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Research Article

Prospective Cohort of Fetal Lung Maturity in Twins Based on Birth Order, Birth Weight and Gender

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Abstract

Objective: Evaluate fetal lung maturity (FLM) tests in twins based on birth order, birth weight and gender. Our hypothesis is these variables are poor predictors of FLM correlation within twin pairs.

Design: Prospective cohort of twins was conducted (ClinicalTrials.gov:NCT01385267).

Methods: During cesarean delivery, amniotic fluid from each amniotic sac was aspirated, and analyzed for FLM index and lamellar body count (LBC). Established cutoff values were used to classify each infant as mature or not. Pregnancy and newborn outcomes were abstracted from the medical records.

Results: 42 patients with 84 twins were included. Median gestational age at delivery was 36 6/7 weeks (IQR=2.43). A ROC curve analysis in twin pregnancy was similar to singleton maturity threshold values (FLM index > 55mg/g and LBC>29 μL). There was good correlation for the numeric maturity values between twins A and B for FLM index (r=0.60) and moderate correlation for LBC (r=0.35). The positive predictive value (PPV) for twin B predicting twin A's mature result was 78% for FLM index and 64% for LBC. The PPV for the larger twin predicting the smaller twin's mature result was 67% for FLM index and 82% for LBC. There was no difference within twin pairs in the incidence of respiratory distress syndrome based on birth order, birth weight, or gender.

Conclusion: There is no variable (birth weight, birth order or gender) that is a good clinical predictor of which twin is likely to be mature or immature based on amniotic fetal lung maturity studies.

Keywords: Amniocentesis; FLM index test; Lamellar body count; Neonatal respiratory distress syndrome; Twins

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Introduction

Twin gestations accounted for 33.9 per 1000 births in 2014 in the USA; however, in that same year, over 58.7% of twins were born at less than 37 weeks [1]. ACOG does not provide specific recommendations about timing of a twin delivery but rather broad range of weeks based on concurrent diagnosis [2]. A recent NICHD workshop advocates delivery of uncomplicated dichorionic twins at 38 weeks and diamniotic-monochorionic twin pregnancies at 34 0/7 to 37 6/7 weeks [3]. These recommendations are based on observed rate of fetal death where the nadir of perinatal mortality for dichorionic twins is 38 completed weeks gestation [4-5]. However when a dichorionic twin pregnancy is complicated intrauterine growth restriction and with a concurrent conditions the range of 4 weeks is stated for optimal timing of delivery at 32 0/7 to 34 6/7 weeks [2].

Historically, when amniocentesis was performed to guide the decision for timing of delivery of twins prior to the NIHCD workshop guidelines, the question as to whether it is sufficient to sample only one amniotic sac for Fetal Lung Maturity (FLM) tests, based on birth order and estimated fetal weight. A common practice in the past of amniocenteses for FLM in twins is to only sample the larger twin, or the nonpresenting twin B, because this twin is more likely to develop Respiratory Distress Syndrome (RDS) than twin A, or the smaller, "more stressed" twin. This practice is based on older studies that concluded that only one twin sac need be aspirated for FLM at greater than 32 weeks gestation [6-8]. They utilized the Lecithin/Sphingomyelin ratio (L/S) test, which is limited in use because it is time consuming, expensive, and difficult to perform. The L/S ratio has been replaced by the FLM index and Lamellar Body Count (LBC) [9-12]. There has not been a contemporary prospective study evaluating fetal lung maturity in twins with the FLM index and LBC.

The aim of this study is to evaluate discordance in amniotic fluid fetal lung maturity studies and respiratory distress syndrome in diamniotic twins based on birth order, gender and birth weight. Our hypothesis is that birth order, birth weight, and gender are poor predictors for fetal lung maturation within twin pairs. A secondary objective is to affirm if the threshold values for fetal lung maturity tests established for singletons can be applied to twin pregnancies.

Materials and Methods

This was a prospective cohort of pregnant women with diamniotic twin gestations at greater than 24 weeks who were not in labor

and scheduled for cesarean delivery or amniocentesis for fetal lung maturity at Good Samaritan Hospital in Cincinnati OH, USA. IRB approval was obtained at TriHealth and the trial was registered through ClinicalTrials.gov identifier: NCT01385267. The study period for collection of amniotic samples was from July 2009 to August 2011. All patients signed written informed consent. Exclusion criteria included any non-English speaking patients, presence of gross blood or meconium in the amniotic sample, known fetal anomaly or aneuploidy, Twin-to-Twin Transfusion Syndrome (TTTS), subsequent vaginal delivery following consent, premature rupture of membranes, or emergent cesarean delivery.

At the time of cesarean delivery, the hysterotomy was made with the attempt not to rupture the membranes. The bulging amniotic sacs were then aspirated by the obstetrician using a 20-gauge needle on a 20-mL syringe. The amniotic fluid specimens were labeled and sent to the Good Samaritan Hospital laboratory where they were filtered and also centrifuged if a bloody appearance was noted. The laboratory determined if the specimen was acceptable for analysis per their standard quality procedures. The determination of the FLM index was made with the TDx FLM assay (Abbott Laboratory, Abbott Park IL) and LBC was performed on the platelet channel of the hematologic counter (H1 Technicon, Siemens, Washington D.C.) A twin was classified as mature if the FLM index was greater than 55 mg/g for non-diabetics and greater than 70 mg/g for diabetics, and if the LBC was greater than 29,000/**μ**L [10,11].

Demographic information, maternal obstetrical and medical history, newborn and fetal data, indications for cesarean delivery and twin A and B outcomes were extracted from the electronic medical record OB TraceVue (Philips Healthcare, Andover MA). If a twin was admitted to the NICU, their outcomes were obtained from their paper medical record. The diagnosis of Respiratory Distress Syndrome (RDS) was made by the attending neonatologist with supporting radiologic evidence. The clinical diagnosis of RDS in preterm infants is based on signs of symptoms of breathing difficulty occurring at birth or within the first few hours, including: tachypnea, retractions and flaring of the nostrils. A chest X-ray demonstrating diffuse opacification of the lungs consistent with surfactant deficiency confirms the clinical diagnosis. Discordant growth was defined as greater than 20% difference of the ratio (larger twin-smaller twin)/larger twin.

Due to the exploratory nature of this study and the relatively low prevalence of twin pregnancies, the goal for enrollment was set at 50 twin pairs. Differences in demographic and clinical characteristics between twin A and twin B were assessed. The correlations of the FLM index and LBC values between twins A and B were calculated using Pearson's *r*.

Receiver Operating Characteristic (ROC) curves were used to determine if the current cutoff values for fetal lung maturity for singletons were appropriate in the study's twin population; diabetics (pregestational and gestational) were excluded from this analysis due to established differences in lung maturity values between diabetics and non-diabetics and the low prevalence of diabetes in the study population. The analysis was then limited to 34 to 38 6/7 weeks as this is the gestational age range quoted by ACOG for optimal timing of twin delivery, depending on concurrent conditions [2].

Sensitivity, specificity, Positive Predictive Values (PPV), and Negative Predictive Values (NPV) were calculated in order to determine the validity of using the fetal lung maturity classification of twin B as a predictor of the maturity of twin A, larger twin (by birth weight) to predict the maturity of the smaller twin, and of using the male twin to predict the female twin's maturity in opposite sex twins. Further analyses of validity were completed after stratifying the study population according to the following characteristics: whether the patient received steroids, sex of the twins, gestational age, and presence of discordant growth. All analyses were done for both the FLM index and LBC values. All data were analyzed using IBM SPSS® Statistics Version 21 (SPSS Inc., an IBM Company, Chicago, IL, USA).

Results

The patient flow sheet from consent to analysis is shown in Figure 1. There were 87 patients consented for the study and 49 sets of twins with acceptable amniotic fluid samples and delivery information available. For the main components of this analysis, only twins born between 34 0/7 and 38 6/7 weeks were included. The demographic and maternal characteristics are shown in Table 1. The maternal baseline characteristics are the same as expected with twins. As expected, the values for the FLM index and the LBC tests increase with advancing gestational age and birth weights (Data not shown).

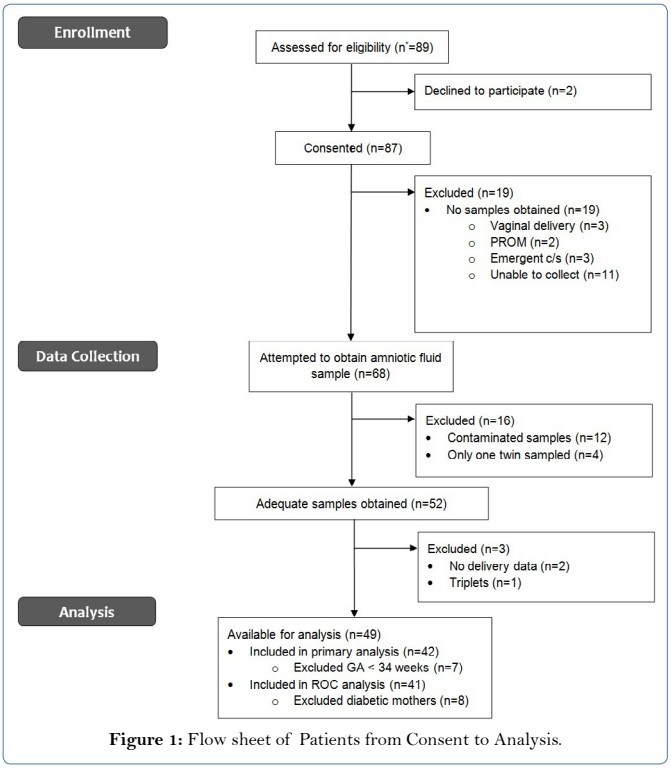

Figure 1: Flow sheet of Patients from Consent to Analysis.

To validate the established cutoffs of the FLM index and LBC for maturity in singletons and to see if they are applicable in our twin population, we used an ROC curve which is shown in Figure 2. All 82 non-diabetic samples were included for this analysis. The Area Under the Curve (AUC) for FLM index and LBC was not significantly different (FLM: 0.852 LBC: 0.824; p=0.660), so both tests perform equally. Using the established singletons cutoffs for maturity in our 82 twins, FLM index (greater than 55 mg/g for non-diabetics) and LBC (greater than 29 **μ**L) have a PPV of 90% for FLM index (sensitivity 56.3%, specificity 77.8%) and PPV of 90.2% for LBC (sensitivity 57.8%, specificity 77.8%), respectively. These same thresholds were used in this twin study for prediction of respiratory distress syndrome. The ability

		Mean	SD	P*
Maternal Age		32.31	5.84	
Birth Weight (g)	Twin A	2687.6	439.5	0.074
	Twin B	2555.7	528.5	
		Median	**IQR†**	**P‡**
Gravidity		2	2	
Gestational Age (weeks)		36.86	2.43	
Percent Discordance		11.25	14.17	
1 min Apgar	Twin A	8	1	0.914
	Twin B	8	1	
5 min Apgar	Twin A	9	0	0.705
	Twin B	9	0	
		N	**%**	**P§**
Race	White	33	78.6	
	African American	7	16.7	
	Other	2	4.8	
Diabetes	Pre-gestational	3	7.1	
	Gestational	5	11.9	
Preeclampsia		10	23.8	
Hypertension		10	23.8	
Corticosteroid usage		22	52.4	
Preterm labor		11	26.2	
Tocolytic usage		12	28.6	
Gestational age > 37 weeks		20	47.6	
Discordant Growth (> 20%)		9	21.4	
Zygosity	Dizygotic	37	88.1	
	Monozygotic	5	11.9	
Male	Twin A	19	45.2	0.523
	Twin B	23	54.8	
Admitted to NICU	Twin A	9	22.0	>0.999
	Twin B	9	22.0	
Respiratory Distress Syndrome	Twin A	1	2.4	0.375
	Twin B	4	9.5	

Table 1: Demographic and Clinical Characteristics of the 42 twin pairs.

*P-value for paired samples t-test; †IQR = Interquartile Range; ‡P-value for related samples Wilcoxon signed rank test; §P-value for related samples McNemar test

of twin B and the larger twin to predict twin A and the smaller twin's maturity is demonstrated in Table 2. When twin B is used to predict the maturity of twin A, the PPV is 77.8% for FLM index and 63.6% for the LBC. Similar values were found when the larger twin was used to predict the maturity of the smaller twin; the PPV was 58.3% for FLM index and 82.3% for LBC. Additional comparisons of PPVs between subsets of patients are shown in Table 3. The PPV of the male twin having a mature value and predicting the female co-twin to have a mature value is only 58.3% for FLM index and 55.6% for LBC.

The relationship between FLM index and LBC values of twin A and twin B and the larger versus smaller twin are shown in Figure 3. The correlation coefficient of twins A and B for FLM index was 0.597, p<0.001 and for LBC was 0.349, p=0.024; for the large and small twins, the correlation coefficient was 0.519, p<0.001 and 0.349, p=0.024 for FLM and LBC, respectively. However, when the twins were categorized as mature or not mature, there were 17 twin pairs with non-matching lung maturity classifications for the FLM index and 15 for LBC.

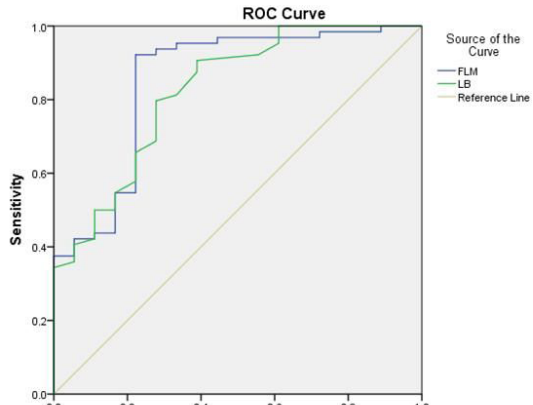

Figure 2: ROC curve excluding diabetics (GDM and DM).

The incidence of respiratory distress syndrome is shown in Table 4. There were a total of 5 twins that were diagnosed with respiratory distress syndrome for an incidence of 6.0%. There was no difference in RDS based on birth weight, birth order or gender. There was one twin at 35 weeks that experienced RDS despite having lung maturity values in the mature range for both FLM index and LBC.

Discussion

This prospective study of FLM tests in twin gestation answers our research question, "Can fetal lung maturity in twins be predicted based on birth weight, birth order and gender?" The positive predictive values of the presenting twin or the larger twin in predicting fetal lung maturity in twin B or the smaller twin was only 58 to 82%, depending on the test used. There was no difference in positive predictive values between FLM index and LBC tests. Therefore, there is no variable (birth weight, birth order or gender) that is a good clinical predictor of which twin is likely to be mature or immature.

A secondary objective was to affirm if the threshold values for fetal lung maturity tests established for singletons can be applied to our twin population. As evidenced by the ROC curve, the established cutoffs for both tests have a 90% PPV (FLM index greater than 55 mg/g for non-diabetics and LBC greater than 29,000/**µ**L). Both tests of fetal lung maturity perform well equally. This is similar to the result of 29,500/µL that was reported by Tsuda et al., where the primary objective was to determine if chorionicity affects the LBC value and incidence of RDS and TTN [13].

Our results and conclusions differ from prior studies on twin gestation and fetal lung maturity that found that only one twin could be sampled to assess for fetal lung maturity, depending on gestational age at the time of amniocentesis or the amount of discordance. These older studies were retrospective in nature and reported on the L/S ratio and were published from 1977-2002 [6-8]. The sample size of the studies ranged from nine to 92 twin sets. The study by Whitworth [7] obtained the amniotic fluid by amniocentesis; however, the mean interval from amniocentesis to delivery was nine days.

	FLM %				LBC %			
Subset (no. of pairs)	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Twin B predicting Twin A (42)	51.85	73.33	77.78	45.83	66.67	61.90	63.64	65.00
Large Twin predicting Small Twin (42)	66.67	52.38	58.33	61.11	53.85	81.25	82.35	52.00
Male Twin predicting Female Twin (22)	58.33	50.00	58.33	50.00	50.00	66.67	55.56	61.54

Table 2: Validity of Birth Order, Birth Weight, and Gender as Predictors of Co-Twin Lung Maturity.

		Twin B predicting Twin A		Large Twin predicting Small Twin	
	Subset (no. of pairs)	**PPV (%)**	**P***	**PPV (%)**	**P***
FLM	Steroids (22)	83.33	>0.999	50.00	0.678
	No Steroids (20)	75.00		64.29	
LBC	Steroids (22)	66.67	>0.999	75.00	0.576
	No Steroids (20)	61.54		88.89	
FLM	Same Sex (20)	80.00	>0.999	66.67	0.680
	Different Sex (22)	75.00		50.00	
LBC	Same Sex (20)	69.23	0.662	90.00	0.537
	Different Sex (22)	55.56		71.43	
FLM	GA 34 0/7 - 35 6/7 (11)	50.00	0.405	20.00	0.122
	GA 36 0/7 - 38 6/7 (31)	81.25		68.42	
LBC	GA 34 0/7 - 35 6/7 (11)	66.67	>0.999	66.67	0.465
	GA 36 0/7 - 38 6/7 (31)	63.16		85.71	
FLM	Discordant Growth (9)	50.00	0.405	25.00	0.272
	No Discordant Growth (33)	81.25		65.00	
LBC	Discordant Growth (9)	66.67	>0.999	66.67	0.465
	No Discordant Growth (33)	63.16		85.71	

Table 3: PPV comparisons within clinical groups.

GA = Gestational Age; * P-value for chi-squared or Fisher's exact test

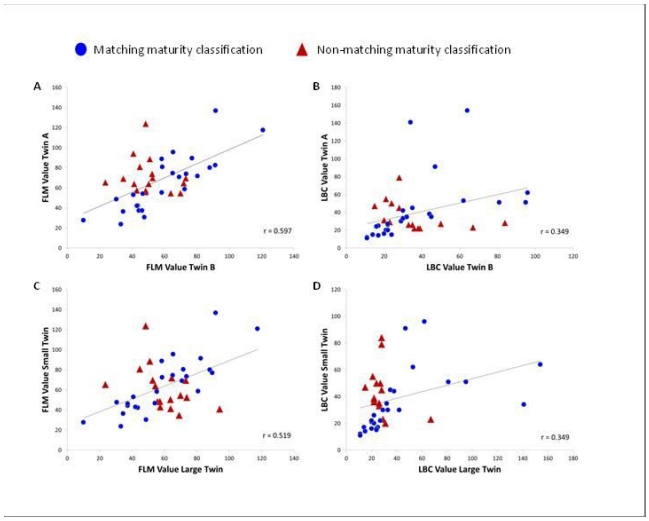

Figure 3: A: Relationship between FLM values of twin A and twin B. **B:** Relationship between LBC values of twin A and twin B. (x and y axes are LBC values x 1000/μL). **C:** Relationship between FLM values of large twin and small twin. **D:** Relationship between LBC values of large twin and small twin (x and y axis are LBC values x 1000/μL).

	RDS		P*
	n	%	
Twin A (42)	1	2.4	0.375
Twin B (42)	4	9.5	
Small Twin (42)	2	4.8	>0.999
Large Twin (42)	3	7.1	
Male in m-f twins (22)	2	9.1	>0.999
Female in m-f twins (22)	1	4.5	

Table 4: Comparison of Respiratory Distress Syndrome.

RDS = respiratory distress syndrome; m-f = male-female; *P-value for McNemar test

Mackenzie et al., retrospectively studied 92 twin pairs and analyzed lung maturity concordance based on gestational age, concordance of estimated fetal weights, and gender. They concluded that only a single amniocentesis is necessary if the gestational age is greater than 35.9 weeks, or if the gestational age is 33-35.9 weeks and the twins are gender concordant or fetal weight discordance is greater than 20% or less than 10% [14]. The prior studies all provide different caveats and "disclaimers" about the prediction of one twin's fetal lung maturity, based on the other twin's value that is difficult to remember and follow in practice. The retrospective nature of the prior studies also can lead to a selection bias as not all twins underwent an amniocentesis prior to delivery. None of the studies reported on respiratory distress syndrome as we did in our study.

The strengths of our prospective study are that respiratory morbidity was reported in our twin population and there was no interval from FLM tests and delivery, since all our samples were collected at the time of cesarean delivery. Also, we were able to validate that the singleton cutoffs for FLM tests can be applied to our twin population. The limitation of this pilot study is the sample size. If we assume that

a diagnostic test has a sensitivity of 80% with a specificity of 90% with a desired precision of 0.05, the number needed to achieve these values is 77 twin pairs, based on our prevalence of RDS of 6% in our twins at 34 0/7 to 38 6/7 weeks [15]. Our ROC curve values for fetal lung maturity may not be generalized to other populations. Another limitation is that following the completion of the study, the FLM index test is no longer commercially available in the United States. However, the results of this quantifiable test for fetal lung maturity are still valid and likely will not be repeated. The ROC curves for FLM index and LBC demonstrate that both tests perform equally well, and the LBC test is still widely available in any lab with a hematologic counter.

The American College of Obstetricians and Gynecologists has stated in Committee Opinion No. 560 that, "Amniocentesis for determination of fetal lung maturity in well-dated pregnancies generally should not be used to guide the timing of delivery" [3]. Our results indicate that there is no variable (birth weight, birth order or gender) that is a good clinical predictor of which twin is likely to be mature or immature based on our amniotic fetal lung maturity results.

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References

1. Hamilton BE, Martin AJ, Osterman MJK, Curtin SC, Mathews TJ (2015) Births: Final Data for 2014. National Vital Statistics Reports 64: 1-48.

2. Spong CY, Mercer BM, D'alton M, Kilpatrick S, Blackwell S, et al. (2011) Timing of indicated late-preterm and early-term birth. Obstet Gynecol 118: 323-333.

3. American College of Obstetricians and Gynecologists (2013) ACOG committee opinion no. 560: Medically indicated late-preterm and early-term deliveries. Obstet Gynecol 121: 908-910.

4. American College of Obstetricians and Gynecologists Committee on Practice Bulletins-Obstetrics; Society for Maternal-Fetal Medicine; ACOG Joint Editorial Committee (2004) ACOG Practice Bulletin #56: Multiple gestation: complicated twin, triplet, and high-order multifetal pregnancy. Obstet Gynecol 104: 869-883.

5. Chasen ST, Madden A, Chervenak FA (1999) Cesarean delivery of twins and neonatal respiratory disorders. Am J Obstet Gynecol 181: 1052-1056.

6. Leveno KJ, Quirk JG, Whalley PJ, Herbert WN, Trubey R (1984) Fetal lung maturation in twin gestation. Am J Obstet Gynecol 148: 405-411.

7. Whitworth NS, Magann EF, Morrison JC (1999) Evaluation of fetal lung maturity in diamniotic twins. Am J Obstet Gynecol 180: 1438-1441.

8. Spellacy WN, Cruz AC, Buhi WC, Birk SA (1977) Amniotic fluid L/S ratio in twin gestation. Obstet Gynecol 50: 68-70.

9. Liu KZ, Shaw RA, Dembinski TC, Reid GJ, Ying SL (2000) Comparison of infrared spectroscopic and fluorescence depolarization assays for fetal lung maturity. Am J Obstet Gynecol 183: 181-187.

10. Karcher R, Sykes E, Batton D, Uddin Z, Ross G (2005) Gestational age-specific predicted risk of neonatal respiratory distress syndrome using lamellar body count and surfactant-to-albumin ratio in amniotic fluid. Am J Obstet Gynecol 193: 1680-1684.

11. Kesselman EJ, Figueroa R, Garry D, Maulik D (2003) The usefulness of the TDx/TDxFLx fetal lung maturity II assay in the initial evaluation of fetal lung maturity. Am J Obstet Gynecol 188: 1220-1222.

12. Herbert WN, Chapman JF, Schnoor MM (1993) Role of the TDx FLM assay in fetal lung maturity. Am J Obstet Gynecol 168: 808-812.

13. Tsuda H, Kotani T, Sumigama S, Mano Y, Kawabata I, et al. (2015) Amniotic lamellar body count: predicting and distinguishing neonatal respiratory outcomes in twin pregnancies. Clinica Chimica Acta 441: 75-78.

14. Mackenzie MW (2002) Predicting concordance of biochemical lung maturity in the preterm twin gestation. J Mat-Fetal Neonatal Med 12: 50-58.

15. Malhotra RK, Indrayan A (2010) A simple nomogram for sample size for estimating sensitivity and specificity of medical tests. Indian J Ophthal 58: 519-522.