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Neonatal adverse outcome in twin pregnancies complicated by small-for-gestational age: twin *vs* singleton reference chart

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Short title Twin versus singleton charts and neonatal outcomes.

Key words: twin pregnancy; birthweight; chorionicity; specific; singleton; reference charts; ultrasound; neonatal outcomes; estimated fetal weight

Contribution

What are the novel findings of this work?

We present a comparison of twin specific estimated fetal weight and birthweight charts versus singleton reference charts and their ability to predict adverse neonatal outcomes in small for gestational age twins. Twin specific charts were more strongly associated with neonatal adverse outcomes than singleton standard charts.

What are the clinical implications of this work?

This study presents further evidence that twin specific charts are better predictors of adverse neonatal outcomes. The use of these charts may reduce misclassification and improve identification of infants who may not be at increased risk of adverse outcomes despite being labelled as small for gestational age by singleton standards.

ABSTRACT

Objective

The use of twin-specific versus singleton charts in the assessment of twin pregnancies has been controversial. The aim of the study was to assess whether a diagnosis of small for gestational age (SGA) made using twin specific estimated fetal weight (EFW) and birthweight (BW) charts is more strongly associated with adverse neonatal outcomes compared to singleton charts in twin pregnancies.

Methods

This was a cohort study of twin pregnancies delivered at St George's Hospital in London between January 2007 and May 2020. Twin pregnancies complicated by intrauterine demise of one or both twins; aneuploidy or major fetal abnormality, twin-to-twin transfusion syndrome or twin anemia polycythemia sequence (TAPS); and those delivered before 32 weeks' gestation, were excluded. SGA was defined as EFW or BW below the 10th centile. The main study outcome was composite neonatal morbidity, which was stratified to mild or severe for sensitivity analysis. Mixed-effects logistic regression analysis with random pregnancy level intercepts was used to test the association between different SGA classifications and adverse neonatal outcomes.

Results

A total of 1329 twin pregnancies were identified, and 913 twin pregnancies (1826 infants) included in the analysis. Of these, 723 (79.2%) were dichorionic and 190 (20.8%) monochorionic. Using the singleton charts, 33.3% and 35.7% were classified as SGA by the singleton chart when using EFW and BW, respectively. The corresponding figures were 5.9% and 5.8% when using the twin specific charts. EFW SGA according to the twin charts, had a significant association with neonatal morbidity (OR 4.78, 95% CI 1.47-14.7, P=0.007), when compared to AGA twins. However, EFW below the 10th percentile according to singleton standards did not have a significant association with neonatal morbidity (OR 1.36, 95% CI 0.63-2.88, P=0.424).

SGA classification of EFW using twin specific standards significantly better model fit than using singleton standard (P<0.001, likelihood ratio test). When twin charts were used for BW classification, BW SGA was significantly associated with 9.2 times increased odds of neonatal morbidity (P<0.001). Neonates classified as SGA only with singleton BW standard, but not with twin specific charts, had a significantly lower rate of adverse outcomes (OR 0.24, 95% CI 0.07-0.66, P=0.009), when compared to AGA twins.

Conclusion

The singleton charts classified one third of twins as SGA, both prenatally and postnatally. SGA infants according to the twin specific charts, but not the singleton charts, had a significantly increased risk of adverse neonatal outcomes. This study provides further evidence that twin specific charts are better predictors of adverse neonatal outcomes; the use of these charts may reduce misclassification of twins as SGA and improve identification of those infants who are truly growth restricted.

INTRODUCTION

When compared to singletons, twin pregnancies are at increased risk of developing neonatal complications. Small for gestational age (SGA) infants, defined internationally as an infant whose birthweight is less than the 10th centile for gestational age, are at greater risk of neonatal complications, including hypoglycemia, necrotizing enterocolitis (NEC), sepsis, polycythemia, intraventricular hemorrhage (IVH) and prolonged hospitalization [1–3].

It is common practice to assess the growth of twins using population-based singleton growth charts. However, recent evidence suggests that it may be more accurate to use twin specific growth charts, as singleton standards label a large proportion of twin pregnancies as SGA, due to the differences in growth trajectory between twins and singletons [4]. Overestimating the risk of morbidity and mortality potentially leads to increased iatrogenic intervention, increased maternal anxiety, and the subsequent risks associated with preterm birth [1,5,6]. A number of twin specific growth references have been published [7–9]. However, it is essential to assess thoroughly twin references and their ability to predict adverse outcomes when compared to singleton reference standards, primarily because it is unknown why twin growth trajectories deviate from their singleton counterparts. This difference may be due to a physiological adaptation for twin pregnancies or possibly due to true placental insufficiency [10,11]. If twin pregnancies represent true placental insufficiency, the introduction of twin specific reference charts may miss some high-risk pregnancies, potentially leading to an increased rate of neonatal morbidity and mortality.

The objective of this study was to compare the performance of recently published twin growth and birthweight charts [12,13] to previously published modern singleton reference charts[14] in identifying SGA twin infants at risk of neonatal morbidity.

METHODS

Study Population and data variables

This was a cohort study of twin pregnancies followed up prenatally and delivered at St George's Hospital, London, UK, between January 2007 and May 2020. Available monochorionic (MC) and dichorionic (DC) twin pregnancy records were identified by searching the electronic maternity and neonatal records (United Kingdom National Neonatal Research Database, BadgerNet, Clevermed Ltd and ViewPoint version 5.6.26.148, ViewPoint Bildverarbeitung GMBH, Wessling, Germany). Maternal data (age in years, parity, body mass index (BMI) in kg/m^2, self-reported ethnicity, mode of conception, alcohol and tobacco consumption during pregnancy) and perinatal outcomes (gestational age at birth, mode of birth, birthweight, and adverse maternal and neonatal outcomes) were collected from the maternity database and neonatal records. Twin pregnancies complicated by intrauterine demise (IUD) of one or both twins; aneuploidy or major fetal abnormality, twin-to-twin transfusion syndrome or twin anemia polycythemia sequence (TAPS); those with missing data regarding gestational age at birth or neonatal outcome; and those delivered before 32 weeks' gestation, were excluded.

Hypertensive disorders of pregnancy (HDP) included gestational hypertension and preeclampsia and were defined by guidelines of the International Society for the Study of Hypertension in Pregnancy (ISSHP) [15]. Gestational age was determined according to the crown-rump length (before 14 weeks' gestation) or head circumference (after 14 weeks' gestation) of the larger fetus in cases of spontaneous conception and according to the embryonic age from fertilization when in-vitro fertilization was carried out [16–18]. Chorionicity was determined prenatally using the presence or absence of the lambda sign at the intertwin membrane–placenta junction at 11–14 weeks, or the number of placentas and the fetal gender after 14 weeks' gestation[16,19]. The last prenatal ultrasound examination reporting estimated fetal weight (EFW) was used for the analysis. EFW was calculated using Hadlock's formula including the following biometry: head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL)[20]. The centiles of EFW were calculated adopting the singleton chart by Nicolaides et al**.** [14] and the twin specific charts by Stirrup et al**.**[13], while birthweight centiles were assessed using the singleton standard reported by Nicolaides et al. [14] and twin chorionicity-specific reference standards reported by Briffa et al. [12] Fetuses with EFW less than 10th centile and newborns with birthweight less than the 10^{th} centile were defined as SGA.

The follow-up of twin pregnancies was performed in line with both the National Institute of Health and Care Excellence (NICE) and International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guidelines. Uncomplicated DC twin pregnancies had scans every 4 weeks thereafter second trimester while complicated DC twins were scanned more frequently, depending on the condition and its severity. Delivery was planned starting from 37 weeks' gestation in uncomplicated DC twin pregnancies. Uncomplicated MC twins were scanned biweekly after 16 weeks. Scanning period was individulized in complicated MC twins. Delivery was planned starting from 36 weeks' gestation in uncomplicated MCDA twin pregnancies and starting from 32 weeks' in MCMA twin pregnancies.

The study outcomes were composite neonatal morbidity, which included the following adverse outcomes; oxygen supplementation or continuous positive airway pressure (CPAP) for <72 hours, hypoglycemia, hypocalcemia, hyperbilirubinemia, or IVH, NEC, bronchopulmonary dysplasia, respiratory distress syndrome (RDS), mechanical ventilation, or neonatal death. We also planned a sensitivity analysis according to severity of adverse outcomes. Mild composite morbidity consisted of oxygen supplementation or CPAP for <72 hours, hypoglycemia, hypocalcemia, hyperbilirubinemia, or IVH grade I/II. Severe morbidity included NEC, IVH grade III/IV, bronchopulmonary dysplasia, mechanical ventilation, RDS, or neonatal death.

Statistical Analysis

Categorical data were presented as number (%) and compared using Chi-squared test. The Shapiro-Wilk test tested the normal distribution of continuous variables, and continuous data were presented as median (interquartile range, IQR). Mixed-effects logistic regression analyses with random pregnancy level intercepts were used to test the association between the different SGA classifications and adverse neonatal outcomes. Pregnancy level intercepts were used to account for the dependency structure between twins. Independent variables were scaled to help model convergence, and appropriate optimization algorithms were employed where needed. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. P-values below 0.05 were considered statistically significant. The statistical analysis was performed using RStudio (Version 1.0.136, Rstudio, Inc.) statistical software.

RESULTS

A total of 1329 twin pregnancies were identified, and 913 twin pregnancies (1826 infants) met the inclusion criteria (Figure1). Of these, 723 (79.2%) were DC and 190 (20.8%) MC. The demographic and obstetric characteristics of the participants are shown in Table 1. The median (IQR) gestational age at birth was 37.4 (36.1–38.4) weeks, and the median (IQR) gestational age at last ultrasound was 35.1 (34.3–36.0) weeks; the time difference between the last ultrasound and birth was 1.4 (0.9-2.3) weeks.

According to the EFW calculated at the last ultrasound examination, 1218 (66.7%) fetuses were appropriate for gestational age (AGA), and 608 (33.3%) were classified as SGA by the singleton chart, while 107 (5.9%) fetuses were considered SGA according to the twin chart. Similar proportions were obtained for birthweight centiles; 1175 (64.3%) newborns were classified as AGA, 651 (35.7%) as SGA according to singleton birthweight charts, and 103 (5.6%) classified as SGA according to the twin specific chart. All those identified as SGA, whether using EFW or birthweight, by the twin chart were also identified as SGA using the singleton chart.

Table 2 shows the proportions of neonatal adverse outcomes in SGA fetuses defined prenatally using EFW by the twin charts, singleton charts, and only by singleton standard (not by twin one). Composite morbidities were significantly more frequent in SGA than in AGA fetuses when classified using the twin (32.7% vs. 8.4%, P<0.001) or singleton (13.6% vs. 7.9%, P<0.001) charts to define SGA (Table 2). However, twins classified as SGA by singleton standard only did not have an increased rate of composite neonatal morbidity when compared to AGA fetuses (9.6 vs 7.9%, P=0.247). The results of the sensitivity analysis were similar for severe neonatal morbidity (Table S1). Fetuses classified as SGA by twin standards had a significantly higher risk of severe neonatal morbidity (14.0% vs 3.3%, P<0.001) compared to AGA fetuses while fetuses classified as SGA by singleton standard only did not (P=0.315).

Table 3 shows the proportions of neonatal adverse outcomes in SGA babies classified using birthweight twin charts, singleton charts, and only by singleton standard (not by twin one). Composite neonatal morbidity was significantly more frequent in SGA fetuses than in AGA fetuses using the twin (24.3% vs. 8.9%, P<0.001) but not the singleton chart (9.4% vs. 10.0%, P=0.643). Moreover, neonates classified as SGA by the singleton standard only had a significantly lower rate of composite neonatal morbidity compared to AGA babies (6.6% vs 10.0%, P=0.018). Results of the sensitivity analysis showed similar results regarding severe neonatal morbidity (Table S2). Severe neonatal morbidity was almost two-fold higher in SGA babies diagnosed using the twin standard when compared to AGA, but the difference did not

reach statistical significance (6.8% vs 3.8%, P=0.126). There was no significant difference in the incidence of severe neonatal morbidity when the twins were classified using the singleton standard (4.0% vs 3.8%, AGA and SGA, respectively, P=0.866)

Mixed-effects regression analysis was undertaken to account for dependency structure between twin pairs. The analysis showed baseline characteristics such as maternal age, parity, ethnicity, smoking, alcohol use or BMI were not associated with mild or severe neonatal adverse composite outcomes (P>0.05 for all, Table 4). Neither were pregnancy level effects such as chorionicity, method of conception, or hypertensive disorders of pregnancy associated with neonatal morbidity (P>0.05 for all). Gestational age at birth was significantly associated with neonatal morbidity (OR 0.11, 95% CI 0.06-0.19, P<0.001). SGA using EFW according to the twin charts was significantly associated with neonatal morbidity (OR 4.78, 95% CI 1.47-14.7, P=0.007). SGA using EFW according to singleton standard did not have a significant association with neonatal morbidity (OR 1.36, 95% CI 0.63-2.88, P=0.424). Fetuses labeled as SGA by the singleton standard only were not at significantly increased risk of neonatal morbidity (OR 0.69, 95% CI 0.28-1.61, P=0.408). According to the likelihood ratio test, SGA classification using twin specific standards performed significantly better than singleton standard (P<0.001). Similar results were obtained for the classification of birthweight according to twin specific and singleton standards. The likelihood ratio test showed SGA according to the twin specific charts was significantly better than according to singleton standards (P<0.001). When twin charts were used for classification, SGA using birthweight was associated with ~9.2 times increased odds of neonatal morbidity (P<0.001). The singleton standard was associated with ~1.1 times decreased odds of adverse outcomes without statistical significance (P=0.790). Neonates classified as SGA using the singleton birthweight standard but not with twin specific charts had a significantly lower rate of adverse outcomes (OR 0.24, 95% CI 0.07-0.66, P=0.009).

We performed sensitivity analyses according to the severity of neonatal morbidity (mild versus severe) (Table 4). SGA according to the twin chart was significantly associated with mild composite adverse outcomes (OR 4.15, 95% CI 1.21-13.1, P=0.018). SGA according to singleton standards did not have a significant association with mild composite adverse outcomes (OR 1.31, 95% CI 0.54-3.05, P=0.529). The insignificant association was in the reverse direction for fetuses labeled as SGA by singleton standards only (OR 0.62, 95% CI: 0.22-1.63, P=0.358). According to the likelihood ratio test, SGA classification using EFW twin specific standards performed significantly better than singleton standard labeling (P<0.001). Similar results were obtained for classifying the birthweight according to twin specific and singleton standards. The likelihood ratio test showed that SGA labeling according to twin specific charts was significantly better than labeling with singleton standards (P<0.001). When

twin charts were used for classification, birthweight below the 10th centile was associated with 17.0 times increased odds of mild composite adverse outcomes (P<0.001). The singleton standard was associated with ~1.04 times increased odds of mild adverse outcomes without statistical significance (P=0.935). Neonates classified as SGA using the singleton birthweight standard but not with twin specific charts had a significantly lower rate of mild adverse outcomes (OR 0.12, 95% CI 0.02-0.45, P=0.004). Neither twin nor singleton chart SGA diagnoses had a significant association with severe adverse neonatal outcomes (P>0.05 for all) in this subgroup.

DISCUSSION

Summary of main findings

Both prenatally and postnatally, singleton charts classified a greater proportion of infants as SGA compared with twin specific EFW and birthweight charts. Those labeled as SGA using the twin charts had significantly increased risk of adverse neonatal outcomes than those identified as AGA. Those classified as SGA by the singleton charts only, and not by the twin charts, were at reduced risk of adverse neonatal outcomes. A similar pattern was observed classifying SGA using EFW when the analysis was stratified for mild and severe neonatal morbidity. SGA classification using BW was not significantly associated with severe morbidity, regardless of charts used.

Interpretation of study findings and comparison with published literature

Our findings suggest that, compared to the singleton reference, twin specific charts can more accurately identify those infants who have the highest risk of developing adverse neonatal outcomes. This superior accuracy of twin charts has also been demonstrated in previous studies investigating other perinatal outcomes[1,21,22]. A similar link between twin birthweight and neonatal mortality was identified by *Gielene et al*. [22] Our previous study identified that these twin specific charts also outperformed singleton charts at predicting abnormal fetal Dopplers[21], suggesting that the twin charts were better at identifying fetal growth restriction, and might therefore be expected to correlate better with adverse perinatal outcomes. In this study we have shown that twin charts are better than singleton reference in identifying the twins at increased risk of neonatal morbidity. We used both birth weight and estimated fetal weight classification compared to previous studies that mostly used birth weight classification.[23,24] Estimated fetal weight based classification and its association with adverse outcomes are clinically more relevant than birth weight classification from the obstetricians perspective. Finally, gestational age at birth was more strongly associated with the risk of severe neonatal morbidity than was a diagnosis of SGA, whether made with twin or singleton charts, a finding consistent with the existing literature [25,26].

Clinical and research implications

Our study supports the growing evidence that moving from a ‘one size fits all’ approach with singleton charts to the use of twin specific reference charts will lead to fewer twins categorized as SGA. A key finding is that infants identified as SGA using the singleton reference alone, and not with twin charts, have similar outcomes to twins classified as AGA by both references. This evidence provides reassurance that the use of twin specific charts will not miss any twins

at increased risk of neonatal adverse outcomes, denying them the increased surveillance afforded to those categorized as SGA. Our findings suggest that the divergence of growth seen between twin pregnancies and singleton pregnancy in the third trimester may result from typical physiological adaptation rather than a pathological process [5]. Our study suggests that the use of these singleton references has the potential to inappropriately label a significant proportion of twins as growth restricted, leading to increased antenatal surveillance and, most importantly, increased iatrogenic interventions, potentially including iatrogenic preterm birth. Finally, we have built on previous research that concluded that twin specific charts are better predictors of abnormal fetal Dopplers, neonatal morbidity and mortality, and in the current study have shown a stronger association with adverse neonatal outcomes, strengthening the evidence that twin references may be safer to use.

Considering this body of evidence, it would be prudent to perform more extensive prospective studies to determine the potential harm associated with the use of singleton charts in the assessment of fetal growth in twin pregnancies. This potential harm is mainly secondary to unnecessary medical intervention and iatrogenic preterm birth. The use of ultrasound scan to screen for fetal growth restriction in twin pregnancies is a screening tool and its false positive, as well as false negative, should be taken into account. Furthermore, the role of fetal Doppler assessment in twin pregnancies is yet to be fully ascertained. A prospective observational study could be performed by implementing twin specific standards and comparing adverse outcome trends in before and after fashion. However, a double-blind randomized trial comparing the two standards would provide more direct and conclusive evidence. It is technically possible to conceal group allocation (twin-specific vs. singleton), though clinicians are usually familiar with weight percentiles across the gestational age spectrum and effective blinding may not be possible.

Strengths and limitations

Our study's main strength is that we analyzed a large and diverse cohort of twin pregnancies, using contemporary twin specific and singleton reference charts, which were not used in the clinical management of these pregnancies, reducing the risk of intervention bias. Moreover, the singleton reference chart we opted to use was developed from a very similar cohort and from a large sample size.

The limitations include the relatively small number of infants labeled as having one of the adverse neonatal outcomes measured. These small numbers means that the analysis might have been underpowered. More extensive multicenter studies would be required to overcome this. While some of our findings are significant, there is considerable overlap in the 95%

confidence interval between the twin and singleton charts. These significant findings are representative of the cohort assessed. Therefore, further studies are required to assess the generalizability of these findings to a large population. Finally, evidence suggests that SGA infants are at risk of chronic conditions, including cardiovascular, endocrine and neurological sequelae [2,27–30], important outcomes to be investigated in future studies. Finally, existing twin specific standards (plain, chorinicity specific, further customized etc.) should be compared to understand which one would be most beneficial to use in future studies.

Conclusion

Our study shows that the twin specific EFW and birthweight charts reduce the number of twins labeled as SGA. Both the EFW and birthweight twin specific charts are more specific at identifying SGA infants at greatest risk of developing adverse neonatal outcomes. Importantly, twins categorized as SGA by singleton charts but not by twin standards did not a have greater risk of adverse neonatal outcomes. Consequently, classification as SGA using twin specific charts rather than singleton charts will avoid some pregnancies being subjected to unnecessary iatrogenic interventions and increased risk of iatrogenic preterm birth.

REFERENCES

1. Kalafat E, Sebghati M, Thilaganathan B, Khalil A; Southwest Thames Obstetric Research Collaborative (STORK). Predictive accuracy of Southwest Thames Obstetric Research Collaborative (STORK) chorionicity-specific twin growth charts for stillbirth: a validation study. Ultrasound Obstet Gynecol. 2019;53:193-199. doi: 10.1002/uog.19069.

2. Unterscheider J, O'Donoghue K, Daly S, Geary MP, Kennelly MM, McAuliffe FM, Hunter A, Morrison JJ, Burke G, Dicker P, Tully EC, Malone FD. Fetal growth restriction and the risk of perinatal mortality-case studies from the multicentre PORTO study. BMC Pregnancy Childbirth. 2014;14:63. doi: 10.1186/1471-2393-14-63.

3. Society for Maternal-Fetal Medicine (SMFM) Martins JG, Biggio JR, Abuhamad A. Society for Maternal-Fetal Medicine Consult Series #52: Diagnosis and management of fetal growth restriction: (Replaces Clinical Guideline Number 3, April 2012). Am J Obstet Gynecol. 2020;223:B2-B17. doi: 10.1016/j.ajog.2020.05.010.

4. Kalafat E, Abiola A, Thilaganathan B, Bhide A, Khalil A. The Association Between Hypertension in Pregnancy and Preterm Birth with Fetal Growth Restriction in Singleton and Twin Pregnancy: Use of Twin Versus Singleton Charts. J Clin Med. 2020;9:2518. doi: 10.3390/jcm9082518.

5. Grantz KL, Grewal J, Albert PS, Wapner R, D'Alton ME, Sciscione A, Grobman WA, Wing DA, Owen J, Newman RB, Chien EK, Gore-Langton RE, Kim S, Zhang C, Buck Louis GM, Hediger ML. Dichorionic twin trajectories: the NICHD Fetal Growth Studies. Am J Obstet Gynecol. 2016;215:221.e1-221.e16. doi: 10.1016/j.ajog.2016.04.044.

6. Herruzo AJ, Martínez L, Biel E, Robles R, Rosales MA, Miranda JA. Perinatal morbidity and mortality in twin pregnancies. Int J Gynaecol Obstet. 1991;36:17-22. doi: 10.1016/0020-7292(91)90172-2.

7. Shivkumar S, Himes KP, Hutcheon JA, Platt RW. An ultrasound-based fetal weight reference for twins. Am J Obstet Gynecol. 2015;213:224.e1-9. doi: 10.1016/j.ajog.2015.04.015.

8. Premkumar P, Antonisamy B, Mathews J, Benjamin S, Regi A, Jose R, Kuruvilla A, Mathai M. Birth weight centiles by gestational age for twins born in south India. BMC Pregnancy Childbirth. 2016;16:64. doi: 10.1186/s12884-016-0850-y.

9. Ananth CV, Vintzileos AM, Shen-Schwarz S, Smulian JC, Lai YL. Standards of birth weight in twin gestations stratified by placental chorionicity. Obstet Gynecol.

1998;91:917-24. doi: 10.1016/s0029-7844(98)00052-0.

10. Stefos T, Deter RL, Hill RM, Simon N. Individual growth curve standards in twins: growth in the second trimester. J Clin Ultrasound. 1989;17:641-6. doi: 10.1002/jcu.1870170905.

11. Deter RL, Lee W, Yeo L, Erez O, Ramamurthy U, Naik M, Romero R. Individualized growth assessment: conceptual framework and practical implementation for the evaluation of fetal growth and neonatal growth outcome. Am J Obstet Gynecol. 2018;218:S656-S678. doi: 10.1016/j.ajog.2017.12.210.

12. Briffa C, Stirrup O, Huddy C, Richards J, Shetty S, Reed K, Khalil A. Twin chorionicity-specific population birth-weight charts developed with adjustment for estimated fetal weight. Ultrasound Obstet Gynecol. 202. doi: 10.1002/uog.23606. Epub ahead of print.

13. Stirrup OT, Khalil A, D'Antonio F, Thilaganathan B; Southwest Thames Obstetric Research Collaborative (STORK). Fetal growth reference ranges in twin pregnancy: analysis of the Southwest Thames Obstetric Research Collaborative (STORK) multiple pregnancy cohort. Ultrasound Obstet Gynecol. 2015;45:301-7. doi: 10.1002/uog.14640.

14. Nicolaides KH, Wright D, Syngelaki A, Wright A, Akolekar R. Fetal Medicine Foundation fetal and neonatal population weight charts. Ultrasound Obstet Gynecol. 2018;52:44-51. doi: 10.1002/uog.19073.

15. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S; International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension. 2018;72:24-43. doi: 10.1161/HYPERTENSIONAHA.117.10803.

16. Khalil A, Rodgers M, Baschat A, Bhide A, Gratacos E, Hecher K, Kilby MD, Lewi L, Nicolaides KH, Oepkes D, Raine-Fenning N, Reed K, Salomon LJ, Sotiriadis A, Thilaganathan B, Ville Y. ISUOG Practice Guidelines: role of ultrasound in twin pregnancy. Ultrasound Obstet Gynecol. 2016;47:247-63. doi: 10.1002/uog.15821. Erratum in: Ultrasound Obstet Gynecol. 2018;52:140.

17. Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol. 1975;82:702-10. doi: 10.1111/j.1471-0528.1975.tb00710.x.

18. Dias T, Ladd S, Mahsud-Dornan S, Bhide A, Papageorghiou AT, Thilaganathan B.

Systematic labeling of twin pregnancies on ultrasound. Ultrasound Obstet Gynecol. 2011;38:130-3. doi: 10.1002/uog.8990.

19. Poon LC, Tan MY, Yerlikaya G, Syngelaki A, Nicolaides KH. Birth weight in live births and stillbirths. Ultrasound Obstet Gynecol. 2016;48:602-606. doi: 10.1002/uog.17287.

20. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985;151:333-7. doi: 10.1016/0002-9378(85)90298-4.

21. Giorgione V, Briffa C, Di Fabrizio C, Bhate R, Khalil A. Perinatal Outcomes of Small for Gestational Age in Twin Pregnancies: Twin vs. Singleton Charts. J Clin Med. 2021;10:643. doi: 10.3390/jcm10040643.

22. Gielen M, Lindsey PJ, Derom C, Loos RJ, Souren NY, Paulussen AD, Zeegers MP, Derom R, Vlietinck R, Nijhuis JG. Twin-specific intrauterine 'growth' charts based on cross-sectional birthweight data. Twin Res Hum Genet. 2008;11:224-35. doi: 10.1375/twin.11.2.224.

23. Nowacka U, Kosińska-Kaczyńska K, Krajewski P, Saletra-Bielińska A, Walasik I, Szymusik I. Predictive Accuracy of Singleton Versus Customized Twin Growth Chart for Adverse Perinatal Outcome: A Cohort Study. Int J Environ Res Public Health. 2021; 18: 2016.

24. Lin D, Rao J, Fan D, Huang Z, Zhou Z, Chen G, Li P, Lu X, Lu D, Zhang H, Luo C, Guo X, Liu Z. Should singleton birth weight standards be applied to identify small-for-gestational age twins?: analysis of a retrospective cohort study. BMC Pregnancy Childbirth. 2021; 21: 446.

25. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA, Kennedy KA, Poindexter BB, Finer NN, Ehrenkranz RA, Duara S, Sánchez PJ, O'Shea TM, Goldberg RN, Van Meurs KP, Faix RG, Phelps DL, Frantz ID 3rd, Watterberg KL, Saha S, Das A, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126:443-56. doi: 10.1542/peds.2009-2959.

26. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371:261-9. doi: 10.1016/S0140-6736(08)60136-1.

27. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med. 1999;340:1234-8. doi:

10.1056/NEJM199904223401603.

28. Cruz-Lemini M, Crispi F, Valenzuela-Alcaraz B, Figueras F, Sitges M, Bijnens B, Gratacós E. Fetal cardiovascular remodeling persists at 6 months in infants with intrauterine growth restriction. Ultrasound Obstet Gynecol. 2016;48:349-56. doi: 10.1002/uog.15767.

29. Tolsa CB, Zimine S, Warfield SK, Freschi M, Sancho Rossignol A, Lazeyras F, Hanquinet S, Pfizenmaier M, Huppi PS. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. Pediatr Res. 2004;56:132-8. doi: 10.1203/01.PDR.0000128983.54614.7E.

30. Gidi NW, Goldenberg RL, Nigussie AK, McClure E, Mekasha A, Worku B, Siebeck M, Genzel-Boroviczeny O, Muhe LM. Comparison of neonatal outcomes of small for gestational age and appropriate for gestational age preterm infants born at 28-36 weeks of gestation: a multicentre study in Ethiopia. BMJ Paediatr Open. 2020;4:e000740. doi: 10.1136/bmjpo-2020-000740.

Figure legends

Figure 1. Study flow chart

Table 1: Baseline demographics and pregnancy characteristics of the study cohort

Twin Pregnancies (n=913)	
Maternal age in years, median (IQR)	34.0 (30.0 – 38.0)
Maternal body mass index at booking in kg/m2, median (IQR)	24.6 (22.1 - 27.9)
Gestational age at delivery in weeks, median (IQR)	37.0 (36.1 - 37.4)
Gestational age at last scan in weeks, median (IQR)	35.1 (34.3 – 36.0)
Difference between last scan and delivery in weeks, median (IQR)	1.43 (0.86 - 2.29)
Nulliparity, n (%)	755 (82.7)
Self-reported ethnicity, n (%) ○ Caucasian ○ Black ○ South Asian ○ East Asian ○ Other/Mixed ○ Missing	 603 (66.1) 119 (13.0) 118 (12.9) 20 (2.2) 50 (5.5) 3 (0.3)
Alcohol use, n (%)	23 (2.5)
Smoking, n (%)	33 (3.6)
Chorionicity, n (%) ○ Dichorionic ○ Monochorionic	 723 (79.2) 190 (20.8)
Assisted conception, n (%)	274 (30.0)
Hypertensive Disorders of Pregnancy, n (%) ○ Preeclampsia ○ Gestational hypertension	 60 (6.6) 49 (5.4)
Vaginal delivery of both twins, n (%)	247 (27.1)

IQR: interquartile range

Table 2. Neonatal adverse outcomes in twins classified as appropriate (AGA) or small-for-gestational age (SGA) according to estimated fetal weight status using singleton and twin charts

	Estimated fetal weight status							
Adverse neonatal outcome	**AGA by twin chart (n=1719)**	**SGA by twin chart (n=107)**	***P value****	**AGA by singleton (n=1218)**	**SGA by singleton (n=608)**	**SGA only by singleton (n=501)**	***P value†***	***P value ‡***
Any composite morbidity, n (%)	144 (8.4)	35 (32.7)	<.001	96 (7.9)	83 (13.6)	48 (9.6)	<.001	.247
– CPAP < 72 hours, n (%)	49 (2.9)	3 (2.8)		36 (3.0)	16 (2.6)	13 (2.6)		
– Hypoglycaemia, n (%)	18 (1.0)	6 (5.6)		7 (0.6)	17 (2.8)	11 (2.2)		
– Hypocalcemia, n (%)	1 (0.1)	0 (0.0)		1 (0.1)	0 (0.0)	0 (0.0)		
– Hyperbilirubinemia, n (%)	60 (3.5)	23 (21.5)		37 (3.0)	46 (7.6)	23 (4.6)		
– IVH grade I/II, n (%)	4 (0.2)	3 (2.8)		2 (0.2)	5 (0.8)	2 (0.4)		
– IVH Grade-III/IV, n (%)	0 (0.0)	1 (0.9)		0 (0.0)	1 (0.2)	0 (0.0)		
– NEC, n (%)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)		
– Bronchopulmonary dysplasia, n (%)	1 (0.1)	1 (0.9)		1 (0.1)	1 (0.2)	0 (0.0)		
– Respiratory distress syndrome, n (%)	54 (3.1)	12 (11.2)		34 (2.8)	32 (5.3)	20 (4.0)		
– Mechanical ventilation, n (%)	19 (1.1)	11 (10.3)		14 (1.1)	16 (2.6)	5 (1.0)		
– Neonatal death, n (%)	0 (0.0)	1 (0.9)		0 (0.0)	1 (0.2)	0 (0.0)		

* AGA by twin chart vs SGA by twin chart; † AGA by singleton vs SGA by singleton; ‡ AGA by singleton vs SGA only by singleton

EFW: Estimated fetal weight, SGA: small for gestational age; AGA: appropriate for gestational age; CPAP: Continuous positive airway pressure, IVH: Intraventricular hemorrhage; NEC: necrotising enterocolitis

Table 3. Neonatal adverse outcomes in twins classified as appropriate (AGA) or small-for-gestational age (SGA) according to birthweight status using singleton and twin charts

	Birthweight status							
Adverse neonatal outcome	**AGA by twin chart (n=1721)**	**SGA by twin chart (n=103)**	***P value****	**AGA by singleton (n=1175)**	**SGA by singleton (n=651)**	**SGA only by singleton (n=548)**	***P value†***	***P value ‡***
Any composite morbidity, n (%)	154 (8.9)	25 (24.3)	<.001	118 (10.0)	61 (9.4)	36 (6.6)	.643	.018
– CPAP < 72 hours, n (%)	50 (2.9)	2 (3.8)		38 (3.2)	14 (2.1)	12 (2.2)		
– Hypoglycaemia, n (%)	19 (1.1)	5 (20.8)		15 (1.3)	9 (1.4)	4 (0.7)		
– Hypocalcemia, n (%)	1 (0.1)	0 (0.0)		1 (0.1)	0 (0.0)	0 (0.0)		
– Hyperbilirubinemia, n (%)	70 (4.1)	13 (15.7)		55 (4.7)	28 (4.3)	15 (2.7)		
– IVH grade I/II, n (%)	6 (0.4)	1 (14.3)		4 (0.3)	3 (0.5)	2 (0.4)		
– IVH Grade-III/IV, n (%)	0 (0.0)	1 (1.0)		0 (0.0)	1 (0.2)	0 (0.0)		
– NEC, n (%)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)		
– Bronchopulmonary dysplasia, n (%)	1 (0.1)	1 (1.0)		1 (0.1)	1 (0.2)	0 (0.0)		
– Respiratory distress syndrome, n (%)	62 (3.6)	4 (3.9)		45 (3.8)	21 (3.2)	17 (3.1)		
– Mechanical ventilation, n (%)	23 (1.3)	7 (6.8)		15 (1.3)	15 (2.3)	8 (1.4)		
– Neonatal death, n (%)	0 (0.0)	1 (1.0)		0 (0.0)	1 (0.2)	0 (0.0)		

* AGA by twin chart vs SGA by twin chart; † AGA by singleton vs SGA by singleton; ‡ AGA by singleton vs SGA only by singleton

EFW: Estimated fetal weight, SGA: small for gestational age; AGA: appropriate for gestational age; CPAP: Continuous positive airway pressure, IVH: Intraventricular hemorrhage; NEC: necrotising enterocolitis

Table 4. Mixed-effects regression results for mild and severe composite adverse outcomes

	Outcome: Any (mild or severe) neonatal morbidity		**Outcome: Mild neonatal morbidity***		**Outcome: Severe neonatal morbidity†**	
Variables	**OR (95% CI)**	**P value‡**	**OR (95% CI)**	**P value‡**	**OR (95% CI)**	**P value‡**
Maternal age in years	1.11 (0.65 – 1.95)	.686	1.15 (0.65 – 2.12)	.627	0.99 (0.49 – 2.09)	.672
Multiparity	1.04 (0.35 – 3.00)	.941	1.20 (0.38 – 3.83)	.746		
Self-reported ethnicity						
- Caucasian	Reference		Reference		Reference	
- Afro-Caribbean	0.81 (0.48 – 1.74)	.810	0.75 (0.46 – 1.63)	.762	0.98 (0.06 – 6.54)	.985
- Asian	0.66 (0.58 – 1.47)	.629	0.57 (0.49 – 1.49)	.568	0.83 (0.05 – 5.47)	.867
- Other-mixed	1.17 (0.59 – 2.21)	.717	1.54 (0.56 – 2.35)	.690	1.24 (0.02 –12.9)	.883
Smoker	0.76 (0.06 – 8.43)	.863	0.26 (0.01 – 6.64)	.622	1.54 (0.01 – 1.92)	.793
Alcohol use	0.87 (0.02 – 13.1)	.941	NE	NA	2.34 (0.01 – 34.1)	.618
Maternal body mass index in kg/m2	1.17 (0.63 – 1.94)	.517	1.21 (0.68 – 1.98)	.464	1.07 (0.49 – 1.97)	.846
Chorionicity						
- Dichorionic	Reference		Reference		Reference	
- Monochorionic	2.03 (0.60 – 6.24)	.224	2.29 (0.64 – 7.58)	.176	1.20 (0.20 –5.32)	.818
Assisted conception	0.81 (0.22 – 2.54)	.730	0.76 (0.17 – 2.65)	.689	0.87 (0.14 – 3.97)	.866
Hypertensive disorders of pregnancy						
- None	Reference		Reference			

- GH	2.06 (0.18 – 12.3)	.862	3.07 (0.32 – 17.6)	.748	1.12 (0.01 – 8.89)	.770
- Preeclampsia	0.81 (0.03 – 5.54)	.483	0.63 (0.10 – 5.57)	.244	0.55 (0.02 – 10.0)	.935
Gestational age at delivery in weeks	0.11 (0.06 – 0.19)	<.001	0.25 (0.12 – 0.42)	<.001	0.32 (0.17 – 0.56)	<.001
EFW <10th centile – twin chart	4.78 (1.47 –14.7)	.007	4.15 (1.21 – 13.1)	.018	2.18 (0.40 – 9.42)	.327
EFW <10th centile – singleton chart	1.36 (0.63 – 2.88)	.424	1.31 (0.54 – 3.05)	.529	1.38 (0.48 – 3.85)	.533
EFW <10th centile – singleton chart only	0.69 (0.28 – 1.61)	.408	0.62 (0.22 – 1.63)	.358	1.02 (0.32 –2.96)	.971
BW <10th centile – twin chart	9.27 (2.86 – 30.0)	<.001	17.0 (4.98 – 61.3)	<.001	0.51 (0.05 – 3.62)	.543
BW <10th centile – singleton chart	0.89 (0.36 – 2.10)	.790	1.04 (0.39 – 2.68)	.935	0.72 (0.20 – 2.29)	.587
BW <10th centile – singleton chart only	0.24 (0.07 – 0.66)	.009	0.12 (0.02 – 0.45)	.004	0.89 (0.24 – 2.90)	.846

All continuous variables are scaled, and odds ratios (OR) correspond to one standard unit change in each variable.

*Mild adverse outcomes included continuous positive airway pressure <72 hours, hypoglycaemia, hypocalcaemia, hyperbilirubinemia, Grade I or II intraventricular haemorrhage

†Severe adverse outcomes included bronchopulmonary dysplasia, mechanical ventilation, respiratory distress syndrome, necrotising enterocolitis, Grade III or IV intraventricular haemorrhage or neonatal death

‡Mixed effects generalised logistic regression with pregnancy level random intercepts

OR: odds ratio, CI: confidence interval, EFW: Estimated fetal weight, BW: birth weight, GH: gestational hypertension