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Causes of Delivery and Outcomes of Very Preterm Twins Stratified to Zygosity

Juliane Spiegler,[1] Christoph Härtel,[1] Lena Schulz,[1] Nicole von Wurmb-Schwark,[2] Thomas Hoehn,[3] Angela Kribs,[4] Helmut Küster,[5] Jens Siegel,[6] Christian Wieg,[7] Jan Weichert,[8] Egbert Herting,[1] Wolfgang Göpel,[1] and the German Neonatal Network (GNN)

[1]Department of Pediatrics, University Hospital of Schleswig-Holstein, Luebeck, Germany
[2]Department of Forensic Medicine, University Hospital of Schleswig-Holstein, Kiel, Germany
[3]Department of Pediatrics, University Düsseldorf, Düsseldorf, Germany
[4]Department of Pediatrics, University Köln, Köln, Germany
[5]Department of Pediatrics, University of Göttingen, Göttingen, Germany
[6]Department of Pediatrics, Auf der Bult, Hannover, Germany
[7]Aschaffenburg Department of Pediatrics, Aschaffenburg, Germany
[8]Department of Gynecology and Obstetrics, University Hospital of Schleswig-Holstein, Luebeck, Germany

The increasing rates of preterm birth among twins implicate that solid data on associated risks and outcomes are required. Assessment of zygosity is often based on clinical criteria (evaluation of placenta; same gender, birth weight discordance as surrogate criteria for monochorionic/monozygotic twins). The aim of this study was to compare clinical versus genetic assessment of zygosity and to compare causes of preterm delivery as well as outcome data of very-low-birth-weight (VLBW; birth weight <1,500 g) twins stratified to zygosity. In a multicenter study, we selected $n = 176$ sets of same gender twins and determined zygosity genetically. In a subgroup of 123 sets of twins, the attending physicians at the study centers were asked to document the parameter 'zygosity' (monozygotic/dizygotic) on the basis of their clinical judgment. Concordance between genetic and clinical assessment was 62.7% for monozygotic twins and 88.9% for dizygotic twins, respectively. Outcome parameters (death, BPD, ROP, NEC, IVH) were comparable in both groups. Genetically dizygotic twins were significantly more often born due to intrauterine infection (33% vs. 20% in monozygotic twins, $p < .01$) and antenatal antibiotics were more frequently given to mothers of dizygotic twins (62% vs. 47% in monozygotic twins, $p < .01$). Obstetric complications such as twin-twin-transfusion-syndrome were only seen in monozygotic twins as expected. The unexpected increase of antenatal antibiotic treatment and birth due to intrauterine infection in dizygotic twins should be confirmed in additional VLBW twin-cohorts.

■ **Keywords:** very preterm infants, twins, zygosity, outcome, causes of delivery, intrauterine infection

Preterm twin birth rates have been increasing over the years (Hartley & Hitti, 2010). They are associated with significant long-term morbidity and mortality. Monozygotic twins are traditionally thought to suffer more complications mainly due to the risk for twin-twin-transfusion-syndrome (TTTS). Assessment of zygosity is often based on clinical criteria (evaluation of placenta; same gender, birth weight discordance as surrogate criteria for monochorionic/monozygotic twins). The rising rates of preterm birth among twins implicate that solid data on associated risks and outcome are required.

Papiernik et al. (2010, MOSAIC-Study Group) compared very preterm twins ($n = 1,254$) and singletons ($n = 3,585$) with regard to prenatal and postnatal complications. In singletons (<32 weeks of gestation), the investigators noted a higher rate of pregnancy complications (hypertension, hemorrhage) and growth restriction. While twins born between 24 and 27 weeks of gestation had a higher morbidity and mortality than singletons, twins between 28 and 31 weeks of gestation had a better outcome than singletons. In their cohort, same sex twins with a discordant weight had

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ADDRESS FOR CORRESPONDENCE: Dr. Juliane Spiegler, Department of Pediatrics, University Hospital of Schleswig-Holstein, Ratzeburger Allee 160, 23538 Luebeck, Germany. E-mail: uni@dr-spiegler.de

significantly higher mortality rates and an increased risk for IVH (intraventricular hemorrhage) grade III and IV. Their data did not include genetic assessment of zygosity.

The objective of this study was to compare the validity of clinical and genetic assessment of zygosity. Our second objective was to compare outcome data of very-low-birth-weight (VLBW, birth weight <1,500 g) twins stratified to zygosity to test the hypothesis that monozygotic VLBW twins have more postnatal complications compared to dizygotic twins and VLBW singletons.

In different gender twins prenatal risk factors are identical since they share the same womb; they are born due to the same reasons and are treated in the same neonatal intensive care unit (NICU). Therefore, this group is very interesting to study gender effects. As a third objective, we compared dizygotic different gender VLBW twins to study the influence of gender in dizygotic twins.

Material and Methods

Subjects

Data from VLBW infants born between 24+0 and 36+6 weeks of gestation and a birth weight of less than 1,500 g were collected from 28 centers in Germany. Between 2003 and 2009, a total of 3,708 VLBW infants with DNA samples were included in our study of influences of genetic polymorphisms on long-term morbidity and mortality. DNA of all infants was isolated from buccal swabs or umbilical cord tissue. Proper assessment of clinical data was ensured by on-site-monitoring. In a subgroup of twins enrolled between 2003 and 2007 (123 sets of twins), the attending physicians at the local study centers were asked to document the parameter 'zygosity' (monozygotic/dizygotic). Sets of twins classified as 'unknown' were excluded. Within the cohort of 1,173 multiples (31% of the total cohort), we selected 176 twin pairs (352 infants) of the same gender and both with a birth weight below 1,500 g and determined zygosity genetically.

Definitions

Zygosity was determined as described by Wurmb-Schwark et al. (2004). Bronchopulmonary dysplasia (BPD) was defined as need for supplemental oxygen at 36 weeks postmenstrual age. Mechanical ventilation was defined as any kind of mechanical ventilation during hospital stay. IVH was per definition any bleeding into the cerebral germinal matrix or the ventricles. Sepsis was defined as any sepsis with clinical symptoms, laboratory findings, and positive blood culture during the stay in the hospital. Surgery for retinopathy of prematurity (ROP) was defined as any ROP treated with laser — or cryocoagulation. Surgery for NEC/FIP was defined as any surgery necessary for necrotizing enterocolitis or focal intestinal perforation, surgery for PDA was any surgical ligation for persistent ductus arteriosus.

Statistical Analyses

Data analysis was performed using SPSS 17.0 data analysis package (IBM). Hypotheses were evaluated with Fisher Exact test. Quantitative data were compared with *t*-test. A p value $<.05$ was considered statistically significant.

Ethics

The study was approved by the local committee on research in human subjects of the University of Luebeck, and the local committees of all participating centers.

Results

Genetic assessment of zygosity was successful in 165 of 176 sets of twins (330 infants, 94%). Eighty-seven sets of twins (174 infants) were monozygotic and 78 sets of twins (156 infants) were dizygotic. 62.7% ($n = 32$ of 51) of monozygotic twins were clinically correctly classified as monozygotic, while 37.3% ($n = 19$ of 51) were erroneously thought to be dizygotic twins. Regarding dizygotic twins, 88.9% ($n = 64$ of 72) were clinically classified correctly as dizygotic while 11.1% ($n = 8$ of 72) were erroneously thought to be monozygotic twins. Misclassifications were significantly more frequent in monozygotic twins compared to dizygotic twins (37% vs. 11%, $p < .01$).

Clinical data of same gender twins and a control group of 2,535 VLBW singletons are given in Tables 1 and 2. Singletons were used as a control group since they represent the 'overall risks of prematurity' in the participating centers.

As expected, TTTS was only noted in monozygotic twins. Antenatal antibiotics were applied more often to dizygotic twins as compared to monozygotic twins or singletons. With regard to causes of preterm delivery, suspected intrauterine infection was noted more often in dizygotic twins. Major outcome parameter did not differ between the two groups (Table 2). Outcome parameter of different gender twins stratified to gender is given in Table 3. Regarding major outcome parameter, there were no significant gender differences.

Discussion

Preterm twin birth rates have been increasing over the last decades for several reasons. Maternal age is higher in developed countries and assisted reproduction is more often necessary at an advanced age. Regarding twin pregnancies, Prapas et al. (2006) showed that maternal age is a risk factor for having VLBW twins. Hartley and Hitti (2010) demonstrated that the rate of preterm twin births increased from 1980 to 2005. While the rate of preterm birth at a very low gestational age (24–31 weeks) remained stable, the rate of preterm twins born between 32 and 36 weeks increased along with increasing requirement for respiratory support. Preterm twins are a highly susceptible group in the NICUs and their special risks need to be identified more clearly (Spiegler et al., 2008).

TABLE 1

Clinical Data of VLBW Twins Stratified to Zygosity

Parameter	Monozygotic twins $N = 174$ (87 sets of twins)	Dizygotic same gender twins $N = 156$ (78 sets of twins)	p	Singletons $N = 2,535$
Gestational age (weeks)	28.6 ± 2.0	28.7 ± 2.3	n.s.	28.6 ± 2.7
Male gender (%)	90/174 (51.7%)	94/156 (60.3%)	n.s.	1313/2534 (51.8%)
Birth weight (g)	1,086 ± 263	1,139 ± 276	n.s.	1,038 ± 300
Twin-twin transfusion syndrome	12/44 (27%)	0/36 (0%)		0
Spontaneous delivery	6/138 (4%)	3/120 (2%)	n.s.	319/2,128 (15%)
Cesarian section	130/138 (94%)	110/120 (92%)	n.s.	1,746/2,128 (82%)
Emergency cesarian section	1/138 (1%)	7/120 (6%)	n.s.	63/2,128 (3%)
Prenatal steroids	166/174 (95%)	140/156 (90%)	n.s.	321/2,526 (87%)
Prenatal antibiotics	81/174 (47%)	96/156 (62%)	$p = .008$	1,064/2,492 (43%)
Tocolytics	112/174 (64%)	112/156 (72%)	n.s.	1,263/2,511 (50%)
Reason of preterm birth				
Premature labor	76/174 (44%)	71/156 (46%)	n.s.	754/2,534 (30%)
Suspected intrauterine infection	35/174 (20%)	51/165 (33%)	$p = .012$	543/2,534 (21%)
EPH-Gestosis	2/174 (1%)	4/156 (3%)	n.s.	289/2,534 (11%)
Non-reassuring heart rate tracing	32/174 (18%)	22/156 (14%)	n.s.	698/2,534 (27%)
Abruptio placentae	6/174 (3%)	8/156 (5%)	n.s.	221/2,533 (9%)

Note: Data are presented as mean and SD (±) or as total numbers and percentage.

TABLE 2

Outcome of VLBW Twins Stratified to Zygosity

Parameter	Monozygotic twins $N = 174$	Dizygotic same gender twins $N = 156$	p	Singletons $N = 2,535$
Gestational age (weeks)	28.6 ± 2.0	28.7 ± 2.3	n.s.	28.6 ± 2.7
Male gender (%)	90/174 (51.7%)	94/156 (60.3%)	n.s.	1313/2534 (51.8%)
Birth weight (g)	1,086 ± 263	1,139 ± 276	n.s.	1,038 ± 300
Death	3/174 (2%)	2/156 (1%)	n.s	83/2,535 (3.3%)
IVH I-II°	23/173 (13%)	23/155 (15%)	n.s.	299/2,527 (12%)
IVH III-IV°	15/173 (8.6%)	18/155 (11.6%)	n.s.	152/2,527 (6%)
ROP surgery	6/172 (3.5%)	7/153 (4.5%)	n.s.	93/2,504 (3.7%)
Ventilation	95/174 (54.6%)	75/156 (48%)	n.s.	1,197/2,535 (47%)
BPD	17/167 (10%)	19/145 (13%)	n.s.	355/2,336 (15%)
Sepsis (pos. Blood culture)	20/170 (12%)	21/155 (13.5%)	n.s.	396/2,494 (16%)
Surgery for NEC/FIP	8/173 (4.6%)	7/156 (4.5%)	n.s.	124/2,522 (5%)

Note: Data are presented as mean and SD (±) or as total numbers and percentage; BPD — bronchopulmonary dysplasia, Sepsis — blood-culture-proven sepsis, IVH — intraventricular hemorrhage, NEC — necrotizing enterocolitis, ROP — retinopathy of prematurity.

TABLE 3

Outcome of VLBW Different Gender Twins Stratified to Gender

Parameter	Dizygotic different gender twin male $N = 118$	Dizygotic different gender twin female $N = 118$	p	Singletons $N = 2,535$
Gestational age (weeks)	28.4 ± 2.2	28.4 ± 2.2	n.s.	28.6 ± 2.7
Birth weight (g)	1,119 ± 279	1,075 ± 289	n.s.	1,038 ± 300
Death	4/118 (3.4%)	2/118 (1.7%)	n.s.	83/2,535 (3.3%)
IVH total	22/116 (19%)	30/117 (25.6%)	n.s.	299/2,527 (12%)
IVH III-IV°	10/116 (8.6%)	14/117 (12.1%)	n.s.	152/2,527 (6%)
ROP surgery	3/116 (2.6%)	8/117 (6.8%)	n.s.	93/2,504 (3.7%)
Ventilation	58/118 (49.2%)	52/118 (44.1%)	n.s.	1,197/2,535 (47%)
BPD	11/118 (10.2%)	11/117 (10.3%)	n.s.	355/2,336 (15%)
Sepsis (pos. Blood culture)	22/114 (19.3%)	16/118 (13.6%)	n.s.	396/2,494 (16%)
Surgery for NEC/FIP	4/116 (3.4%)	5/118 (4.2%)	n.s.	124/2,522 (5%)

Note: Data are presented as mean and SD (±) or as total numbers and percentage; BPD — bronchopulmonary dysplasia, Sepsis — blood-culture-proven sepsis, IVH — intraventricular hemorrhage, NEC — necrotizing enterocolitis, ROP — retinopathy of prematurity.

All data in our study are limited by the relatively small number in each group. As in Sudanese twins (Elshibly & Schmalisch, 2010) and in other studies, girls weighed less than boys in our cohort; however, this did not reach statistical significance. In their study, twins were more prone to be growth restricted than singletons. In our study, birth weight of all groups of twins is very similar to singletons. However, the Sudanese twins were significantly less premature (average gestational age 37 weeks, compared to 28 weeks in our study). Interestingly, none of the major outcome parameters

of different gender twins reached significance even though strong gender effects have been shown in large groups of preterm babies (Tyson et al., 2008).

In our cohort, causes of preterm delivery were similar between mono- and dizygotic twins. Monozygotic twins received prenatal steroids more frequently (95% in monozygotic twins, 90% in dizygotic twins, and 87% in singletons). This trend did not reach statistical significance; however, this trend might reflect that monozygotic twins are supposed to be more at risk than dizygotic twins or singletons. Myles et al. (1997) reported that twin pregnancies with premature rupture of membranes (PROM) had a shorter latency until birth but were less likely to suffer from amniotic infection. Mazor et al. (1996) demonstrated that the prevalence of intraamniotic infection was similar in twin and singleton pregnancies with preterm labor. In our study, dizygotic twins were more prone to receive antenatal antibiotics and birth was more likely due to suspected intrauterine infection with preterm labor and rupture of membranes. PROM is seen more often in twin gestations. Our cohort was not monitored for PROM.

However, even a higher rate of PROM does not explain why dizygotic twins are more prone to suspected infection than monozygotic twins. Dizygotic same gender twins should have a similar risk of infection as different gender twins. In our cohort, we saw a similar high rate of antenatal antibiotics in different gender twins compared to dizygotic same gender twins (60% vs. 62%) but less suspected intrauterine infection (22% vs. 33%). If premature labor does not respond to tocolytics and antenatal antibiotics, intrauterine infection is a frequent cause of preterm delivery. Despite receiving more tocolytics (75%), premature labor was the most common reason for preterm birth in different gender twins (59% vs. monozygotic twins 44%, same gender dizygotic twins 46%, singletons 30%). Larger cohorts are needed to confirm whether or not dizygotic twins are more prone to infection than monozygotic twins.

Cesarean section is the standard delivery mode in most German delivery wards for VLBW infants, while the MOSAIC group reported rates of spontaneous delivery up to 52%. There is an ongoing discussion whether or not a cesarean section improves outcome in VLBW infants (Paul et al., 2002; Riskin et al., 2008).

Papiernik et al. (2010) showed in a large cohort (MOSAIC group) that twins at 24–27 weeks of gestation had an increased neonatal mortality and higher rates of intracranial hemorrhage if they were of the same gender and had discordant birth weights. This suggests an influence of zygosity (and suspected TTTS) on the observed morbidity. However, no genetic determination of zygosity was done. In our cohort, clinical assessment of zygosity alone revealed inconsistent results in 10–30%. Therefore, clinical assessment of zygosity on its own may not lead to proper counseling with regard to outcome of twins. After correcting for zygosity, we observed no significant differences in major outcome parameters (including IVH) stratified to zygosity (Table 2). Larger cohorts are necessary to confirm our observation, given the fact that our data contradict previous data (Papiernik et al., 2010).

In 1999, Mizrahi et al. reported that twins do better than comparable singletons regarding peripartal complications. Kirkby et al. (2010) compared outcome of VLBW twins and higher multiples to VLBW singletons and discovered no significant differences. The MOSAIC-Group showed differences in same gender twins between 24 and 27 weeks of gestation with a discordant birth weight, but otherwise similar outcomes of twins and singletons. Together with our data, there are now four cohorts that show comparable outcome data for VLBW twins and singletons in North America, different areas in Europe (MOSAIC-Group), Israel, and Germany (German Neonatal Network). Our data are useful and reassuring for counseling parents of VLBW twins. The unexpected observation of a higher rate of intrauterine infection in dizygotic VLBW twins should be confirmed in a larger cohort.

Conclusion

Clinical assessment of zygosity in preterm twins on its own was unreliable and may be a significant confounder of data. Therefore, genetic assessment of zygosity has the potential to optimize data quality in VLBW twins. Consequently, prognostic counseling of parents on mortality and long-term morbidity of preterm twins may be improved. If data of VLBW-twin pregnancies are analyzed for scientific purposes, genetic determination of zygosity is highly recommended. Major outcome parameters show no differences between monozygotic twins, dizygotic twins, and singletons. Regarding prenatal complications, we found a higher risk for suspected intrauterine infection and subsequent premature delivery in dizygotic twins that needs to be confirmed in larger cohorts.

References

Elshibly, E. M., & Schmalisch, G. (2010). Differences in anthropometric measurements between Sudanese newborn twins and singletons. *Twin Research and Human Genetics*, *13*, 88–95.

Hartley, R. S., & Hitti, J. (2010). Increasing rates of preterm twin births coincide with improving twin pair survival. *Journal of Perinatal Medicine*, *38*, 297–303.

Kirkby, S., Genen, L., Turenne, W., & Dysart, K. (2010). Outcomes and milestone achievement differences for very low-birth-weight multiples compared with singleton infants. *American Journal of Perinatology*, *27*, 439–444.

Mazor, M., Hershkovitz, R., Ghezzi, F., Maymon, E., Horowitz, S., & Leiberman, J. R. (1996). Intraamniotic infection in patients with preterm labor and twin pregnancies. *Acta Obstetrica Gynecologica Scandinavica*, *75*, 624–627.

Mizrahi, M., Furman, B., Shoham-Vardi, I., Vardi, H., Maymon, E., & Mazor, M. (1999). Perinatal outcome and

peripartum complications in preterm singleton and twins deliveries: A comparative study. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *87*, 55–61.

Myles, T. D., Espinoza, R., Meyer, W., & Bieniarz, A. (1997). Preterm premature rupture of membranes: Comparison between twin and singleton gestations. *Journal of Maternal and Fetal Medicine*, *6*, 159–163.

Papiernik, E., Zeitlin, J., Delmas, D., Blondel, B., Künzel, W., Cuttini, M., Weber, T., Petrou, S., Gortner, L., Kollée, L., Draper, E. S., & MOSAIC Group. (2010). Differences in outcome between twins and singletons born very preterm: Results from a population-based European cohort. *Human Reproduction*, *25*, 1035–1043.

Paul, D. A., Sciscione, A., Leef, K. H., & Stefano, J. L. (2002). Cesarean delivery and outcome in very low birthweight infants. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *42*, 41–45.

Prapas, N., Kalogiannidis, I., Prapas, I., Xiromeritis, P., Karagiannidis, A., & Makedos, G. (2006). Twin gestation in older women: Antepartum, intrapartum complications, and perinatal outcomes. *Archives of Gynecology and Obstetrics*, *273*, 293–297.

Riskin, A., Riskin-Mahiah, S., Bader, D., Kugelmann, A., Lerner geva, L., Boyko, V., & Reichman, B. (2008). Delivery mode and severe intraventricular hemorrhage in single, very low birth weight, vertex infants. *Obstetrics and Gynecology*, *112*, 21–28.

Spiegler, J., Kattner, E., Vochem, M., Küster, H., Möller, J., Müller, D., Kribs, A., Segerer, H., Wieg, C., Nikischin, W., von der Wense, A., Gebauer, C., Herting, E., & Göpel, W. (2008). Very low birth weight infants as a model to study genetic influences on neonatal weight gain. *Journal of Pediatric Gastroenterology and Nutrition*, *46*, 113–116.

Tyson, J. E., Parikh, N. A., Langer, J., Green, C., & Higgins, R. D. (2008). Intensive care for extreme prematurity - moving beyond gestational age. *New England Journal of Medicine*, *358*, 1672–1681.

Wurmb-Schwark, N., Schwark, T., Christiansen, L., Lorenz, D., & Oehmichen, M. (2004). The use of different multiplex PCRs for twin zygosity determination and its application in forensic trace analysis. *Legal Medicine*, *6*, 125–130.