

Association Between Fetal Pulmonary Doppler Indices and Neonatal Respiratory Distress in Patients Undergoing Elective Cesarean Section Between 38 and 39 Weeks Gestation

Diaa Mohammed Elsayed Morshedy ^{1,*} M.B.B.Ch, Abd Almonem Mohammed Zakarya ¹ MD and Ahmed Mohammed Saeed ¹ MD.

*Corresponding Author:

Diaa Mohammed Elsayed Morshedy
the_crims_diaa_2010@yahoo.com

Received for publication March 28, 2022; Accepted October 23, 2022; Published online October 23, 2022.

doi: 10.21608/aimj.2022.130194.1885

Citation: Diaa M. , Abd Almonem M. and Ahmed M. Association Between Fetal Pulmonary Doppler Indices and Neonatal Respiratory Distress in Patients Undergoing Elective Cesarean Section Between 38 and 39 Weeks Gestation. AIMJ. 2022; Vol.3-Issue10 : 73-78.

¹Obstetrics and Gynecology Department, Faculty of Medicine, Al-Azhar University Cairo, Egypt.

ABSTRACT

Background: Respiratory distress syndrome (RDS) is a frequent source of respiratory distress in newborns that manifests within hours after birth, usually just after delivering. Preterm newborns and, on rare occasions, mature babies are affected with RDS. Numerous studies have established the value of Doppler as a non-invasive means of assessing pulmonary artery pressure in newborns and adults. Furthermore, the pulmonary vasculature grows with the lung during pregnancy, with both the actual number of pulmonary arteries and the pulmonary artery vascular resistance decreasing somewhat.

Aim of the work: To see whether fetal pulmonary indices might predict newborn respiratory distress syndrome in patients undergoing elective cesarean section between 38- and 39-weeks' gestation.

Patients and methods: This was a cross-sectional prospective research which was carried in al- Hussein hospital from October 2020 till April 2021. This study included 100 pregnant women.

Results: The sensitivity, specificity and accuracy of different variables of U/S to predict RDs showed that umbilical artery RI and PI had sensitivity 78.0%; 65.0%, specificity 70.0' 70.0 and accuracy 75.0%; 67% respectively. Middle cerebral artery RI and PI to predict RDs had sensitivity 80.0%; 75.0%, specificity 75.0; 70.0 and accuracy 76.0%; 72% respectively. The At/Et ratio showed a sensitivity 95.0%, specificity 90.0 and accuracy was 93.0%.

Conclusion: The current investigation found that fetal PA-derived Ultrasonographic characteristics may accurately predict neonatal RDS in term newborns. The RI, PI, PSV, and At/Et ratio are among these metrics.

Keywords: Fetal Pulmonary Doppler; RDS; Cesarean Section.

Disclosure: The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors.

Authorship: All authors have a substantial contribution to the article.

Copyright: The Authors published by Al-Azhar University, Faculty of Medicine, Cairo, Egypt. Users have the right to read, download, copy, distribute, print, search, or link to the full texts of articles under the following conditions: Creative Commons Attribution-Share Alike 4.0 International Public License (CC BY-SA 4.0).

INTRODUCTION

Respiratory distress syndrome (RDS) in newborns is a prevalent medical condition. It's one of the main sources of newborn illnesses and death, especially in premature babies.¹

Immature lung growth and a absence of pulmonary surfactant synthesis are the main causes of the syndrome.² Maternal drug use, maternal diabetes, and genetic vulnerability are all risk factors. The intricacy of the underlying illness, difficult identifying in certain instances, and absence of particular management options are all factors that contribute to the high morbidity and fatality rates associated with newborn RDS.³

Predicting RDS development may assist to enhance the care of afflicted infants, given its clinical and

economic implications. Many biochemical and sonographic techniques were examined in this area. Amniotic fluid surfactant/albumin proportion, lecithin/sphingomyelin ratio, and lamellar body count, amniotic fluid lamellar bodies count, and umbilical cord blood variably produced peptides were all included in the first group.⁴

Ultrasonographic imaging is helpful in identifying a variety of prenatal and neonatal disorders. The prenatal pulmonary artery [PA] indices and prenatal lung capacity were the most important ultrasonographic data. The PA indicators that were studied were as follows: pulmonary artery resistance index, At/Et ratio.⁵

The most basic, ubiquitous, non-invasive, and valuable method for regular prenatal screening scanning is ultrasound.⁶

The bi-parietal diameter, femur length, epiphyseal centers of the lower limb, placental grading, colon grading, and free-floating particles in the amniotic fluid were all ultrasonography characteristics employed in many studies to determine fetal lung maturity.⁷

For additional efforts to determine fetal lung maturity, ultrasound can evaluate prenatal lung shape and measure prenatal thalamic echogenicity.⁸

Researchers used a pulsed-wave Doppler study that included the uterine artery, umbilical artery, baby middle cerebral artery, and baby main pulmonary artery to see whether there was a connection between maternal-fetal Doppler velocimetry measures and fetal lung maturity indicators.⁹

The researchers wanted to see whether fetal pulmonary indices might predict newborn respiratory distress syndrome in women who had an elective caesarean delivery between 38 and 39 weeks of pregnancy.

PATIENTS AND METHODS

This was cross-sectional prospective research which was carried in al- Hussein hospital from October 2020 till April 2021. This study includes 100 pregnant women.

Inclusion criteria: Women with singleton pregnancy, with an accurate gestational age, medically free women, only fetus delivered within 24h of admission will included, and pregnant women undergoing elective cesarean section with gestational age between 38 - 39 week.

Exclusion criteria: Multifetal pregnancy, known fetal chromosomal or substantial structural anomalies, and fetus with structural anomalies identified after birth were all ruled out, cesarean section due to fetal distress, post term pregnancy, and patient who don't know her last menstrual period.

The following tests were administered to all research participants:

Formal consent: was obtained.

Full history taken including: Name, age, occupation and address.

Date of last menstrual history to determine gestational age.

A general checkup is performed to rule out medical problems, such as vital signs, height, weight, and BMI.

Obstetric palpation.

Ultra sound examination including:

Number of fetus (exclusion of multiple pregnancies).

Position of placenta.

Biometry, gestational age and presentation.

Estimated fetal weight using Hadlock formula depending on BPD, HC, AC, and FL for assessment of normal growth, Macrosomia and IUGR.

Umbilical artery Doppler flowmetry for assessment of fetal condition.

Follow the major pulmonary artery until halfway between the pulmonary valve and the bifurcation of the right and left branches in a systemic fashion (the four-chamber view and the three-vessel view) with the baby at rest and no baby breath activities in the axial view of the thorax.

Statistical analysis:

The IBM SPSS software program version 24.0 was used to feed the data into the computer. Number and percent were used to describe qualitative data. The Chi-square test was used to see how various groups compared on category variables. For regularly distributed data, mean and standard deviation were used, whereas abnormally distributed data was reported using average, minimum, and maximum. For normally distributed data, independent t-tests were used to compare two independent populations, whereas F-tests (ANOVA) were used to compare more than two populations. The results of significance tests are expressed as two-tailed probability. The significance of the acquired findings was assessed at a 5% level. When comparing the averages of distinct sample groups, the student (unpaired-sample) "t" test is utilized. The Chi-Square test examines the relationship between qualitative nominal variables, with a focus on frequencies. It assesses if the observed and predicted frequencies are considerably different. P values >0.05 were regarded substantial.

RESULTS

Maternal Age (years)	RDS		t-test P
	No "n=87"	Yes "n=13"	
Range	20.00-35.00	20.00-30.00	1.445
Median	25.91	24.77	0.232 N.S.
S.D.	3.18	3.17	

T= student t-test P was substantial if < 0.05 N.S. = Not substantial

Table 1: Comparison between patients with and without RDS regarding maternal age.

Table (1) shows analyses of patients with and without RDS regarding maternal age. Maternal age of patients with RDS ranged from 20-30 with mean value 24.77±3.17 and maternal age of patients without RDS ranged from 20-35 with mean value 25.91±3.18. There was no statistical substantial variance between patients with and without RDS regarding maternal age (P > 0.05).

	RDs		P
	No "n=87"	Yes "n=13"	
At/Et ratio:	0.21-0.60	0.21-0.30	26.236
Range	0.41	0.26	0.0001*
Mean	0.11	0.03	
S.D.			
Neonatal birth weight:	2500.00-4170.00	3130.00-3490.00	0.010
Range	3351.65	3265.38	0.921 N.S.
Mean	494.60	91.98	
S.D.			
APGAR score at 1Minute	5.00-10.00	3.00-6.00	47.580
Range	7.91	4.62	0.0001*
Mean	1.65	1.19	
S.D.			
APGAR score at 5Minute	7.00-10.00	6.00-9.00	9.291
Range	8.53	7.54	0.003*
Mean	1.06	1.27	
S.D.			

* = significant difference. At/Et ratio = ratio of acceleration time to ejection time.

Table 2: Comparison of patients with and without RDS in terms of the acceleration-to-ejection time ratio (At/Et ratio), newborn birth weight, and APGAR score.

There was statistical substantial variance between patients with and without RDS regarding At/Et ratio ($P < 0.05$). There was statistical no substantial variance between patients with and without RDS regarding neonatal birth weight ($P > 0.05$). There was statistical substantial variance between patients with and without RDS regarding APGAR score at 1minute and APGAR score at 5minute ($P < 0.05$) (Table 2).

	RDs		P
	No "n=87"	Yes "n=13"	
Umbilical artery measurements using U/S:			
Umbilical artery RI			
Range	0.41-.86	0.41-.71	6.015
Mean	0.66	0.57	0.016*
S.D.	0.13	0.10	
Umbilical artery PI	0.40-1.44	0.47-1.03	5.356
Range	0.89	0.689	0.023*
Mean	0.31	0.18	
S.D.			
Middle cerebral:			
Middle cerebral artery RI	0.62-1.00	0.70-1.74	3.301
Range	0.82	1.10	0.021*
Mean	0.12	0.33	
S.D.			
Middle cerebral artery PI	1.00-1.80	1.15-2.22	3.648
Range	1.38	1.76	0.011*
Mean	0.26	0.42	
S.D.			
Pulmonary artery:			
Pulmonary artery RI	0.71-0.95	0.80-0.94	1.558
Range	0.83	0.88	0.102
Mean	0.07	0.05	
S.D.			
Pulmonary artery PI	1.21-2.53	1.49-2.45	1.401
Range	1.81	2.09	0.213
Mean	0.38	0.30	
S.D.			
Pulmonary artery S/D ratio	4.12-15.91	4.97-20.44	2.021
Range	10.26	13.33	0.029*
Mean	3.60	5.57	
S.D.			
Pulmonary artery PSV	50.66-116.04	72.53-108.32	3.166
Range	79.21	92.43	0.015*
Mean	18.55	12.15	
S.D.			

* = significant difference

Table 3: Comparison between patients with and without RDS regarding umbilical artery measurements using U/S, Middle cerebral and pulmonary artery.

There was statistical substantial variance between patients with and without RDS regarding umbilical artery measurements ($P < 0.05$). There was statistical substantial variance between patients with and without RDS regarding middle cerebral ($P < 0.05$). There was no statistical substantial variance between patients with and without RDS regarding pulmonary artery RI and pulmonary artery PI ($P > 0.05$), while there was statistical substantial variance regarding pulmonary artery S/D and pulmonary artery PSV ($P < 0.05$) (Table 3).

Test Result Variable(s)	Area	Cut off values	P values	Sensitivity	specificity	accuracy	Asymptotic 95% C.I.	
							Lower Bound	Upper Bound
Umbilical artery RI	0.719	0.61	0.011*	78.2	70.0	75.0	0.598	0.839
Umbilical artery PI	0.701	0.71	0.020*	65.0	70.0	67.0	0.580	0.821

Table 4: Sensitivity, specificity and accuracy of umbilical artery value in predict RDS.

Sensitivity, specificity and accuracy of umbilical artery value in predict RDS. The area under the curve for umbilical artery RI was 0.719, and at cut off 0.61, the sensitivity was 78.2, specificity was 70% and accuracy was 75%, while umbilical artery PI the area under the curve was 0.71 and at cut off value 0.71 the sensitivity was 65% while the specificity was 70% and accuracy was 67% (Table 4).

Test Result Variable (s)	Area	Cut off value	P value	Sensitivity	specificity	accuracy	Asymptotic 95% C.I.	
							Lower Bound	Upper Bound
Middle cerebral artery RI	0.803	0.92	0.003*	80.0	75.0	76.0	0.773	1.000
Middle cerebral artery PI	0.767	1.41	0.012*	75.0	70.0	72.0	0.605	0.930

Table 5: Sensitivity, specificity and accuracy of middle cerebral artery value in predict RDS.

Sensitivity, specificity and accuracy of middle cerebral artery value in predict RDS. Middle cerebral artery RI sensitivity was 80%, specificity was 75% and accuracy was 76%, while Middle cerebral artery PI was 75%, 70% and 72% respectively (Table 5).

	Area	Cut off value	P value	Asymptotic 95% C.I.	
				Lower Bound	Upper Bound
	0.91	0.31	0.0001	0.837	0.959
Sensitivity				95.0	
Specificity				90.0	
Accuracy				93.0	

Table 6: Sensitivity, specificity and accuracy of At/Et ratio in predict RDS.

Sensitivity, specificity and accuracy of At/Et ratio in predict RDS. The area under the curve was 0.91, at the cut off value 0.31 the sensitivity was 95%, specificity was 90% and accuracy was 93% (Table 6).

DISCUSSION

The findings of this research indicated that 13 patients (13.0%) was RDs, on comparing the two groups of neonates with RDs and without RDs it was found that the maternal age, maternal data which include parity, previous CS and gestational age in the two groups was matched without substantial change. Additionally, no substantial change in U/S measures was detected between the two groups, this result was important to eliminate the effect of basic demographic and clinical data on the outcome.

In our results, the umbilical artery measurements using U/S showing that the umbilical artery RI and umbilical artery PI was substantially greater in patient group without RDs more than RDs group. On the other hand, the middle cerebral artery RI and PI was substantially greater in patients with RDs more than patients' group without RDs. Pulmonary artery S/D ratio and PSV showed a significant

increase in patients with RDs more than patients without RDs. At/Et ratio shows a highly significant increase in patients without RDs.

The sensitivity, specificity and accuracy of different variables of U/S to predict RDs showed that umbilical artery RI and PI had sensitivity 78.0%; 65.0%, specificity 70.0% 70.0 and accuracy 75.0%; 67% respectively.

Middle cerebral artery RI and PI to predict RDs had sensitivity 80.0%; 75.0%, specificity 75.0; 70.0 and accuracy 76.0%; 72% respectively.

Finally the At/Et ratio showed a sensitivity 95.0%, specificity 90.0 and accuracy was 93.0%.

In agreement with our findings, Alsheikh et al.¹⁰ conducted the link of fetus doppler pulmonary artery measures and neonatal respiratory distress condition in newborn infants and discovered that all variables, such as pulmonary artery RI, PI, PSV, and At/Et ratio, performed well as predictors of RSD advancement in the newborns surveyed.

In agreement with our findings, Laban et al.¹¹ conducted research on 80 women. 13.8 percent of infants born in their study experienced RDS. The authors recognized the predictive significance of PA-RI in terms of infants with RDS. They identified a RI of 0.74 in the pulmonary artery as a major predictor of RDS. Additionally, they said that baby lung volume might be employed consistently for the same reason.

By contrast, Kim et al.¹² found the pulmonary artery At/Et ratio as a substantial predictor of RDS in their study of 42 infants, including 11 who acquired RDS. Notably, this research included a diverse cohort of term and premature infants born during singleton or twin pregnancies.

Similarly, Guan et al.¹³ found that only the pulmonary artery acceleration time [at] and the At/Et ratio could accurately predict newborn RDS in 43 preterm infants. Büke et al.¹⁴ on 105 women and Duncan et al.¹⁵ on 95 premature infants recently recognized the prognostic value of the pulmonary artery At/Et ratio.

Azpurua et al.⁹ found an adverse connection of the amniotic fluid lecithin/sphingomyelin ratio, a fetal-lung advancement measure, and the At/Et ratio in the fetal PA in a study of 29 newborns. This explains why the At/Et ratio is important in the prediction of RDS. It suggests that inadequate prenatal lung maturation plays a role in RDS etiology.

In contrast to our results and those of previous research, Güngör et al.¹⁶ found no substantial variations in fetal PA Doppler indices of neonates who advanced RDS and those who did not, even after steroid treatment, in a study of 40 preterm newborns. This is most likely due to the fact that newborns in various trials have varying demographic and clinical features.

Moety et al.¹⁷ analyze the baby pulmonary artery Doppler indices to predict newborn respiratory distress disorder, which agrees with our findings. The findings of this research revealed that babies with newborn RDS had considerably lower At/Et and PSV, as well as greater PI and RI, as compared to babies who did not acquire RDS.

In comparison to babies that do not develop RDS, babies who develop RDS have increased pulmonary vascular resistance and pressure, as well as decreased pulmonary blood flow. MPA At/Et was shown to be considerably lower in premature babies with RDS in two earlier investigations, which agrees with our findings.¹³

After adjusting for GA, the variation between the two groups for At/Et, PI, and RI in this research remained considerable. This suggests that these three indicators may be used to predict RDS development independently.

MPA At/Et and PSV were favorably connected in this research, but PI and RI were adversely correlated with GA. The most significant link was discovered between GA and At/Et. The S/D ratio did not alter appreciably during the course of the pregnancy. The

findings of Chaoui et al.¹⁸, who reported Doppler echocardiography of the major stems of the pulmonary arteries in a normal human fetus, are completely congruent.

According to Schenone et al.¹⁹, MPA At/Et and the TDx- FLM-II (reported in the amniotic fluid) were positively connected, indicating that a higher At/Et is associated with a more developed lung and a lower likelihood of suffering RDS, which is consistent with our results.

At/Et might predict RDS development in premature babies with a sensitivity of 71.4 percent and a specificity of 93.1 percent, according to Guan et al.¹³. In a study of 43 infants, Schenone et al.¹⁹ discovered that a cutoff value of 0.3 for At/Et had a sensitivity of 73% and a specificity of 93% for predicting immature surfactant/albumin ratio findings. However, the authors found no link between At/Et and clinical RDS.

Scopesi et al.²⁰ conducted to examine the association of umbilical artery RI Doppler velocimetry and prenatal and newborn distress in order to discover if umbilical artery RI is a predictor of RDS and whether fetus parameter is the most predictive of newborn respiratory distress disorder. They showed that higher UA RI values (> 0.70) can be used as a cut-off value for predicting which babies are at low/high risk of developing respiratory problems, and that Doppler velocimetry analysis can provide more reliable information about prenatal situations and predict when delivery and unit care admission should be scheduled.

FLM was determined by lamellar body count (LBs) in amniotic fluid by amniocentesis in research by Piazze et al.²¹ where fetal lung maturity (FLM) indices were linked with Doppler velocimetry results. They discovered that MCA PI and LBs had a favorable relationship. In infants with RDS, MCA PI exhibited a tendency toward lower values (1.36 ± 0.5 vs. 1.69 ± 0.4 , NS). They also discovered that a median Ut RI > 0.64 was associated with a delayed biophysical FLM represented as a reduction in LBs, with 90.9 percent sensitivity and 90.3 percent specificity.

CONCLUSION

The current investigation found that fetal PA-derived ultrasonographic characteristics may accurately predict neonatal RDS in term newborns. The RI, PI, PSV, and At/Et ratio are among these metrics.

Conflict of interest : none

REFERENCES

1. Niemarkt HJ, Hütten MC, Kramer BW. Surfactant for respiratory distress syndrome: new ideas on a familiar drug with innovative applications. *Neonatal*. 2017; 111(4): 408-14.
2. Rubarth LB, Quinn J. Respiratory development and respiratory distress syndrome. *Neonatal Netw*. 2015; 34(4): 231-8.

3. Chi M, Mei YB, Feng ZC. A review on neonatal acute respiratory distress syndrome. *Zhongguo Dang Dai Er Ke Za Zhi*. 2018; 20(9):724-8.
4. Hu Y, Wang J, Zhou Y, Xie H, Yan X, et al. Peptidomics analysis of umbilical cord blood reveals potential preclinical biomarkers for neonatal respiratory distress syndrome. *Life Sci*. 2019; 236: 116737. DOI: 10.1016/j.lfs.2019.116737.
5. Laban M, Mansour GM, Elsafty MS, Hassanin AS, EzzElarab SS. Prediction of neonatal respiratory distress syndrome in term pregnancies by assessment of fetal lung volume and pulmonary artery resistance index. *Int J Gynaecol Obstet*. 2015; 128(3):246-50.
6. Pallavi L, Sushil K, Lakhkar DL, Soniya D, Abhijeet I, et al. Assessment of fetal lung maturity by ultrasonography. *Ann Int Med Den Res*. 2017; 3(4): 1-4.
7. Loret de Mola JR, Judge N, Entsminger C, DeViney M, Muise KL, et al. Indirect prediction of fetal lung maturity. Value of ultrasonographic colonic and placental grading. *J Reprod Med*. 1998; 43(10):898-902.
8. Palacio M, Bonet-Carne E, Cobo T, Perez-Moreno A, Sabrià J, et al. Prediction of neonatal respiratory morbidity by quantitative ultrasound lung texture analysis: a multicenter study. *Am J Obstet Gynecol*. 2017; 217(2): 191-6.
9. Azpurua H, Norwitz ER, Campbell KH, Funai EF, Pettker CM, et al. Acceleration/ejection time ratio in the fetal pulmonary artery predicts fetal lung maturity. *Am J Obstet Gynecol*. 2010; 203(1): 40-7.
10. Alsheikh AM, Elsadek AM, Gebreel SA. Relation Between Fetal Doppler Pulmonary Artery Indices and Neonatal Respiratory Distress Syndrome In Term Neonates. *International Journal of Medical Arts*. 2021; 3(1): 1046-52.
11. Laban M, Mansour GM, El-Kotb A, Hassanin A, Laban Z, et al. Combined measurement of fetal lung volume and pulmonary artery resistance index is more accurate for prediction of neonatal respiratory distress syndrome in preterm fetuses: a pilot study. *J Matern Fetal Neonatal Med*. 2019; 32(4):626-32.
12. Kim SM, Park JS, Norwitz ER, Hwang EJ, Kang HS, et al. Acceleration time-to-ejection time ratio in fetal pulmonary artery predicts the development of neonatal respiratory distress syndrome: a prospective cohort study. *Am J Perinatol*. 2013; 30(10): 805-12.
13. Guan Y, Li S, Luo G, Wang C, Norwitz ER, et al. The role of Doppler waveforms in the fetal main pulmonary artery in the prediction of neonatal respiratory distress syndrome. *J Clin Ultrasound*. 2015; 43(6): 375-83.
14. Büke B, Destegül E, Akkaya H, Şimşek D, Kazandi M. Prediction of neonatal respiratory distress syndrome via pulmonary artery Doppler examination. *J Matern Fetal Neonatal Med*. 2019; 32(10):1640-5.
15. Duncan JR, Tobiasz AM, Dorsett KM, Aziz MM, Thompson RE, et al. Fetal pulmonary artery acceleration/ejection time prognostic accuracy for respiratory complications in preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med*. 2020; 33(12): 2054-8.
16. Güngör ES, İlhan G, Gültekin H, Zebitay AG, Cömert S, et al. Effect of Betamethasone on Fetal Pulmonary and Umbilical Artery Doppler Velocimetry and Relationship With Respiratory Distress Syndrome Development. *J Ultrasound Med*. 2017; 36(12): 2441-5.
17. Moety GA, Gaafar HM, El Rifai NM. Can fetal pulmonary artery Doppler indices predict neonatal respiratory distress syndrome? *J Perinatol*. 2015; 35(12):1015-9.
18. Chaoui R, Taddei F, Rizzo G, Bast C, Bollmann R. Doppler echocardiography of the main stems of the pulmonary arteries in the normal human fetus. *Ultrasound Obstet Gynecol*. 1998; 11: 173-9.
19. Schenone MH, Samson JE, Jenkins L, Suhag A, Mari G. Predicting fetal lung maturity using the fetal pulmonary artery Doppler wave acceleration/ejection time ratio. *Fetal Diagn Ther*. 2014; 36: 208-14.
20. Scopesi F, Gazzolo D, Cortez R, Nigro M, Pittaluga C, et al. Neonatal respiratory distress syndrome screening by behavioural state and umbilical artery Doppler velocimetry. *J Perinat Med*. 1994; 22(1):176-8.
21. Piazzze JJ, Anceschi MM, Picone G, Cerekja A, La Torre R, et al. Association between maternal-fetal Doppler velocimetry and fetal lung maturity. *J Perinat Med*. 2003; 31(6):484-8.