

OBSTETRICS

The randomized Tracheal Occlusion To Accelerate Lung growth (TOTAL)-trials on fetal surgery for congenital diaphragmatic hernia: reanalysis using pooled data



Ben Van Calster, PhD; Alexandra Benachi, MD, PhD; Kypros H. Nicolaides, MD; Eduard Gratacos, MD, PhD; Christoph Berg, MD, PhD; Nicola Persico, MD, PhD; Glenn J. Gardener, MD, PhD; Michael Belfort, MD, PhD; Yves Ville, MD, PhD; Greg Ryan, MB; Anthony Johnson, MD, DO; Haruhiko Sago, MD, PhD; Przemysław Kosiński, MD, PhD; Pietro Bagolan, MD, PhD; Tim Van Mieghem, MD, PhD; Philip L. J. DeKoninck, MD, PhD; Francesca M. Russo, MD, PhD; Stuart B. Hooper, PhD; Jan A. Deprest, MD, PhD

BACKGROUND: Two randomized controlled trials compared the neonatal and infant outcomes after fetoscopic endoluminal tracheal occlusion with expectant prenatal management in fetuses with severe and moderate isolated congenital diaphragmatic hernia, respectively. Fetoscopic endoluminal tracheal occlusion was carried out at 27⁺⁰ to 29⁺⁶ weeks' gestation (referred to as "early") for severe and at 30⁺⁰ to 31⁺⁶ weeks ("late") for moderate hypoplasia. The reported absolute increase in the survival to discharge was 13% (95% confidence interval, -1 to 28; $P=.059$) and 25% (95% confidence interval, 6–46; $P=.0091$) for moderate and severe hypoplasia.

OBJECTIVE: Data from the 2 trials were pooled to study the heterogeneity of the treatment effect by observed over expected lung-to-head ratio and explore the effect of gestational age at balloon insertion.

STUDY DESIGN: Individual participant data from the 2 trials were reanalyzed. Women were assessed between 2008 and 2020 at 14 experienced fetoscopic endoluminal tracheal occlusion centers and were randomized in a 1:1 ratio to either expectant management or fetoscopic endoluminal tracheal occlusion. All received standardized postnatal management. The combined data involved 287 patients (196 with moderate hypoplasia and 91 with severe hypoplasia). The primary endpoint was survival to discharge from the neonatal intensive care unit. The secondary endpoints were survival to 6 months of age, survival to 6 months without oxygen supplementation, and gestational age at live birth. Penalized regression was used with the following covariates: intervention (fetoscopic endoluminal tracheal occlusion vs expectant), early balloon insertion (yes vs no), observed over expected lung-to-head ratio, liver herniation (yes vs no), and trial (severe vs moderate). The interaction between intervention and the observed over expected lung-to-head ratio was evaluated to study treatment effect heterogeneity.

RESULTS: For survival to discharge, the adjusted odds ratio of fetoscopic endoluminal tracheal occlusion was 1.78 (95% confidence interval, 1.05–3.01; $P=.031$). The additional effect of early balloon insertion was highly uncertain (adjusted odds ratio, 1.53; 95% confidence interval, 0.60–3.91; $P=.370$). When combining these 2 effects, the adjusted odds ratio of fetoscopic endoluminal tracheal occlusion with early balloon insertion was 2.73 (95% confidence interval, 1.15–6.49). The results for survival to 6 months and survival to 6 months without oxygen dependence were comparable. The gestational age at delivery was on average 1.7 weeks earlier (95% confidence interval, 1.1–2.3) following fetoscopic endoluminal tracheal occlusion with late insertion and 3.2 weeks earlier (95% confidence interval, 2.3–4.1) following fetoscopic endoluminal tracheal occlusion with early insertion compared with expectant management. There was no evidence that the effect of fetoscopic endoluminal tracheal occlusion depended on the observed over expected lung-to-head ratio for any of the endpoints.

CONCLUSION: This analysis suggests that fetoscopic endoluminal tracheal occlusion increases survival for both moderate and severe lung hypoplasia. The difference between the results for the Tracheal Occlusion To Accelerate Lung growth trials, when considered apart, may be because of the difference in the time point of balloon insertion. However, the effect of the time point of balloon insertion could not be robustly assessed because of a small sample size and the confounding effect of disease severity. Fetoscopic endoluminal tracheal occlusion with early balloon insertion in particular strongly increases the risk for preterm delivery.

Key words: congenital diaphragmatic hernia, fetal surgery, fetoscopic endoluminal tracheal occlusion, fetoscopy, prenatal diagnosis, preterm premature rupture of the membranes, pulmonary hypoplasia, randomized controlled trial, ultrasound

Cite this article as: Van Calster B, Benachi A, Nicolaides KH, et al. The randomized Tracheal Occlusion To Accelerate Lung growth-trials on fetal surgery for congenital diaphragmatic hernia: reanalysis using pooled data. *Am J Obstet Gynecol* 2022;226:560.e1-24.

0002-9378/\$36.00

© 2021 Elsevier Inc. All rights reserved.

<https://doi.org/10.1016/j.ajog.2021.11.1351>

 Click [Video](#) under article title in Contents at ajog.org

Introduction

Congenital diaphragmatic hernia (CDH) is associated with impaired fetal lung development, leading to neonatal respiratory failure and pulmonary hypertension, which can cause neonatal death. In isolated cases, the survival chances can be predicted prenatally by the measurement of the lung size and the presence of intrathoracic herniation of the liver.¹ The lung area contralateral to

the defect is measured on a standardized 4-chamber view of the heart and is divided by the head circumference to obtain the *observed* lung-to-head ratio (LHR); the LHR is then expressed as a percentage of what is *expected* in a normal fetus of the same gestational age (GA) to obtain the observed/expected lung-to-head-ratio (*o/e* LHR).² Fetuses with a left-sided CDH and an *o/e* LHR <25% are considered to have *severe*

AJOG at a Glance

Why was this study conducted?

Two randomized controlled trials on fetoscopic endoluminal tracheal occlusion (FETO) for isolated fetal congenital diaphragmatic hernia reported a statistically significant improvement in survival in severe hypoplasia but not in moderate hypoplasia.

Key findings

This reanalysis on the basis of pooled data suggests that FETO increases survival and prematurity in both the severity groups.

What does this add to what is known?

The discrepancy in the results between the 2 trials is more likely because of performing FETO at an earlier gestational age in severe hypoplasia than in moderate hypoplasia; there was no evidence that the effect of FETO depends on the disease severity.

pulmonary hypoplasia, because their survival chances are <25%.² When the o/e LHR is 25.0% to 34.9% irrespective of the liver position or 35.0% to 44.9% with intrathoracic liver herniation, the hypoplasia is *moderate*, and the estimated survival chances are around 55%.²

Fetoscopic endoluminal tracheal occlusion (FETO) with a balloon can stimulate lung growth and improve neonatal survival.³ In a large observational study in fetuses with left-sided severe hypoplasia, FETO at a median GA of 27 weeks resulted in a 49% survival, where survival was only 24% in historic controls who did not have fetal therapy.⁴ The significant predictors of survival were GA at delivery and lung size at the time of FETO.⁴ Therefore, along the spectrum of severe hypoplasia, those with the highest o/e LHR were the most likely to survive.⁵ However, FETO also increased the risk for preterm birth, in turn compromising survival chances.^{4,6} Because randomized data were lacking, we designed the Tracheal Occlusion to Accelerate Lung growth (TOTAL)-trials, wherein fetuses were randomized to either FETO or expectant prenatal management, both followed by standardized neonatal care.^{7,8} In fetuses with *severe* lung hypoplasia, the balloon was inserted at 27⁺⁰ to 29⁺⁶ weeks gestation, this time point being further referred to as “early”.⁹ In *moderate* cases, insertion was at 30⁺⁰ to 31⁺⁶ weeks (“late”) to reduce the risks for very

preterm birth and considering the higher survival rate when comparing with severe hypoplasia.¹⁰ In both trials, balloon removal was scheduled at 34⁺⁰ to 34⁺⁶ weeks or earlier if clinically indicated.¹¹ In the trial for moderate hypoplasia, survival to discharge from the neonatal unit was 63% (62/98) in the FETO group and 50% (49/98) in the expectant management group ($P=0.059$; risk difference, 13%; 95% confidence interval [CI], -1 to 28; relative risk [RR], 1.27; odds ratio [OR], 1.72).¹⁰ In the trial for severe hypoplasia, survival to discharge was 40% (16/40) in the FETO group and 15% (6/40) in the expectant management group ($P=0.0091$; risk difference, 25%; 95% CI, $6-46$; RR, 2.67; OR, 3.78).⁹ A reductionist interpretation of these results, in terms of statistical significance, suggests that there is evidence of improved survival when the FETO is <25%, but not when FETO is $\geq 25\%$. Alternative interpretations may be that first, there is an overall beneficial effect of FETO that decreases with increasing o/e LHR, and second, that the beneficial effect of FETO is primarily dependent on earlier balloon insertion rather than on o/e LHR. The objective of this study is to use the pooled data from the 2 TOTAL trials to examine the heterogeneity of the treatment effect by o/e LHR. Despite the strong confounding of the timing of balloon insertion with o/e LHR, we also aim to gain insight into the effect of GA at balloon insertion.

Material and Methods
Study design, setting, and procedures

This is a reanalysis of pooled individual participant data from 2 multicenter trials in which women were randomized in a 1:1 ratio (without stratification factors) to either expectant prenatal management or FETO. The patients were recruited between 2008 and 2020 at 14 experienced FETO centers in 11 countries (Supplemental Table 1). Both trials had a group-sequential design with 5 preplanned interim analyses for superiority. The primary endpoint was survival to discharge from the neonatal unit; in the trial for moderate hypoplasia, survival at 6 months without oxygen dependency was a coprimary endpoint. The trial for moderate hypoplasia was not stopped early, whereas the trial for severe hypoplasia was stopped after the third interim analysis.

The participants in the present study include all 287 randomized patients who did not withdraw consent (196 moderate and 91 severe hypoplasia) (Figure 1). The 91 cases in the severe hypoplasia group comprise of 80 patients included at the third interim analysis and 11 overrunning patients, that is, who were already randomized when the decision to stop the trial was made.

The primary endpoint is survival to discharge from the neonatal intensive care unit (NICU). The secondary endpoints are survival to 6 months of age, survival to 6 months without oxygen dependency, and the GA at live birth.

Analysis population

We performed an intention-to-treat analysis: patients were analyzed according to the arm to which they were randomized, irrespective of postrandomization events (Figure 1 provides the details). For the secondary endpoint GA at live birth, we only used data from live born babies ($n=279$).

Covariates

The key variables were intervention (FETO vs expectant) and o/e LHR as the central prognostic variable which also determined severity of hypoplasia. The additional variables were early balloon

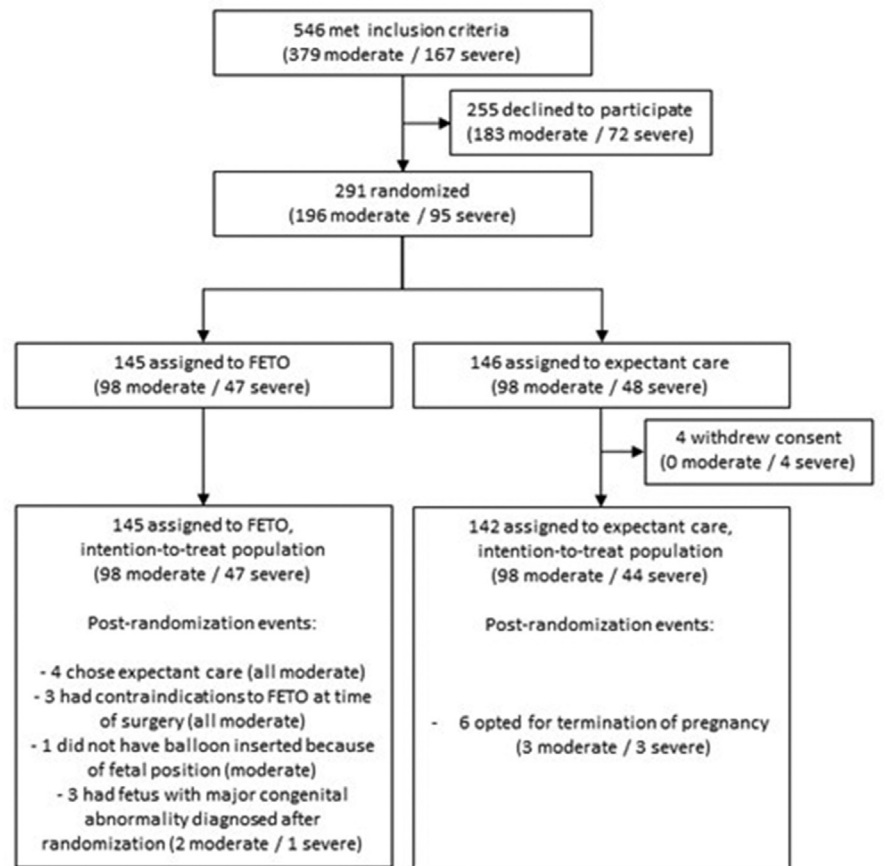
insertion (yes vs no), liver herniation, and the trial to which the participants were recruited (severe vs moderate). The timing of balloon insertion was confounded with o/e LHR, because disease severity determined the fetal surgical protocol. The use of an intervention variable and a timing variable implies the following: the intervention variable estimates the effect of FETO with late balloon insertion and the timing variable assesses the additional effect of early instead of late insertion. The trial covariate aims to capture other sources of heterogeneity between trials apart from the timing of balloon insertion, o/e LHR, and liver herniation. For example, there were differences in the FETO centers (supplemental Table 1) and postnatal care centers. Finally, we evaluated the interaction between intervention and o/e LHR to assess heterogeneity of the effect of FETO (on logit scale).

Statistical methods and sample size

We used logistic regression (binary endpoints) or ordinary least squares regression (GA at live birth), both with penalized maximum likelihood estimation.¹² For ordinary regression, we examined the model diagnostics and concluded that there was no need to transform the outcome variable. The main reasons for using penalized estimation are as follows: (1) the trial for severe hypoplasia stopped early for superiority, which incurs a risk for optimistic effect sizes,^{13,14} (2) the confounding between o/e LHR and the time of balloon insertion and between o/e LHR and liver herniation, (3) the objective of assessing the interaction between intervention and o/e LHR,¹⁵ and (4) the aim to tentatively visualize the predicted outcomes by o/e LHR and liver herniation.

We included all covariates by default without variable selection. We allowed for a nonlinear effect of o/e LHR by using restricted cubic splines with 3 knots.¹² In practice, this added 1 covariate. We evaluated the interaction between intervention and o/e LHR using an alpha of 0.157 (ie, the Akaike criterion).¹⁶ However, this does not imply that $P \leq .157$ was

FIGURE 1
Flow chart combining the 2 randomized TOTAL trials



FETO, fetoscopic endoluminal tracheal occlusion; TOTAL, Tracheal Occlusion To Accelerate Lung growth.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

used to claim statistical significance. We did not specify an alpha level to declare statistical significance and interpreted results more generally, on the basis of uncertainty and the integration with other evidence and considerations. The statistical analysis was performed using R version 4.0.1 (R core team 2021), using the rms package for regression modeling.¹² Information on sample size and further comments about the analysis are provided in the Appendices A-C.

Results

Table 1 summarizes the baseline characteristics and endpoints (Supplemental Tables 2 and 3 provide trial-specific results). In fetuses randomized to FETO, survival to discharge from the neonatal unit was 54% compared with 39% in

those randomized to expectant management. Supplemental Figure 1 displays the GAs at which balloon insertion was done as a function of the o/e LHR at randomization. The adjusted odds ratio (aOR) of FETO with late balloon insertion for survival to discharge was 1.78 (95% CI, 1.05–3.01; $P=.031$) (Table 2). The effect of early vs late balloon insertion was highly uncertain (aOR, 1.53; 95% CI, 0.60–3.91, $P=.370$). When combining these 2 effects, the aOR of FETO with early balloon insertion was 2.73 (95% CI, 1.15–6.49). There was evidence for associations of o/e LHR and liver herniation with survival. Importantly, the interaction between o/e LHR and intervention was weak and it was therefore not added to the model (beta, 0.011; standard error (SE), 0.016;

TABLE 1
Descriptive statistics for baseline characteristics and endpoints in the pooled data

Baseline characteristics	FETO (n=145)	Expectant (n=142)
Maternal age (y)	31.4 (27.5–34.5)	30.9 (27.0–34.3)
Gestational age (wk)	28.1 (26.6–29.3)	27.7 (26.7–29.0)
Nulliparous	70 (48)	68 (48)
Body mass index (kg/m ²)	24.0 (20.9–28.4)	23.1 (21.1–27.4)
Smoking during pregnancy	11 (8)	14 (10)
Alcohol use during pregnancy	1 (1)	1 (1)
Ethnicity		
White	124 (86)	124 (87)
Asian	9 (6)	7 (5)
Black	4 (3)	6 (4)
Other	8 (6)	5 (4)
Ultrasound findings		
Observed/expected lung-to-head ratio (%)	28.0 (24.0–33.0)	28.0 (23.2–33.4)
Liver herniation	121 (83)	116 (82)
Deepest vertical pocket of amniotic fluid (mm)	6.0 (5.0–7.9)	6.3 (5.7–7.6)
Cervical length (mm)	36 (32–40)	36 (32–40)
Placenta anterior	83 (57)	71 (50)
Placenta posterior	56 (39)	66 (46)
Placenta fundal	6 (4)	5 (4)
Endpoints		
Survival to discharge (primary endpoint)	79 (54)	55 (39)
Survival to 6 mo of age	78 (54)	55 (39)
Survival to 6 mo without oxygen supplementation	63 (43)	46 (32)
Gestational age at live birth (wk) (n=279)	35.6 (33.8–37.3)	38.3 (37.0–39.0)

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

$P=502$). The model is visualized in [Figure 2](#). On the basis of this model, [Supplemental Figure 2](#) shows the estimated differences in the probability of survival to discharge for early and late FETO vs expectant management.

The results for survival to 6 months were nearly identical to those of survival to discharge from the NICU ([Table 3](#), [Supplemental Figures 3](#) and [4](#)). Again, the interaction between o/e LHR and intervention was not included (beta, 0.011; SE, 0.016; $P=514$). For survival to 6 months of age without oxygen supplementation, the results were comparable yet more uncertain ([Table 4](#), [Figure 3](#), [Supplemental Figure 5](#)). The

aOR of FETO with late balloon insertion for survival to discharge was 1.63 (95% CI, 0.96–2.76). For FETO with early balloon insertion, the aOR was 2.21 (95% CI, 0.83–5.86). The interaction between o/e LHR and intervention was not included (beta, 0.0063; SE, 0.017; $P=710$). The results for GA at live birth were very different ([Table 5](#), [Figure 4](#)). There was evidence of a strong effect of the timing of balloon insertion. The GA at delivery was on average 1.7 weeks earlier (95% CI, 1.1–2.3) following FETO with late insertion and 3.2 weeks earlier (95% CI, 2.3–4.1) following FETO with early insertion compared with expectant management. There was

little evidence of an association of o/e LHR and liver herniation with the GA at live birth. Again, the interaction between o/e LHR and intervention was not included (beta, -0.012 ; SE, 0.023; $P=612$).

Further analyses (not prespecified) suggested that the effect of FETO on the GA at live birth was partially mediated by preterm premature rupture of membranes (PPROM) (details in [Appendix B](#)) and that live birth was delayed by on average 4 (95% CI, 1–7) days per week delay in balloon insertion ([Figure 5](#); details in [Appendix C](#)). Nevertheless, a large amount of variation in the GA at live birth was not explained

TABLE 2
Multiple logistic regression analysis for the primary endpoint survival to discharge

Covariate	B (SE)	Adjusted OR (95% CI)	Pvalue
Intervention: FETO (late insertion) vs expectant	0.58 (0.27)	1.78 (1.05–3.01)	.031
Early balloon insertion (yes vs no)	0.43 (0.48)	1.53 (0.60–3.91)	.370
Above 2 parameters combined: early FETO vs expectant ^a	1.01 (0.44)	2.73 (1.15–6.49)	
o/e LHR (%)	0.076 (0.039)	1.08 (1.00–1.17)	.0040
o/e LHR, nonlinear	0.016 (0.042)	1.02 (0.94–1.10)	
Liver herniation (yes vs no)	−0.70 (0.32)	0.50 (0.27–0.93)	.028
Trial (severe vs moderate)	−0.62 (0.44)	0.54 (0.23–1.26)	.155

B, regression coefficient; CI, confidence interval; FETO, fetoscopic endoluminal tracheal occlusion; o/e LHR, observed/expected lung-to-head-ratio; OR, odds ratio; SE, standard error.

^a The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. Am J Obstet Gynecol 2022.

by the moment of insertion (adjusted R-squared, 11%).

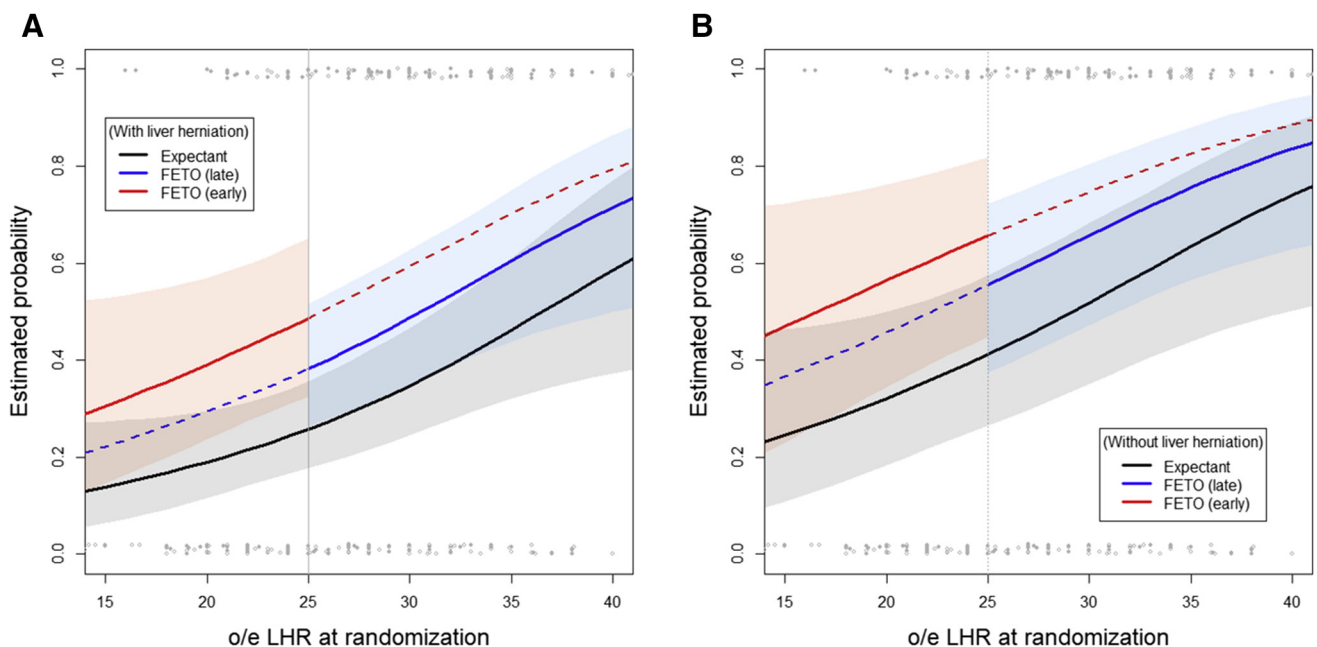
Comment

Principal findings

There are 4 main findings of this individual patient analysis from the 2 TOTAL trials. First, this study suggests that in both moderate and severe lung

hypoplasia, FETO has a beneficial effect on the survival to discharge from the NICU. Second, there is some support for a positive additional effect of early insertion. However, caution is required because of considerable uncertainty in the effects and the strong confounding with disease severity. Third, an adverse consequence of FETO is an increased

risk of preterm birth, which is further increased by early balloon insertion. Fourth, there was no evidence that the effect of FETO depends on the o/e LHR for any endpoint. The discrepancy between the results of the 2 TOTAL trials is therefore more likely caused by the difference in the timing of balloon insertion.

FIGURE 2
Estimated probabilities of survival to discharge from the model in Table 2

Panel A refers to cases with liver herniation and panel B to cases with the liver confined to the abdomen. Dashed lines indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. The raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. Am J Obstet Gynecol 2022.

TABLE 3
Multiple logistic regression analysis for the secondary endpoint survival to 6 months

Covariate	B (SE)	Adjusted OR (95% CI)	Pvalue
Intervention: FETO (late insertion) vs expectant	0.54 (0.27)	1.72 (1.02–2.89)	.043
Early balloon insertion (yes vs no)	0.45 (0.47)	1.57 (0.62–3.97)	.340
Above 2 parameters combined: early FETO vs expectant ^a	0.99 (0.44)	2.69 (1.14–6.35)	
o/e LHR (%)	0.075 (0.038)	1.08 (1.00–1.16)	.0032
o/e LHR, nonlinear	0.020 (0.041)	1.02 (0.94–1.11)	
Liver herniation (yes vs no)	–0.72 (0.32)	0.49 (0.26–0.91)	.024
Trial (severe vs moderate)	–0.60 (0.43)	0.55 (0.24–1.27)	.162

B, regression coefficient; CI, confidence interval; FETO, fetoscopic endoluminal tracheal occlusion; o/e LHR, observed/expected lung-to-head-ratio; OR, odds ratio; SE, standard error.

^a The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

Results in the context of what is known

Extensive animal studies have demonstrated that tracheal occlusion causes fluid retention in the future airways and that this acts as a mechanical stimulus for lung growth (reviewed in Khan¹⁷). At the molecular level, the DNA synthesis rate is most active 48 h after occlusion and remains stable and elevated at 7 days.¹⁸ The net effect on lung development, however, is the result of a complex interaction between when the occlusion is done, for how long, whether the occlusion is reversed, and whether steroids are used.¹⁷ The current analysis cautiously suggests that an earlier occlusion is of larger benefit. This is in line with animal experiments showing a greater response to experimental

tracheal occlusion (TO) in younger than in older fetuses with pulmonary hypoplasia.^{19,20} An earlier occlusion combines 2 factors that potentially determine the intensity of the lung response to occlusion, that is, compliance of the chest, which is higher in younger fetuses and hence permits more expansion and a longer duration of occlusion.²¹ Earlier vs late occlusion can be simulated in animals that have a longer pregnancy duration, such as sheep. In fetal lambs with CDH, a late TO induced lung growth, but its effect was more prominent when TO was done earlier.²² Also of interest is the experimental finding that TO is associated with greater growth of the mesenchymal tissue than that of the airways and hence it results in thicker air/blood gas barriers.²¹ This may, next

to the consequences of prematurity, reduce oxygen uptake across the lungs and could reduce the effect of TO on survival at 6 months without oxygen supplementation.

Also, previous clinical data support the greater effect of early occlusion. In a clinical study on longitudinal changes in lung volume following FETO, the net lung expansion rate, corrected for GA, was on average 1.5% per week after FETO.²³ Remarkably, in that study, GA at FETO was the single independent predictor of the lung volume eventually achieved, and hence, lung size (or the degree of hypoplasia) at baseline was not predictive.²³ On the basis of a model developed on that dataset, late occlusion would result in a net volume increase of 15.2% compared with 35% if the occlusion period started

TABLE 4
Multiple logistic regression analysis for the secondary endpoint survival to 6 months without oxygen dependency

Covariate	B (SE)	Adjusted OR (95% CI)	Pvalue
Intervention: FETO (late insertion) vs expectant	0.49 (0.27)	1.63 (0.96–2.76)	.070
Early balloon insertion (yes vs no)	0.30 (0.52)	1.36 (0.49–3.75)	.558
Above 2 parameters combined: early FETO vs expectant ^a	0.79 (0.50)	2.21 (0.83–5.86)	
o/e LHR (%)	0.052 (0.040)	1.05 (0.97–1.14)	.0052
o/e LHR, nonlinear	0.043 (0.042)	1.04 (0.96–1.13)	
Liver herniation (yes vs no)	–1.16 (0.32)	0.31 (0.17–0.59)	.0003
Trial (severe vs moderate)	–0.95 (0.46)	0.38 (0.16–0.96)	.040

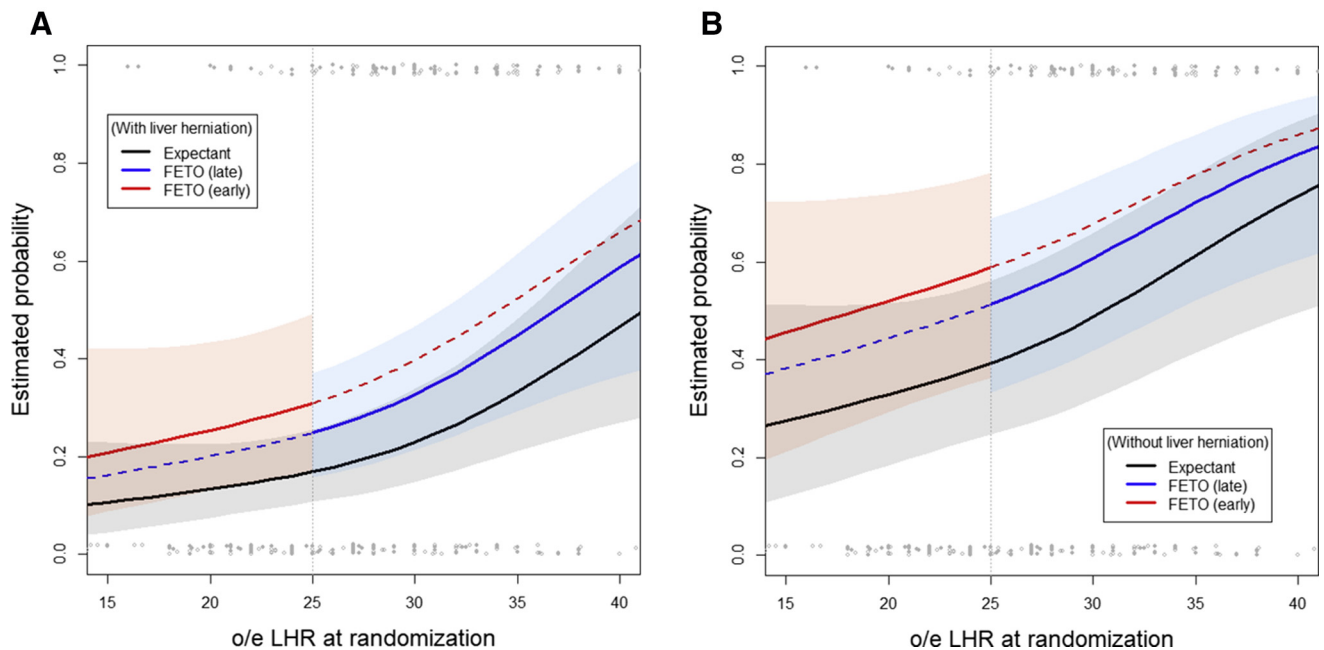
B, regression coefficient; CI, confidence interval; FETO, fetoscopic endoluminal tracheal occlusion; o/e LHR, observed/expected lung-to-head-ratio; OR, odds ratio; SE, standard error.

^a The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

FIGURE 3

The estimated probabilities of survival to 6 months without oxygen supplementation from the model in Table 4



Panel A refers to cases with liver herniation and panel B to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. The raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

earlier and lasted on average for 6 weeks. These *in vivo* data and those from an earlier study²⁴ support the correlation between the duration of occlusion and subsequent lung volume increase. Also in the TOTAL trials, the change in lung size, evidenced by an increase in o/e LHR, was on average +67% over 34 days of occlusion from 27⁺⁰ to 29⁺⁶ weeks onward,

compared with only +32% over 24 days of occlusion from 30⁺⁰ to 31⁺⁶ weeks onwards.^{9,10}

Clinical and research implications

In retrospect, our decision to insert the balloon as late as 30⁺⁰ to 31⁺⁶ weeks of gestation in moderate hypoplasia may have resulted in less lung growth, and

therefore, inadequate improvement in survival, by comparison with the expectantly managed group. The obvious strategy for extending the duration of TO is to insert the balloon in both the severe and moderate hypoplasia groups at 27⁺⁰ to 29⁺⁶ weeks of gestation and accept the inevitable increase in the risk of prematurity. An alternative strategy is to delay

TABLE 5

Multiple linear regression analysis for the secondary endpoint gestational age at live birth

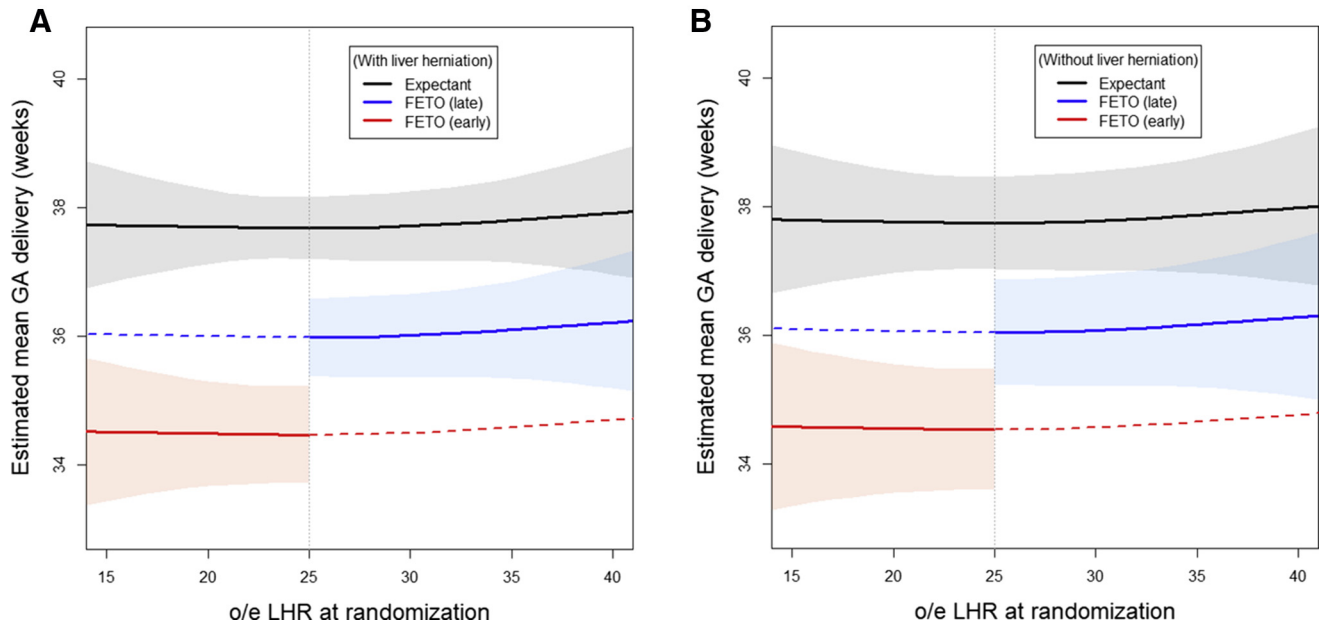
Covariate	B (SE)	Adjusted OR (95% CI)	Pvalue
Intervention: FETO (late insertion) vs expectant	-1.70 (0.31)	-2.30 to -1.09	<.0001
Early balloon insertion (yes vs no)	-1.51 (0.52)	-2.54 to -0.58	.0043
Above 2 parameters combined: early FETO vs expectant ^a	-3.21 (0.46)	-4.11 to -2.31	
o/e LHR (%)	-0.0057 (0.046)	-0.096 to 0.085	.884
o/e LHR, nonlinear	0.020 (0.049)	-0.077 to 0.12	
Liver herniation (yes vs no)	-0.065 (0.36)	-0.76 to 0.63	.885
Trial (severe vs moderate)	0.12 (0.50)	-0.86 to 1.10	.812

B, regression coefficient; CI, confidence interval; FETO, fetoscopic endoluminal tracheal occlusion; o/e LHR, observed/expected lung-to-head-ratio; OR, odds ratio; SE, standard error.

^a The effect of early FETO vs expectant management is derived from the first 2 parameters and their variance-covariance information.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

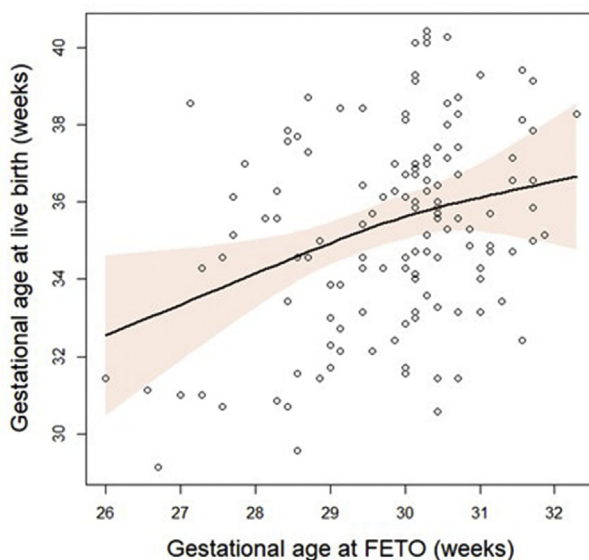
FIGURE 4
Estimated gestational age at live birth, based on the model from Table 5



Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. The *dashed lines* indicate situations that are not included in the Tracheal Occlusion To Accelerate Lung growth trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise, 95% confidence intervals are added as shaded areas. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

FIGURE 5
Gestational age at live birth by gestational age at FETO



A regression line with pointwise 95% confidence intervals is added, see Appendix C for details.

FETO, fetoscopic endoluminal tracheal occlusion.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

the removal of the balloon from 34 to 37 weeks. However, the latter would be unwise, because many women will go into labor before the planned day for removal,¹⁰ necessitating emergency removal of the balloon, which is more complex and risky than an elective procedure.²⁵ In view of these findings, we think that further clinical investigation with an earlier balloon insertion strategy in moderate cases is warranted. Obviously, this should be done with comprehensive documentation including secondary endpoints that cover the potential impact of prematurity while maintaining the same skill set and standards as in the TOTAL trial. Ideally, this would best be addressed by a randomized controlled trial. Given the rarity of the condition, the time it took to complete both TOTAL trials and the controversy on randomizing patients when benefit of fetal surgery is likely may not be evident.^{26–32} Prospective high-quality registries that capture consecutive cases and all known

confounding factors could be considered as second best.³¹ This should also include factors unrelated to the severity of the condition but to the surgery and postnatal management center, including experience and case load, which may also influence outcome.^{33,34} It may be wise to also apply that registration to the rarer forms of CDH, such as right-sided cases.³⁵

Strengths and limitations

Despite the strength of data pooling, this study has limitations. First, the individual trials had limited sample size.^{28,36} This leads to uncertain estimates, evidenced by wide confidence intervals. Second, the severe trial was stopped early for superiority. The effect estimates for trials that are stopped early for superiority can be overoptimistic.^{13,14} When estimating the overall effect of FETO, the potentially overoptimistic estimation of the effect of FETO in severe cases may be counteracted by the following: (1) the relatively large amount of data from moderate cases, (2) the use of penalized regression, and (3) the lesser known fact that group-sequential trials, when not stopped early, may have pessimistic effect sizes.³⁷ Early stopping may have affected the interaction between the intervention and o/e LHR toward finding a larger effect of FETO when o/e LHR is low. However, our results provide little support for the existence of this interaction. Third, the FETO balloon was inserted at an earlier GA in patients with severe hypoplasia to maximize lung growth.^{23,24} This confounding may artificially increase the FETO effect in severe hypoplasia. We accounted for this in the analysis, yet results should be interpreted with caution. Fourth, recruitment by fetal surgery center was imbalanced and different in the 2 trials. Postnatal care centers were even more numerous, so that the number of neonates per center eventually was very small. We used trial as a covariate to address these and other sources of heterogeneity between trials.

Conclusions

Our findings suggest that in CDH, FETO increases the survival chances. There was

no evidence that these effects depend on the o/e LHR. The results also suggest that earlier occlusion may well improve survival compared with later occlusion, but such a policy would be accompanied by an increased prematurity risk. ■

Acknowledgments

We thank all the participants and the multidisciplinary teams of all postnatal management centers that make the Tracheal Occlusion To Accelerate Lung growth trials a success.

References

- Russo FM, Cordier AG, De Catte L, et al. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). *Prenat Diagn* 2018;38:629–37.
- Jani J, Nicolaidis KH, Keller RL, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007;30:67–71.
- Deprest J, Gratacos E, Nicolaidis KH; FETO Task Group. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results. *Ultrasound Obstet Gynecol* 2004;24:121–6.
- Jani JC, Nicolaidis KH, Gratacos E, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. *Ultrasound Obstet Gynecol* 2009;34:304–10.
- Jani JC, Nicolaidis KH, Gratacos E, Vandecruys H, Deprest JA; FETO Task Group. Fetal lung-to-head ratio in the prediction of survival in severe left-sided diaphragmatic hernia treated by fetal endoscopic tracheal occlusion (FETO). *Am J Obstet Gynecol* 2006;195:1646–50.
- Al-Maary J, Eastwood MP, Russo FM, Deprest JA, Keijzer R. Fetal tracheal occlusion for severe pulmonary hypoplasia in isolated congenital diaphragmatic hernia: a systematic review and meta-analysis of survival. *Ann Surg* 2016;264:929–33.
- Reiss I, Schaible T, van den Hout L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. *Neonatology* 2010;98:354–64.
- Snoek KG, Reiss IKM, Greenough A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH euro consortium consensus - 2015 update. *Neonatology* 2016;110:66–74.
- Deprest JA, Nicolaidis KH, Benachi A, et al. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med* 2021;385:107–18.

10. Deprest JA, Benachi A, Gratacos E, et al. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med* 2021;385:119–29.

11. Van der Veecken L, Russo FM, De Catte L, et al. Fetoscopic endoluminal tracheal occlusion and reestablishment of fetal airways for congenital diaphragmatic hernia. *Gynecol Surg* 2018;15:9.

12. Harrell FE Jr. *Regression modeling strategies*. New York, NY: Springer; 2015.

13. Walter SD, Guyatt GH, Bassler D, Briel M, Ramsay T, Han HD. Randomised trials with provision for early stopping for benefit (or harm): the impact on the estimated treatment effect. *Stat Med* 2019;38:2524–43.

14. Schou IM, Marschner IC. Meta-analysis of clinical trials with early stopping: an investigation of potential bias. *Stat Med* 2013;32:4859–74.

15. van Klaveren D, Balan TA, Steyerberg EW, Kent DM. Models with interactions overestimated heterogeneity of treatment effects and were prone to treatment mistargeting. *J Clin Epidemiol* 2019;114:72–83.

16. Heinze G, Wallisch C, Dunkler D. Variable selection - a review and recommendations for the practicing statistician. *Biom J* 2018;60:431–49.

17. Khan PA, Cloutier M, Piedboeuf B. Tracheal occlusion: a review of obstructing fetal lungs to make them grow and mature. *Am J Med Genet* 2007;145C:125–38.

18. Keramidaris E, Hooper SB, Harding R. Effect of gestational age on the increase in fetal lung growth following tracheal obstruction. *Exp Lung Res* 1996;22:283–98.

19. De Paepe ME, Johnson BD, Papadakis K, Luks FI. Lung growth response after tracheal occlusion in fetal rabbits is gestational age-dependent. *Am J Respir Cell Mol Biol* 1999;21:65–76.

20. Wu J, Ge X, Verbeken EK, Gratacos E, Yesildaglar N, Deprest JA. Pulmonary effects of in utero tracheal occlusion are dependent on gestational age in a rabbit model of diaphragmatic hernia. *J Pediatr Surg* 2002;37:11–7.

21. Probyn ME, Wallace MJ, Hooper SB. Effect of increased lung expansion on lung growth and development near midgestation in fetal sheep. *Pediatr Res* 2000;47:806–12.

22. Lipsett J, Cool JC, Runciman SI, Ford WD, Kennedy JD, Martin AJ. Effect of antenatal tracheal occlusion on lung development in the sheep model of congenital diaphragmatic hernia: a morphometric analysis of pulmonary structure and maturity. *Pediatr Pulmonol* 1998;25:257–69.

23. Nawapun K, Eastwood MP, Diaz-Cobos D, et al. In vivo evidence by magnetic resonance volumetry of a gestational age dependent response to tracheal occlusion for congenital diaphragmatic hernia. *Prenat Diagn* 2015;35:1048–56.

24. Cannie MM, Jani JC, De Keyzer F, Allegaert K, Dymarkowski S, Deprest J. Evidence and patterns in lung response after fetal

tracheal occlusion: clinical controlled study. *Radiology* 2009;252:526–33.

25. Jiménez JA, Eixarch E, DeKoninck P, et al. Balloon removal after fetoscopic endoluminal tracheal occlusion for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2017;217:78.e1–11.

26. Basurto D, Russo FM, Van der Veeken L, et al. Prenatal diagnosis and management of congenital diaphragmatic hernia. *Best Pract Res Clin Obstet Gynaecol* 2019;58:93–106.

27. Ville Y. Should we offer fetal surgery for severe congenital diaphragmatic hernia or bring these cases to trial? Difference between chance and hazard. *Ultrasound Obstet Gynecol* 2020;56:491–2.

28. Deprest J. Prenatal treatment of severe congenital diaphragmatic hernia: there is still medical equipoise. *Ultrasound Obstet Gynecol* 2020;56:493–7.

29. Crombag N, Pizzolato D, Dierickx K. Direct access to investigational interventions outside the trial process: ethical reflections on the TOTAL-trial debate. *Ultrasound Obstet Gynecol* 2021;57:177–8.

30. Vergote S, Pizzolato D, Russo F, Dierickx K, Deprest J, Crombag N. The TOTAL trial dilemma: a survey among professionals on equipoise regarding fetal therapy for severe congenital diaphragmatic hernia. *Prenat Diagn* 2021;41:179–89.

31. Verweij EJ, de Vries MC, Oldekamp EJ, et al. Fetoscopic myelomeningocele closure: is the scientific evidence enough to challenge the gold standard for prenatal surgery? *Prenat Diagn* 2021;41:949–56.

32. Provoost V, Tilleman K, D'Angelo A, et al. Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014;29:413–7.

33. Grushka JR, Laberge JM, Puligandla P, Skarsgard ED; Canadian Pediatric Surgery Network. Effect of hospital case volume on outcome in congenital diaphragmatic hernia: the experience of the Canadian Pediatric Surgery Network. *J Pediatr Surg* 2009;44:873–6.

34. Senat MV, Bouchghoul H, Stirnemann J, et al. Prognosis of isolated congenital

diaphragmatic hernia using lung-area-to-head-circumference ratio: variability across centers in a national perinatal network. *Ultrasound Obstet Gynecol* 2018;51:208–13.

35. Russo FM, Cordier AG, Basurto D, et al. Fetal endoscopic tracheal occlusion reverses the natural history of right-sided congenital diaphragmatic hernia: European multicenter experience. *Ultrasound Obstet Gynecol* 2021;57:378–85.

36. Rodrigues HC, van den Berg PP. Randomized controlled trials of maternal-fetal surgery: a challenge to clinical equipoise. *Bioethics* 2014;28:405–13.

37. Marschner IC, Schou IM. Underestimation of treatment effects in sequentially monitored clinical trials that did not stop early for benefit. *Stat Methods Med Res* 2019;28:3027–41.

38. Baron RM, Kenny DA. The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986;51:1173–82.

39. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. *Int J Epidemiol* 2013;42:1511–9.

40. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K, Trinh M, Wong W. Mediation: R package for causal mediation analysis. R package version 4.5.0. Available at: <https://cran.r-project.org/web/packages/mediation/index.html>.

Author and article information

From the Department of Development and Regeneration, Cluster Woman and Child, KU Leuven, Leuven, Belgium (Drs Van Calster, Van Mieghem, DeKoninck, Russo, and Deprest); Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands (Dr Van Calster); EPI-center, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium (Dr Van Calster); Department of Obstetrics and Gynaecology of the Hospital Antoine Béclère, Université Paris Saclay, Clamart, France (Dr Benachi); King's College Hospital, London, United Kingdom (Dr Nicolaidis); Hospital Clinic and Sant Joan de Deu, Barcelona, Spain (Dr Gratacos); University Hospital Bonn, Bonn, Germany (Dr Berg); Hospital Maggiore Policlinico IRCCS, University of Milan, Milan, Italy (Dr Persico); Mater Mother's Hospital, Brisbane, Australia (Dr Gardener); Texas Children's Hospital,

Baylor College of Medicine Houston, TX (Dr Belfort); Hospital Necker, Paris, France (Dr Ville); Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada (Drs Ryan and Van Mieghem); Children's Hermann Memorial Hospital, Houston, TX (Dr Johnson); National Center for Child Health and Development, Tokyo, Japan (Dr Sago); First Department of Obstetrics and Gynecology, Medical University of Warsaw, Warsaw, Poland (Dr Kosiński); Medical and Surgical Department of the Fetus-Newborn-Infant, Bambino Gesù Children's Hospital, Research Institute, Rome, Italy (Dr Bagolan); Clinical Department of Obstetrics and Gynaecology, University Hospitals Leuven, Leuven, Belgium (Drs Russo and Deprest); The Ritchie Centre, Hudson Institute for Medical Research, Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia (Dr Hooper); Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands (Dr DeKoninck); and Institute for Women's Health, University College London Hospital, London, United Kingdom (Dr Deprest).

Received July 15, 2021; revised Nov. 15, 2021; accepted Nov. 15, 2021.

The authors report no conflict of interest.

The setup of the Tracheal Occlusion To Accelerate Lung growth (TOTAL)-trials was funded by the European Commission through its sixth framework program in Life Sciences & Health (CT2006-37409) and later, by the KU Leuven (Belgium; C32/17/054) (both to J.A.D.), the Wellcome Trust (WT101957) and from the Engineering and Physical Sciences Research Council (NS/A000027/1) of the United Kingdom for the "Image-Guided Intrauterine Minimally Invasive Fetal Diagnosis and Therapy" study (both to J.A.D. and K.H.N.), and the Unité de Recherche Clinique Paris APHP Centre, Université de Paris, France (to A.B.) for data management and monitoring. B.V.C. was supported by Internal Funds KU Leuven (C24M/20/064). The funding sources had no role in the study design; collection, analysis, and interpretation of data; writing of the report; or the decision to submit the article for publication.

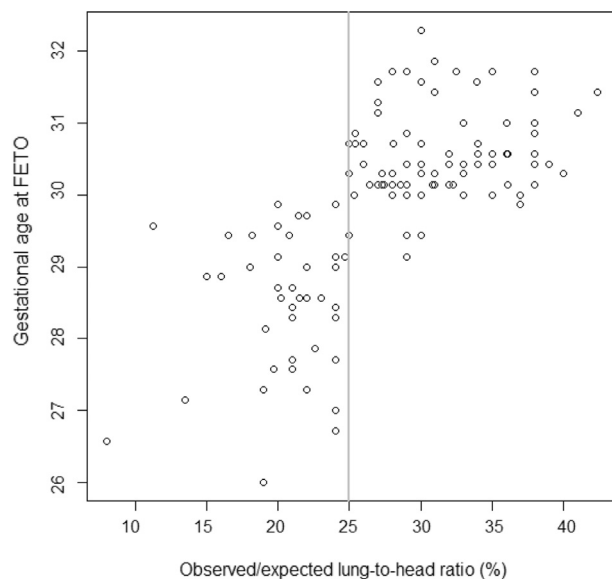
The TOTAL trials were registered at www.clinicaltrials.gov, for the severe trial as ClinicalTrials.gov Identifier: NCT01240057, on November 15, 2010, enrolling the first patient on February 11, 2011 and last on February 16, 2020, and for the moderate trial as ClinicalTrials.gov Identifier: NCT00763737, on October 1, 2008, enrolling the first patient on October 10, 2008 and last on May 2, 2019.

Corresponding author: Jan A. Deprest, MD, PhD. jan.deprest@uzleuven.be

Supplemental Material

SUPPLEMENTAL FIGURE 1

Gestational age at FETO by observed/expected lung-to-head ratio



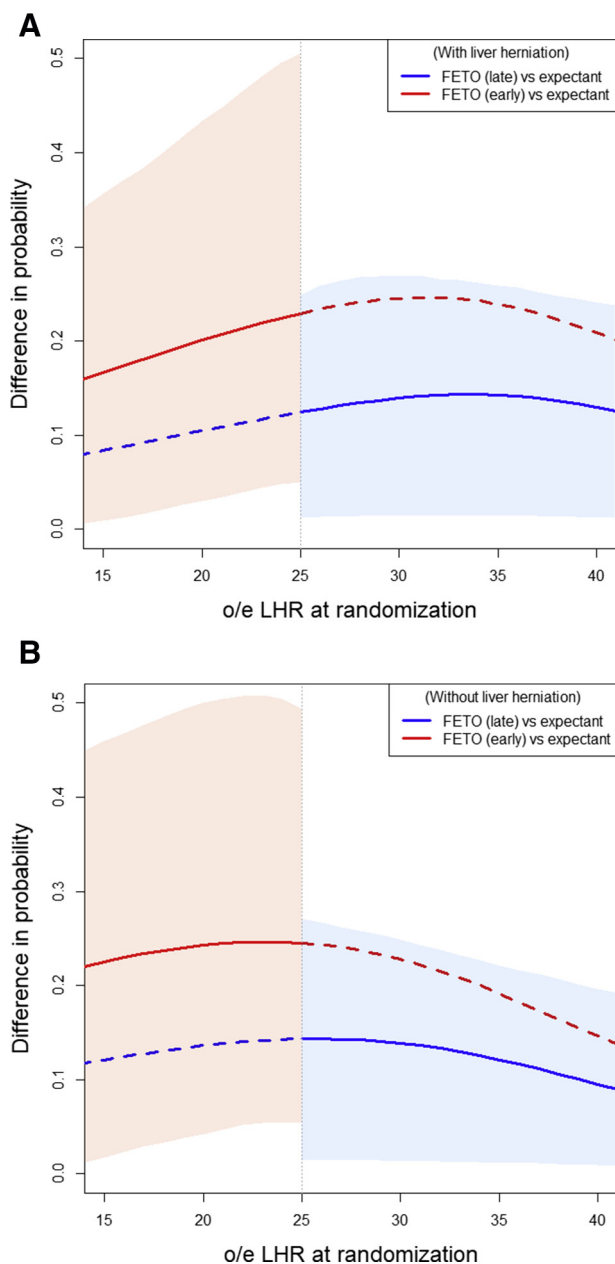
Scatter plot of the observed/expected lung-to-head ratio and gestational age at FETO in patients randomized to FETO and in whom FETO was performed/attempted (n=138). The *vertical line* demarcates the severe and moderate cases.

FETO, fetoscopic endoluminal tracheal occlusion.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL FIGURE 2

Estimated differences in the probability for survival to discharge for FETO vs expectant management



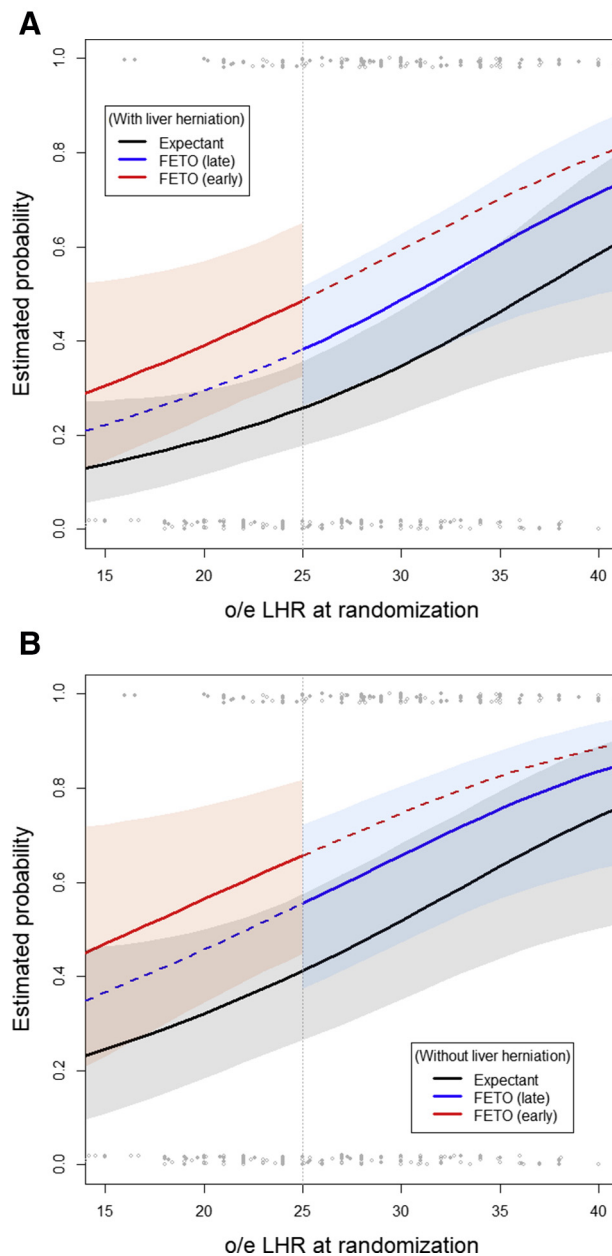
Based on the model in Table 2. Panel **A** refers to cases with liver herniation and panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

FETO, fetoscopic endoluminal tracheal occlusion; TOTAL, Tracheal Occlusion To Accelerate Lung growth.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL FIGURE 3

Estimated probabilities of survival to 6 months of age from the model in Table 3

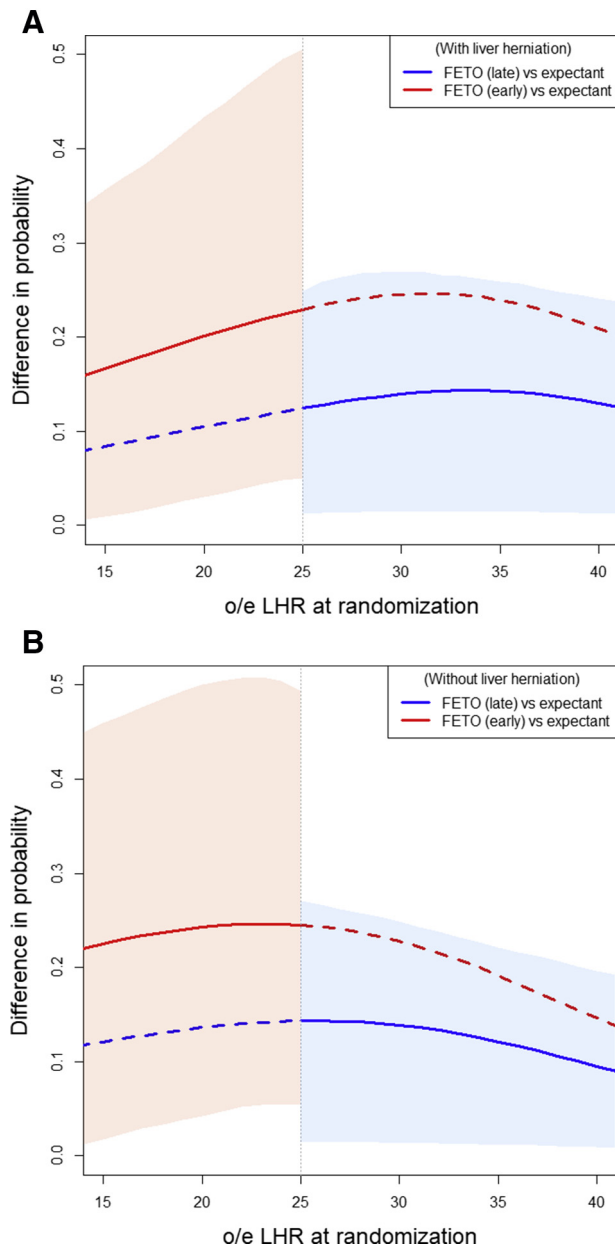


Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas. Raw patient data are indicated on top (survivors) and at the bottom (nonsurvivors) of the plot. Technical detail: to create these plots, we set the trial covariate to 0.5 (halfway between severe and moderate).

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL FIGURE 4

Estimated differences in the probability for survival to 6 months for FETO vs expectant management



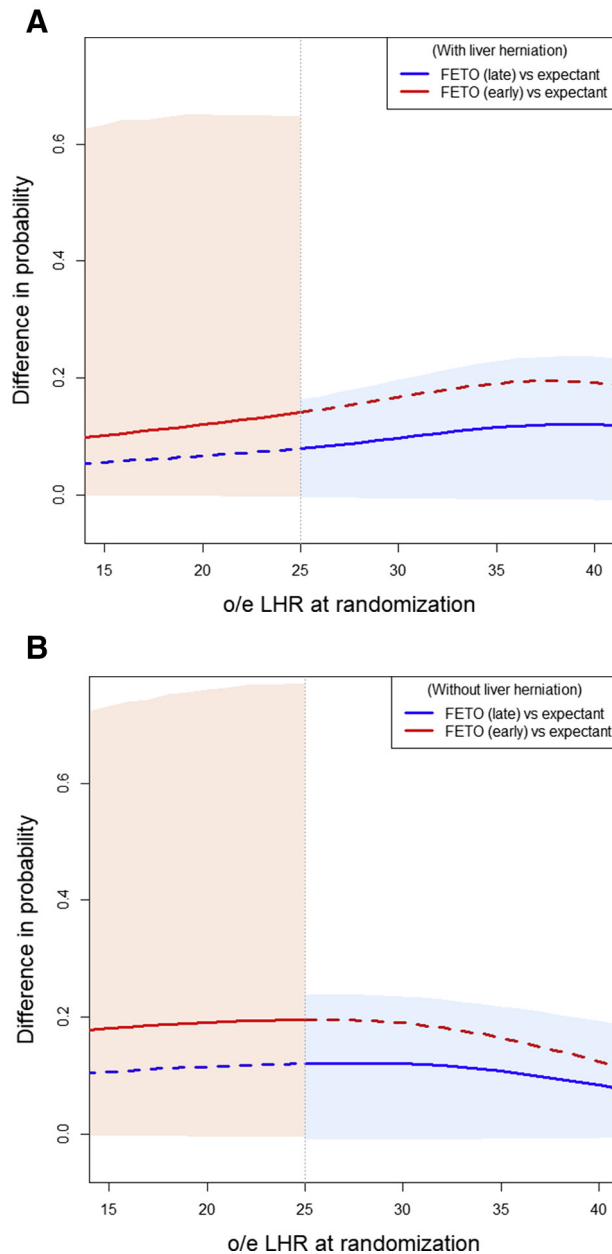
Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

FETO, fetoscopic endoluminal tracheal occlusion; TOTAL, Tracheal Occlusion To Accelerate Lung growth.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL FIGURE 5

Estimated differences in the probability for survival to 6 months without oxygen supplementation for FETO vs expectant management



Panel **A** refers to cases with liver herniation, panel **B** to cases with the liver confined to the abdomen. *Dashed lines* indicate situations that were not present in the TOTAL trials: early insertion in moderate hypoplasia and late insertion in severe hypoplasia. Pointwise 95% confidence intervals are added as shaded areas based on the percentile bootstrap method with 10,000 bootstraps.

FETO, fetoscopic endoluminal tracheal occlusion; TOTAL, Tracheal Occlusion To Accelerate Lung growth.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL TABLE 1

Patients per center and per trial

Center	FETO center location	Pooled, n (%)	RCT moderate, n	RCT severe, n
1	Leuven, Belgium	77 (27)	56	21
2	Paris Antoine Bécclère, France	65 (23)	46	19
3	London, United Kingdom	45 (16)	18	27
4	Barcelona, Spain	35 (12)	34	1
5	Milan, Italy	15 (5)	10	5
6	Bonn, Germany	14 (5)	13	1
7	Toronto, Canada	6 (2)	0	6
8	Brisbane, Australia	5 (2)	5	0
9	Houston, Baylor College, United States	5 (2)	5	0
10	Paris Necker, France	5 (2)	5	0
11	Warsaw, Poland	5 (2)	1	4
12	Tokyo, Japan	5 (2)	0	5
13	Houston UTH, United States	4 (1)	2	2
14	Rome, Italy	1 (0.3)	1	0
	TOTAL	287 (100)	196	91

FETO, fetoscopic endoluminal tracheal occlusion; RCT, randomized controlled trial; UTH, The University of Texas Health Science Center.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL TABLE 2

Descriptive statistics of baseline characteristics and endpoints for the data from the trial on moderate hypoplasia

Baseline characteristics	Fetoscopic endoluminal tracheal occlusion (n=98)	Expectant (n=98)
Maternal age (y)	31.1 (27.5–33.7)	31.6 (27.4–34.9)
Gestational age (wk)	28.4 (26.6–29.6)	28.2 (27.0–29.3)
Nulliparous	45 (46)	48 (49)
Body mass index (kg/m ²)	23.8 (20.4–28.2)	22.2 (21.0–25.9)
Smoking during pregnancy	11 (11)	8 (8)
Alcohol use during pregnancy	1 (1)	1 (1)
Ethnicity		
White	87 (89)	88 (90)
Asian	3 (3)	3 (3)
Black	2 (2)	2 (2)
Other	6 (6)	5 (5)
Ultrasound findings		
Observed/expected lung-to-head ratio (%)	30.9 (28.0–34.0)	31.0 (28.0–34.5)
Liver herniation	79 (81)	78 (80)
Deepest vertical pocket of amniotic fluid (mm)	6.0 (5.0–7.6)	6.4 (5.5–7.7)
Cervical length (mm)	37 (33–40)	36 (31–40)
Placenta anterior	50 (51)	47 (48)
Placenta posterior	43 (44)	47 (48)
Placenta fundal	5 (5)	4 (4)
Endpoints		
Survival to discharge (primary endpoint)	62 (63)	49 (50)
Survival to 6 mo of age	61 (62)	49 (50)
Survival to 6 mo without oxygen supplementation	53 (54)	43 (44)
Gestational age at live birth (wk) (n=191)	35.9 (34.3–37.9)	38.1 (37.0–38.9)

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL TABLE 3

Descriptive statistics of baseline characteristics and endpoints for the data from the trial on severe hypoplasia

Baseline characteristics	Fetoscopic endoluminal tracheal occlusion (n=47)	Expectant (n=44)
Maternal age (y)	32.1 (27.3–35.3)	29.4 (25.9–33.8)
Gestational age (wk)	27.7 (26.6–28.6)	27.0 (26.4–28.0)
Nulliparous	25 (53)	20 (45)
Body mass index (kg/m ²)	24.1 (21.9–29.7)	24.3 (21.7–30.5)
Smoking during pregnancy	0 (0)	6 (14)
Alcohol use during pregnancy	0 (0)	0 (0)
Ethnicity		
White	37 (79)	36 (82)
Asian	6 (13)	4 (9)
Black	2 (4)	4 (9)
Other	2 (4)	0 (0)
Ultrasound findings		
Observed/expected lung-to-head ratio (%)	21.0 (19.9–24.0)	21.0 (18.0–23.0)
Liver herniation	42 (89)	38 (86)
Deepest vertical pocket of amniotic fluid (mm)	6.5 (5.6–8.0)	6.2 (5.8–7.4)
Cervical length (mm)	34 (30–39)	36 (32–39)
Placenta anterior	33 (70)	24 (55)
Placenta posterior	13 (28)	19 (43)
Placenta fundal	1 (2)	1 (2)
Endpoints		
Survival to discharge (primary endpoint)	17 (36)	6 (14)
Survival to 6 mo of age	17 (36)	6 (14)
Survival to 6 mo without oxygen supplementation	10 (21)	3 (7)
Gestational age at live birth (wk) (n=88)	34.6 (31.9–36.7)	38.4 (36.6–39.0)

Data are presented as median (interquartile range) or as number (percentage). There were no missing values for these parameters.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

Appendix A

Additional information about sample size and statistical analysis.

Change to the statistical analysis plan based on reviewers' comments

Because of a reviewer comment, the final approach for the regression models deviated from the initial approach. Initially, we did not include the trial covariate and aimed to adjust for fetal surgery center instead. The fetal surgery center is where the patient was assessed, randomized, and in the case of fetoscopic endoluminal tracheal occlusion, the balloon was also inserted and removed. Because randomization was not stratified, the individual trials did not require an adjustment for center. When pooling data, however,

the distribution of fetal surgery center was different for moderate and severe cases. We did not adjust for center using a random intercept: many centers had few recruits, causing computational difficulties (no convergence). Instead, we used a categorical variable: center 1 (n=77), center 2 (n=65), and the other centers (n=145). We used only 3 levels to avoid spending too many model parameters on this covariate. We also applied variable selection on this covariate only (that is, other covariates were included by default) using an alpha level of 0.157. For all endpoints, the center covariate was omitted because the obtained *P* value was $>.157$. However, using a binary trial covariate to indicate in which trial each participant was recruited (severe vs moderate) may be

a better approach to cover remaining heterogeneity between trials.

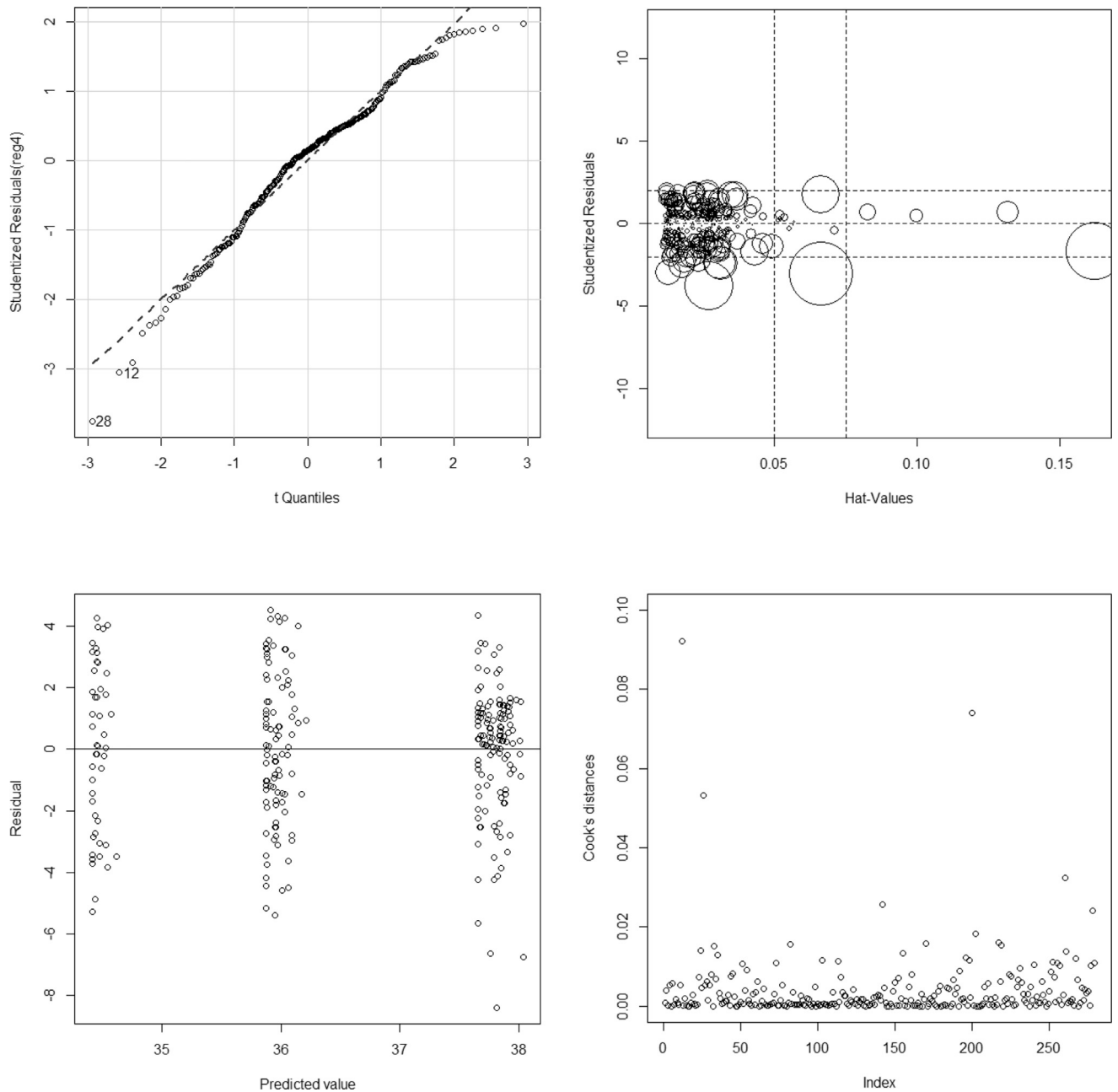
Penalized regression

We used penalized maximum likelihood estimation where the penalty factor lambda is chosen using the corrected Akaike information criterion from a grid of value between 0 and 20 with steps of 0.1. We used the `pen` function from the `rms` R package.¹²

Model diagnostics for the ordinary least squares regression model of gestational age at delivery

We state in the main text that GA at delivery does not need to be transformed. We examined the plots shown in [Supplemental Figure 6](#) and [Supplemental Figure 7](#) (after applying the optimal Box-Cox transformation for GA at delivery).

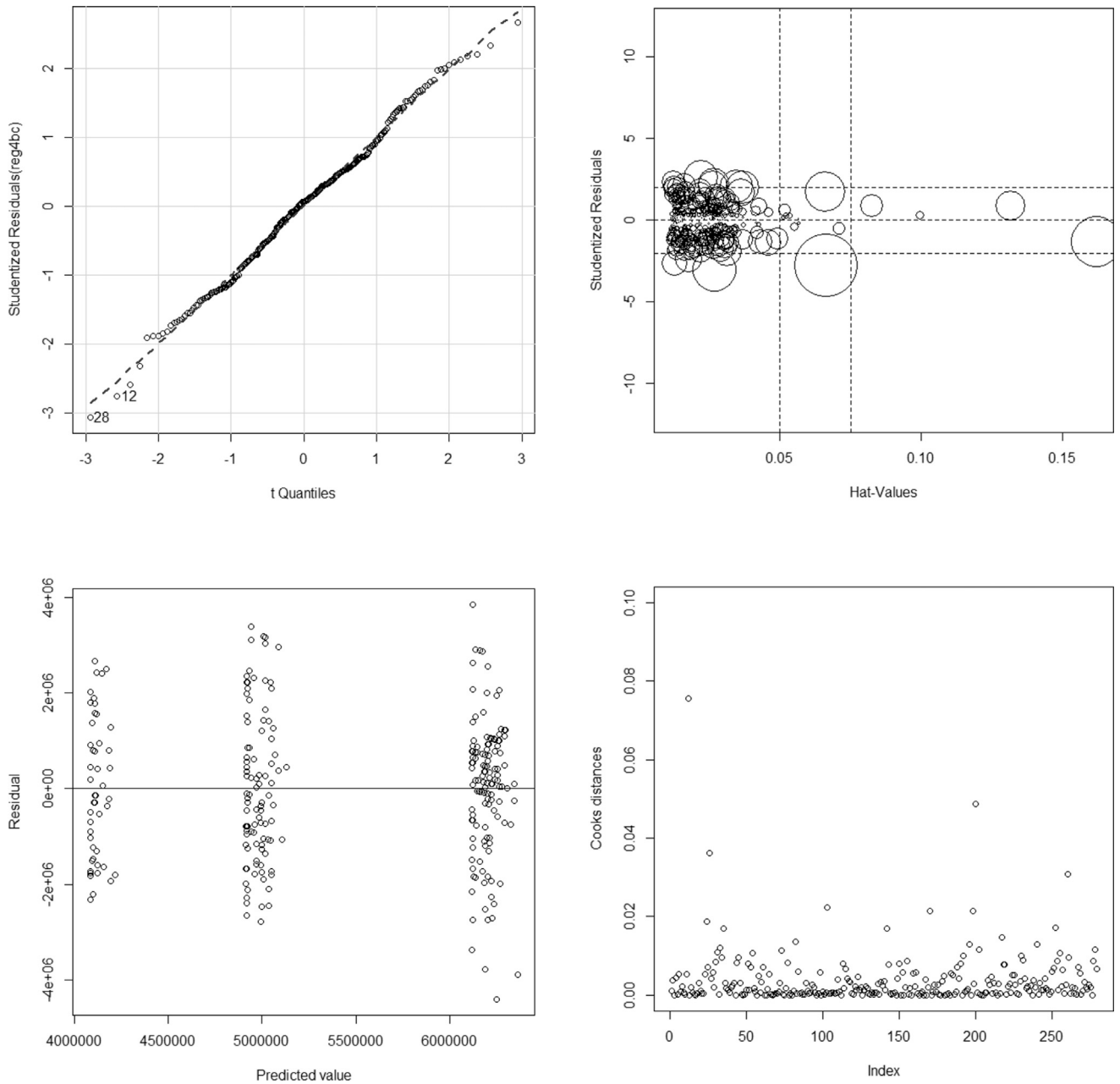
SUPPLEMENTAL FIGURE 6
Diagnostic plots for the model presented in Table 5



Top left presents the qq plot, top right the residual by predicted value plot to evaluate heteroskedasticity, bottom left the influence plot of studentized residuals by hat values, and bottom right the Cook's distance values for all observations.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

SUPPLEMENTAL FIGURE 7
Diagnostic plots for the regression model of gestational age at delivery after the optimal Box-Cox transformation (lambda 4.727)



The top left presents the qq plot, the top right represents the residual by predicted value plot to evaluate heteroskedasticity, the bottom left the influence plot of studentized residuals by hat values and bottom right the Cook's distance values for all observations.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. Am J Obstet Gynecol 2022.

Sample size

We considered 8 parameters in the multivariable analyses. The number of 'events' is defined by the number of cases in the smallest outcome category for binary endpoints and by the sample size for continuous endpoints. The number of 'events' was 134 for survival to discharge (134 survived, 153 did not survive), 133 for survival to 6 months, 109 for survival to 6 months without oxygen dependency, and 279 for gestational age at live birth. For the primary endpoint, there were 134/8=17 events per parameter. For survival to 6 months without oxygen dependency, there were 109/8=14 events per variable. Although the events per

variable is an overly rough criterion that ignores contextual factors, these values are acceptable by most common standards when the focus is on model coefficients rather than on making robust predictions for new patients.¹⁶ After changing the analysis plan following a reviewer comment, the multivariable analyses contained only 7 parameters and hence the number of events per parameter was higher.

Appendix B

This section discusses the mediating role of preterm premature rupture of membranes (PPROM) for the effect of fetoscopic endoluminal tracheal

occlusion on gestational age (GA) at live birth.

To study the potential mediating role of PPRM, we conducted a classical Baron-Kenny mediation analysis.³⁸ First, we repeated the analysis of GA at live birth, but added PPRM before 37 weeks as a binary covariate. We were interested in the effect of PPRM, and in the eventual decrease of the effects of intervention and early balloon insertion after adding PPRM as covariate (Supplemental Table 4). Relative to the model without PPRM (Table 5 in main paper), the coefficient of intervention reduced by 46% (from -1.70 to -0.91), the coefficient for early insertion reduced by 13% (from -1.51 to -1.32).

SUPPLEMENTAL TABLE 4

Linear regression of gestational age at live birth (in weeks) with additional correction for PPRM before 37 weeks (n = 279)

Covariate	B (SE)	Pvalue
Intervention: FETO (late insertion) vs expectant	-0.91 (0.28)	.0014
Early balloon insertion (yes vs no)	-1.32 (0.46)	.0044
PPROM <37 wk (yes vs no)	-2.63 (0.28)	<.0001
o/e LHR (%)	-0.0067 (0.041)	.984
o/e LHR, nonlinear	0.0035 (0.044)	
Liver herniation (yes vs no)	0.0026 (0.31)	.993
Trial (severe vs moderate)	-0.042 (0.44)	.924

B, model coefficient; FETO, fetoscopic endoluminal tracheal occlusion; o/e LHR, observed/expected lung-to-head ratio; PPRM, preterm premature rupture of membranes; SE, standard error. Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. Am J Obstet Gynecol 2022.

Second, we conducted a logistic regression analysis of PPRM using the same covariates as in the other models (intervention, early balloon insertion, o/e LHR with nonlinear parameter, and liver herniation) (Supplemental Table 5).

SUPPLEMENTAL TABLE 5

Logistic regression of preterm premature rupture of membranes <37 weeks (n = 279)

Covariate	B (SE)	Pvalue
Intervention: fetoscopic endoluminal tracheal occlusion (late insertion) vs expectant	1.48 (0.30)	<.0001
Early balloon insertion (yes vs no)	0.39 (0.48)	.417
o/e LHR (%)	-0.0017 (0.036)	.590
o/e LHR, nonlinear	-0.031 (0.041)	
Liver herniation (yes vs no)	0.13 (0.35)	.718
Trial (severe vs moderate)	-0.34 (0.46)	.460

B, model coefficient; o/e LHR, observed vs expected lung-to-head ratio; SE, standard error.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. Am J Obstet Gynecol 2022.

These results suggest that the effect of FETO on GA at live birth is partially mediated by PPROM: PPROM has an effect on GA at live birth and its inclusion in the model halves the coefficient of the intervention variable, and the intervention variable has an effect on PPROM. There was little evidence that the effect of early balloon insertion on GA at live birth was (partly) mediated by PPROM, but this may have been caused by the fact that

we included PPROM as a binary variable.

The classical Baron-Kenny approach to mediation analysis may however be flawed in certain circumstances, such as when there is an interaction between exposure (FETO in this study) and mediator (PPROM).³⁹ We therefore conducted a causal mediation analysis using version 4.5.0 of the mediate package for R.⁴⁰ We allowed for a possible interaction between the exposure (FETO)

and the mediator (PPROM), using robust standard errors and 1000 simulations using the quasi-Bayesian Monte Carlo method. However, we did not use penalized estimation for this analysis. The results confirm the results from the classical analysis, suggesting that 52% of the total effect of FETO on GA at live birth is mediated by PPROM (Supplemental Table 6). The evidence of an interaction between exposure and mediator was weak ($P=.45$).

SUPPLEMENTAL TABLE 6

Linear regression of gestational age at live birth (in weeks) with additional correction for the preterm premature rupture of membranes before 37 weeks (n = 279)

Statistic	Estimate (95% confidence interval)
Total effect	-1.77 (-2.48 to -1.05)
Average causal mediated effect	
Expectant	-1.01 (-1.57 to -0.46)
FETO	-0.85 (-1.31 to -0.44)
Average	-0.93 (-1.38 to -0.48)
Average direct effect	
Expectant	-0.92 (-1.58 to -0.30)
FETO	-0.76 (-1.40 to -0.08)
Average	-0.84 (-1.45 to -0.21)
Proportion mediated (average)	0.52 (0.31 to 0.84)

FETO, fetoscopic endoluminal tracheal occlusion.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

Appendix C

This section discusses the relationship between gestational age (GA) at fetoscopic endoluminal tracheal occlusion (FETO) and GA at live birth.

We observed that FETO and early balloon insertion had an effect on the

gestational age (GA) at live birth, whereas there was very little evidence that the observed/expected lung-to-head-ratio and liver herniation were prognostic for GA at live birth. We therefore conducted a post-hoc simple linear regression of the GA at live birth on the gestational age at FETO in

cases randomized to FETO and in whom FETO was performed (n=138, including the patient where balloon insertion failed because of the fetal position). We again used restricted cubic splines to model the GA at FETO (Supplemental Table 7, Figure 5 in main text).

SUPPLEMENTAL TABLE 7

Linear regression of gestational age at live birth (in weeks) on gestational age at FETO in patients randomized to FETO and who received FETO (n = 138)

Covariate	B (SE)	Pvalue
GA at FETO (wk)	0.80 (0.33)	.017
GA at FETO (wk), nonlinear	−0.20 (0.31)	.536
Trial (severe vs moderate)	−0.083 (0.65)	.899

B, model coefficient; GA, gestational age; FETO, fetoscopic endoluminal tracheal occlusion; SE, standard error.

Van Calster et al. Reanalysis of Tracheal Occlusion To Accelerate Lung growth trials for congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2022.

There was evidence of a relationship between the gestational age (GA) at FETO and the GA at live birth, but considerable interindividual variability was observed. This is evident from Figure 5 (large spread of GA at live birth conditional on gestational age at

FETO), and by the adjusted R-squared value of 11%. The R-squared value estimates the amount of variability in the GA at live birth that is explained by variability in the GA at FETO.

An analysis including only a linear term for gestational age at

FETO yielded a regression coefficient (beta) of 0.59 (standard error, 0.23), suggesting that live birth is delayed by on average 4 days ($7 \times 0.59 = 4.1$; 95% confidence interval, 1.0–7.3) per week delay in balloon insertion.