared with heavier twins, lighter twins had heavier placentas (549.30 ± 218.57 grams *vs.* 482.52 ± 221.02 grams, $p = 0.01$). Similarly, lighter twins had wider and longer placentas compared to heavier twins ($p = 0.01$ for both variables).

In the MC cohort, cord length was found to be significantly shorter among lighter twins (24.90 ± 11.64 cm *vs.* 25.63 ± 12.03 cm, $p = 0.01$) compared with heavier twins. Type of cord insertion was not significantly different in any of the comparisons.

Fetal growth restriction and pathology results

Overall, the majority of twins were AGA (72.7%). Stratified growth restriction by chorionicity showed a statistically significantly different growth restriction in the MC group compared with DC ($p = 0.01$). Overall, 5.8% of twins were SGA. The MC cohort had twice as many SGA twins compared with the DC cohort (8.7% *vs.* 4.9%). The difference between MC and DC was negligible in terms of frequencies of AGA, while LGA in MC gestations was 5% lower than in DC gestations (Table 5).

A further analysis of the result was conducted to determine the association between fetal growth restriction and placental pathology, comparing SGA (n=170) and AGA (n=2,133) cohorts. LGA twins were left out of this comparison (n=629). The SGA cohort had higher frequencies of unequal placenta sharing (48.7%) *vs.* AGA twins (28.7%, $p = 0.01$). Furthermore, the SGA cohort had lighter placenta (455.83 ± 200.33 grams *vs.* 538.51 ± 229.59 grams, $p = 0.01$) and smaller placenta dimensions (length and width, $p = 0.01$ for both) compared to the AGA group. In addition, length of cord (22.46 ± 11.21 cm *vs.* 27.75 ± 12.69 cm, $p = 0.01$) and its distance from the margin of placenta (4.04 ± 1.93 cm *vs.* 4.64 ± 2.30 cm, $p = 0.01$), and the other cord (11.61 ± 5.06 cm *vs.* 14.30 ± 8.45 cm, $p = 0.01$) were statistically significantly shorter than the AGA cohort.

When further analysis was conducted stratifying the data according to chorionicity, a higher frequency of unequal placenta sharing was observed in SGA twins compared to AGA in the DC ($p = 0.02$) cohort but not in MC twins ($p = 0.26$). The placenta was lighter among SGA twins compared to AGA in the MC cohort (532.48 grams ± 200.74 *vs.* 696.24 ± 214.25 grams, $p = 0.01$). Similarly, placenta dimensions were smaller in SGA twins ($p = 0.01$ for both width and length of placenta) compared to AGA twins. A comparable pattern was observed for the DC cohort. SGA twins also had shorter cords compared to their counterparts in both the MC (21.38 ± 11.15 cm *vs.* 26.17 ± 12.46 cm, $p = 0.01$) and DC (23.06 ±

11.24 cm *vs.* 28.25 ± 12.73 cm, $p = 0.01$) cohorts. Cord insertion type, chorionitis, and chorionic inflammatory villi were not significantly different in any of the comparison groups, nor did the composite scores. Small numbers in these comparisons were of concern.

Discussion

The aim of this study was to assess the pathological characteristics of placenta and cord in relation to BWD in twin gestations. The present findings suggest that MC twins had a higher frequency of BWD ≥ 30% than DC twins. The placental origin of growth discordance was due to presence of anastomosis, unequal placenta sharing, and pathological. Other factors significantly associated with growth discordance were inclusive of the size of the placenta, inflammatory reactions, cord length, and cord insertion type.

Twin pregnancies with evidence of growth discordance have a higher risk of adverse outcomes [13-15]. BWD has been attributed to chorionicity in many studies [16-18]. Microscopic and macroscopic examination of the placenta is therefore important in the evaluation of growth discordance [19]. Studies in singleton pregnancies have shown smaller, lighter placentas with morphological changes in villus structure in placentas of fetuses affected by intrauterine growth impairment [20-22]. The impaired fetal growth has been attributed to defective trophoblast invasion [23] and placental infarcts [24] leading to impaired development of the utero-placental circulation.

The pathophysiological changes in the placenta and its relation with BWD have been studied in the literature [24, 25]. However, these studies are limited by relatively small numbers, inadequate control of confounding factors, and inclusion of twins with congenital anomalies. Moreover, in cases of multi-central studies, placenta examination was carried out in different pathology laboratories that applied different approaches in examination of placentas and cords. This could lead to variation between pathology results.

The present data is based on a large cohort of twin data collected over a ten-year period. The authors excluded pathology reports of twins born with congenital anomalies, missing birth weight data and those born at less than 20 weeks of gestation. They have also excluded cases with TTTs, twins with one single stillbirth, twins with smaller than 500 gram weights, and those who underwent reduction procedures in utero. Standardized pathology examination techniques have been carried out in all twin pregnancies in one hospital, hence lower variation in pathological reports is expected in the present study.

Studies in the literature related to pathological findings of BWD twins are scarce. Several studies have focused on MC placentas only and suggested that growth discordance in twin pregnancies is attributed to TTTs [26, 27] while others evaluated a single element, for instance placenta size/placenta weight [28, 29], placenta pathology or cord abnormalities in relation to BWD of twins [30, 31]. The present study provides a comprehensive picture of pathological changes of the placenta and cord in both MC and DC twins with or without BWD.

Anastomosis

In the present hospital-based cohort of 1,466 twin pregnancies the authors have established associations between moderate and severe BWD and chorionicity. There were higher frequencies of placenta anastomosis among MC twins than DC ones. Placenta anastomosis in MC is ascribed as the cause of BWD, while in DC twins discordant growth is linked to differences in placental mass or differences in placental parenchymal lesions [32]. The number of cases of placenta anastomoses in the present study was around 263 (9.0% of total population). This observation is based on data where twins with TTTs were excluded. The present study limitation is a non-differentiation diagnosis between "arterial-to-arterial" anastomosis versus "vein- to-artery" anastomosis.

Unequal placenta sharing

Unequal placenta sharing is considered an abnormality of placentation as it affects the available nutrient delivery, gas exchange, and waste removal which may directly impact fetal growth [5]. Apart from genetic potential, crowding in utero, and placenta insufficiency, unequal sharing of placenta mass is an explanation for discordant fetal growth in DC [24]. Unequal placenta sharing is also reported in MC twins. A retrospective study reported a prevalence of 154 placentas out of 395 MC twin placentas (39%) [32]. The present study provides data for both MC and DC cohorts where the authors showed a higher frequency of this condition in MC twins compared to DC.

The relationship with BWD and unequal placenta sharing was also studied in a prospective cohort of 522 MC/DC twin pregnancies (1997-2003) using Dye injection studies on fresh postpartum placentas. Unequal placenta sharing was defined as one twin receiving blood from > 60% of the placenta. The study revealed that 50% of the cases with BWD > 20% had unequal placenta sharing compared with 10.4% of the cases without unequal placenta sharing ($p = 0.01$) [33]. The phenomenon of increasing placenta territory discordance leading to discordant growth is thought to result from unequal splitting of the initial cell mass [34].

Another small prospective study of 100 MC placentas investigated the relationship between placenta territory and BWD from pregnancies not complicated by TTTs. All samples were from pregnancies with two live born twins. Placental territory discordance was calculated by dividing the venous surface area of the larger placental part by that of the smaller. Unequal placental sharing was defined as a placental territory discordance of ≥ 1.5. Placental territory dis-

cordance increased with BWD ($p = 0.001$) [5].

The present study showed that regardless of the level of BWD severity, a higher percentage of sharing of placenta is expected among twins with BWD compared to concordant twins. The relationship between BWD and placenta sharing remained strong even after adjustment for chorionicity (OR 4.56 *vs*. 4.26 for BWD ≥ 20% and 2.01 *vs*. 2.00 for BWD ≥ 30%). Higher odds for the threshold level of ≥ 20% compared to ≥ 30% could be the result of various depths of anastomosis or type of anastomosis (artery-artery *vs.* artery-vein).

Sex discordance was found to be a significant predictor in the relationship between BWD and placenta characteristics. To the best of the present authors' knowledge, no study to date has investigated this relationship. These results will be discussed in the next section, where they examine the role of sex discordance in relation to placenta characteristics.

Placental lesions

Placental histological examination is critical in the evaluation of intrauterine growth impairment. Singleton pregnancies are shown to have higher rates of morphological changes in villus structure in placentas of fetuses affected by intrauterine growth restriction [20, 22]. The abnormalities in the villus structure are due to defective trophoblastic development leading to impaired utero-placental circulation. An association between placenta infarcts and impaired utero-placental circulation of growth restricted fetuses is reported in singleton pregnancies [23].

In twin pregnancies, such a relationship has also been previously studied [23, 35] but publications on gross and histological placental examination of MC and DC twins are rare. Two retrospective studies evaluating placenta histology have been limited by a rather smaller sample size [24, 25]. A retrospective study of 147 twin pairs (99 DC and 48 MC) suggested that in DC twins a BWD > 20% is attributable to a greater number of placental lesions in the lighter twin than in the heavier twin ($p < 0.05$) [24]. The other study included 388 DC and 89 MC twin pregnancies and found significantly more vascular thrombotic lesions in the placental domains of smaller twins in DC pairs [25].

A prospective study of 668 twin pairs (21.1% MC and 78.9% DC) analysed a composite variable including evidence of infarction, retro-placental hemorrhage, chorangioma, subchorial fibrin, and abnormal villus maturation. These histological abnormalities were more frequent in placentas of smaller twins of BWD pairs ($p = 0.02$) and in placentas of SGA infants ($p = 0.01$). Such associations were observed in DC twins but not in MC ones [31]. The present authors did not find these differences in this study, potentially due to a smaller number of lesions in this study population which could be due to the retrospective nature of the data. The incidence of composite placenta lesions in our study was 54 (13.2%) with a slightly higher frequency in MC compared to DC twins (2.6% *vs.* 1.6%), although this difference was not significant.

Another reason for differences between the present findings and the aforementioned study could be the present higher number of twins, specific differences in defining the composite variable, or measurement bias. The present composite placental pathological lesions included placental infarction, chorangioma, subchorial fibrin deposition, and retro-placental hematoma.

Cord length

Information about cord length in twin pregnancies is scarce. Twins are reported to have, on average, a 7.9 cm shorter umbilical cord compared with singleton pregnancy [36]. A higher incidence of categorised short umbilical cords (less than 35 cm in length) is also reported in twins compared to single pregnancies [37]. In the present research, the average length of umbilical cord was shorter in MC compared to DC twins and in growth discordant twins compared to concordant ones.

The relationship between BWD and length of cord has been investigated in the literature controlling for the impact of gestational age [38]. The present authors investigated such a relationship in this study. Adjusted for gestational age, sex discordance, and chorionicity, the BWD was statistically significantly related to cord length, suggesting that shorter cords by average of 3.13 cm are associated with severe growth discordance. Additionally, the present results suggest that shorter cords yield lighter twins. Such a finding has not been reported in the literature.

A small study of 24 MC and 200 DC placentas [39] showed statistically significant differences between the length of the umbilical cords in MC twins and in DC ones. Unlike the present study, this study included TTTs cases in the analysis. No information about BWD was reported in this study.

Velamentous cord insertion (VCI)

An association between VCI and compromised fetal growth in singleton pregnancies is a longstanding discovery [40]. In twin gestations, the effect of VCI is dependent on chorionicity. A high incidence of VCI in twins (21%) was reported to be significantly more common in MC than DC twin pairs (18% *vs*. 6%; $p = 0.001$) [41]. Moreover, the effect of VCI on BWD has been reported to be 13.5 times greater for MC twin gestations than for DC twins. In the present study, VCI was associated with BWD. Severely growth discordant twins had higher odds of getting their nutrients from a placenta with velamentous cord insertion than a placenta with a central cord. This finding has been reported in previous studies with a smaller sample size of MC twins [42]. A matched case-control study of 47 twin pairs compared the placental characteristics of MC placentas from pregnancies with BWD > 25% with a control group of MC placentas without BWD, matched for gesta-

tional age at birth. The rate of VCI was significantly different in BWD twins compared with concordant ones (30% *vs.* 16%, $p = 0.036$) [10]. In the present study the authors adjusted the impact of gestational age and found similar results.

A prospective study of 319 twins also found a higher frequency of VCI in BWD of ≥ 20% compared with growth concordant twins (22% *vs*. 8%, $p = 0.001$). A total of 369 MC placentas were analyzed in another study of MC placentas, and the frequency of VCI was found to be 36% in BWD twins compared to 21% in normal MC group ($p = 0.06$) [43].

A higher incidence of VCI is reported in twins with TTTs than in non-TTTs placentas. In view of this finding, an etiologic role for VCI in the impaired growth of fetus is linked with TTTs. The authors proposed that TTTs could result from hemodynamic instability due to reduced blood flow to the donor twin with a VCI, hence growth impairment in a donor twin [44]. In the present study, the authors have excluded twins with TTTs and still found a relationship between VCI and BWD despite adjustment for chorionicity. This conclusion is in the light of the fact that the authors were unable to show any association between VCI and placenta sharing, or other placenta lesions. Thus, the reason(s) for association between BWD and VCI remains unknown.

Marginal cord insertion

The present findings suggest that it was more likely to show growth discordance at birth if the cord is inserted into the placenta marginally rather than centrally. This finding is in agreement with the result of a study of 60 MC twins in that when one placental cord insertion site was central and the other was marginal, 33% of twin pairs exhibited a BWD of at least 20% [45]. The study did not separate the marginal and velamentous cord insertion due to low numbers.

A prospective larger study of cord insertion type and growth discordance was also conducted on a large population of 806 twin pairs (165 MC, 651 DC). Monochorionic twins had higher rates of marginal ($p = 0.0068$) placental cord insertion. A significant association between non-central (combined marginal and velamentous) placental cord insertion sites and growth abnormalities was reported in MC twins but not in DC twins [3]. The present study finding is in agreement with this study because the authors' ordinal regression model showed higher odds of BWD in marginal versus velamentous cord insertions. These odds were adjusted for chorionicity, sex discordance, and gestational age.

Conclusions

The present study focused on the relationship between the type of the twin placenta and BWD of the twins in a large cohort study from one major hospital in BC. The large sample size, similar chorionicity, verification in one laboratory, and ability to control for confounding variables such as gestational age and sex discordance enabled the authors to control for several major confounders.

First, MC twins had a higher frequency of BWD ≥ 30% than DC twins, suggesting that the single placenta is less efficient than the DC placenta to nurture twins. Secondly, the authors attempted to assess the placental origin of aberrant growth among twins, mainly in correlating the presence of anastomosis, unequal placenta sharing, and pathological lesions, as well as investigating the size of the placenta, inflammatory reactions, cord length and cord insertion type. From these variables, anastomosis, unequal placenta sharing, cord size, and cord insertion type were found to be the key elements that impacted growth aberration.

The present authors hypothesised that MC placentas compensate for lack of nutritional flow by infiltrating to other placenta surfaces, mainly due to the following findings: 1) higher rates of severe growth discordance were negatively associated with higher frequencies of anastomosis, 2) placentas in MC twins were more likely to have shared arteries/veins. Compensation for lack of vascular sufficiency would mean a fused placenta or sharing more portions of the placenta. This translates to higher rates of unequal placenta sharing among growth discordant twins irrespective of chorionicity, as suggested by the result of multivariable regression models. Other than anastomosis and unequal placenta sharing, the shorter cord length and non-central cord insertion type were associated with BWD.

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