Pediatric Surgery International (2019) 35:1131–1136
https://doi.org/10.1007/s00383-019-04543-9

ORIGINAL ARTICLE

A new approach using image analysis to assess pulmonary hypoplasia in the fetal lamb diaphragmatic hernia model

Takuya Kawaguchi[1] · Kohei Kawaguchi[1] · Juma Obayashi[1] · Kunihide Tanaka[1] · Kei Ohyama[1] · Yasuji Seki[1] · Hideki Nagae[1] · Shigeyuki Furuta[1] · Kevin C. Pringle[2] · Hiroaki Kitagawa[1]

Accepted: 1 August 2019 / Published online: 14 August 2019

Abstract

Background In congenital diaphragmatic hernia (CDH), there is pulmonary hypoplasia (PH) and also pulmonary vascular and bronchial abnormalities. Few studies have investigated bronchial maldevelopment in CDH. We evaluated bronchial area (BA) by bronchography in a fetal lamb DH model to develop a measure of PH.

Methods We created DH in fetal lambs at 75 days gestation, delivering by cesarean section and killing them at term (DH, $n=12$). Normal term fetuses provided controls (C, $n=5$). We measured total lung volume (TLV) and performed barium bronchography. Using image analysis, BA, total lung area (TLA) and bronchial area/lung area ratio (B/L ratio) were calculated. Student's T test ($p<0.05$; significant) and Spearman's correlation coefficient were performed.

Results TLV (ml) was 133.3 ± 41.2 in DH and 326 ± 22.5 in C ($p=0.0000001$). TLA (cm^2) was 78.8 ± 17.4 in DH and 107.1 ± 10.3 in C ($p=0.006$). BA (cm^2) was 39.6 ± 11.9 in DH and 52.2 ± 7.7 in C ($p=0.019$). The B/L ratio was 0.45 ± 0.06 in DH and 0.49 ± 0.05 in C ($p=0.28$). There are correlations in DH between TLV and TLA ($r=0.79$), TLV and BA ($r=0.73$) and in C between TLV and TLA ($r=0.97$) and TLV and BA ($r=0.67$).

Conclusion It may be possible to assess PH on fetal MRI, given the correlation between TLV and TLA, and TLV and BA.

Keywords Congenital diaphragmatic hernia · CDH · Pulmonary hypoplasia · Image analysis · Bronchial abnormalities

Introduction

Congenital diaphragmatic hernia (CDH) occurs approximately once per 2500 births [1]. Though the management for CDH after birth has been improving, there is still a high mortality in CDH, especially for those requiring ECMO [1]. The feature that determines survival in CDH is not just pulmonary hypoplasia (PH), but also the significant abnormalities of the bronchi and the pulmonary arteries [2–4], which contribute to the respiratory failure often seen after birth and greatly affect prognosis [4, 5].

Fetal treatment using fetal endoscopic tracheal occlusion (FETO) reported by Harrison et al. [6] has been attempted, and the TOTAL trial centered on Europe is currently being undertaken [7], but the results are not yet available.

In recent years, prenatal diagnosis using ultrasound or MRI has rapidly advanced [8]. In the case of severe CDH, accurate diagnosis and prognosis are necessary both for assessment of the need for prenatal treatment and to optimize postnatal management. It is known that prolapse of the liver through the diaphragmatic defect is associated with a poor prognosis [9, 10]. Lung/head ratio (LHR) estimated by prenatal ultrasound or MRI has been conventionally used as a prenatal predictor of severity [10].

However, these predictors are not reliable [11]. A study of risk classification using indicators such as lung/liver signal ratio (LLSIR) has also been reported [11]. Even using these indicators, it is still difficult to assess the prenatal pulmonary bronchial development on fetal MRI, though there is the possibility that vascular development may be able to be assessed in the near future.

✉ Takuya Kawaguchi
t2kawaguchi@marianna-u.ac.jp

1 Division of Pediatric Surgery, St. Marianna University School of Medicine, 2-16-1, Sugao, Miyamae-ku, Kawasaki, Kanagawa 2168511, Japan

2 Department of Obstetrics and Gynecology, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand

If we can determine the correlation between total lung volume and pulmonary bronchial development before birth on fetal MRI, we may be able to improve the accuracy of predicting the prognosis of fetuses diagnosed with DH. Given that there is a strong correlation between the development of pulmonary vessels and bronchi [2, 3], it could be possible to predict the pulmonary bronchial development and, possibly, prognosis, using the pulmonary vascular area on fetal MRI.

In our previous study [12], we created a CDH lamb model and assessed lung maturity using both the type 1 alveolar epithelial cell ratio (AT1 ratio) on pathological assessment and image analyzing software. In this study, using the same image analyzing software, we evaluated the correlation between TLA, BA, using X-ray images of the lungs, and TLV, to assess the possibility that the BA is significantly correlated with TLV and TLA in the hope that this may eventually be extrapolated to an assessment of the pulmonary vascular area as an accurate indicator of prognosis in antenatally diagnosed CDH.

Material and methods

The study was approved by the University of Otago, Wellington, Animal Ethics Committee (Wellington, New Zealand, Approval Number AEC 1–16).

Prior to transport from the farm 24–72 h prior to surgery, the ewes were checked by ultrasound to confirm their pregnancy and avoid any unnecessary surgery. The perioperative management and anesthesia management performed on sheep were as previously reported [13, 14].

A left-sided DH was created in 13 fetal lambs at approximately 75 days of gestation (Group DH). The uterus was exposed through an L flank incision under general anesthesia. A transverse hysterotomy was performed and the upper body of the fetus was exposed down to the umbilical cord. An incision was made in the left thorax through the eighth or ninth intercostal space. An incision approximately 1.5 cm long was made in the left hemi-diaphragm. The stomach was pulled into the thoracic cavity through a diaphragmatic incision to make a diaphragmatic hernia. The chest wall was closed, and the fetus was returned to the uterus. The uterus and the abdominal wall of the ewe were closed as we previously reported [13, 14]. The ewes were returned to the pasture and monitored daily. At term (about 145 days of gestation), the fetuses were delivered by caesarean section and killed.

The DH lambs were killed as we reported previously [13, 14]. Five normal fetuses were delivered by cesarean section at term and killed as controls (Group C), using the same protocols. The body weight and crown-to-rump length were measured. The lungs were fixed by tracheal infusion with 10% buffered formaldehyde at 30 cm water and removed. Total lung volume was measured by water displacement. The fixed lungs were taken to the radiology suite and barium was injected into the trachea in a stepwise fashion until the terminal bronchioles were visible on X-ray. The final X-ray was taken ensuring that all the lobes were adequately displayed (Fig. 1).

Image analysis was performed on the radiographs using "Image J" [15]. Several regions of the lung parenchyma and bronchi and other areas were selected to make artificial intelligence (AI) data that distinguished the density of these regions (Fig. 2a, b). The resulting RGB images of all lung and bronchial regions were converted to 8-bit images without the trachea and subjected to automatic thresholding (Fig. 3a, b). Although it would be preferable to calculate these parameters in three dimensions, in this study we examined only two-dimensional X-rays, which is what is generally available when MRI or U/S images are displayed. The AI data created in the first example were applied to all cases, and each image was measured under the same conditions [16]. Using these images, the total lung area (TLA) and bronchial area (BA) were measured, and the bronchial area/lung area ratio (B/L ratio) was calculated. These results were then correlated with the total lung volume (TLV) in each group and these findings were compared between both groups.

The values obtained in each measurement were described as mean ± standard deviation. Student's t test and Spearman's correlation coefficient were used for statistical analysis, with $p < 0.05$ considered to be statistically significant for

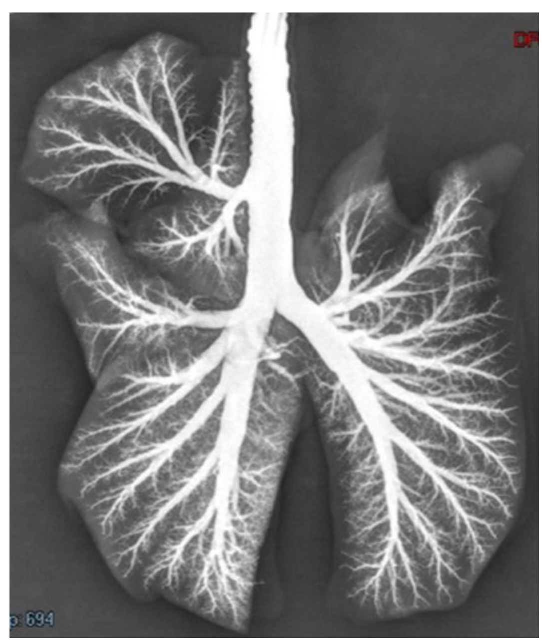

Fig. 1 Lung bronchography radiographs taken after barium injection

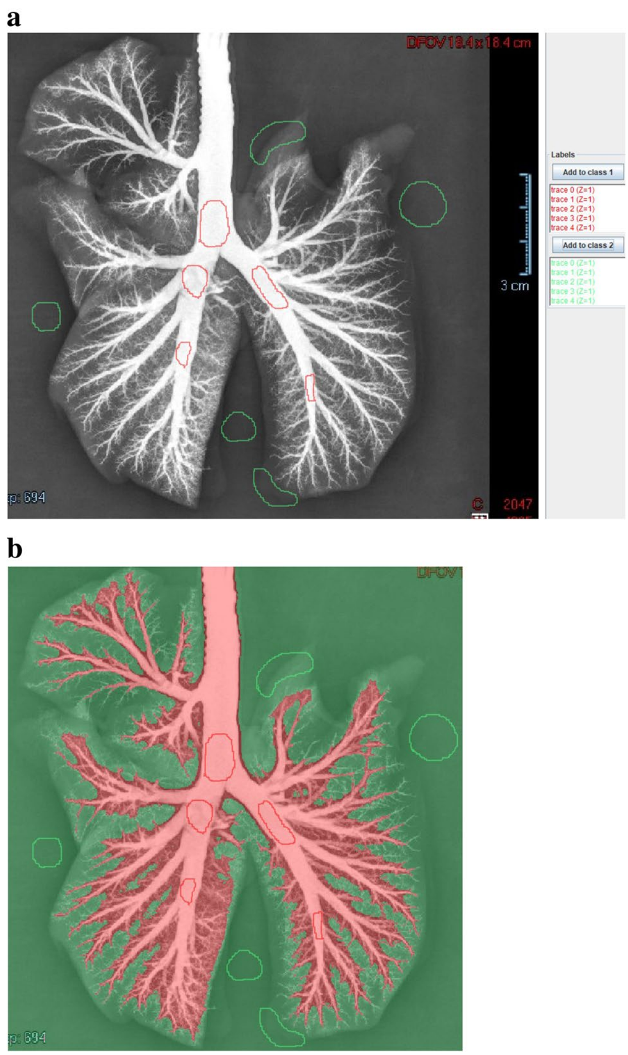

Fig. 2 The bronchial area selected by red circles (**a**). Lung parenchyma area and background selected by green circles (**a**). Classifying this result, only the bronchial area was distinguished in red part (**b**)

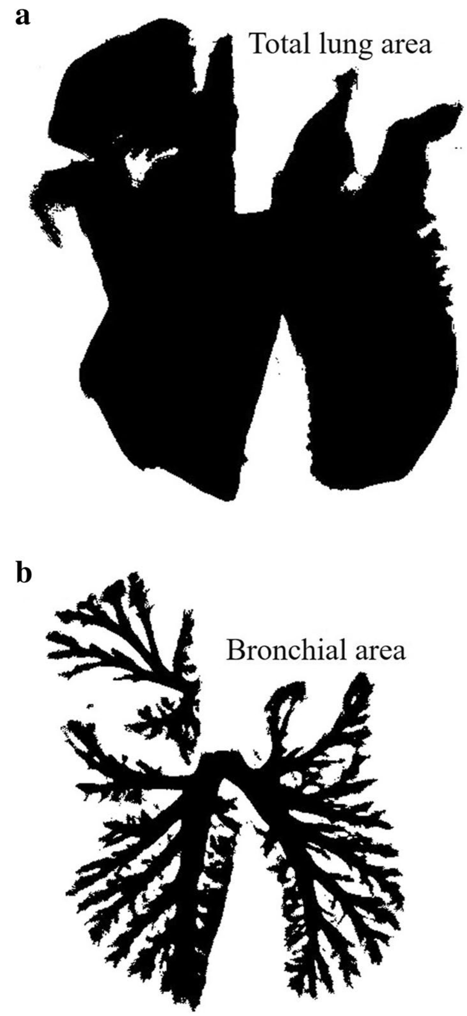

Fig. 3 The 8-bit images of total lung area (**a**) and bronchial area (**b**) converted from RGB images obtained using artificial intelligence data by "image J"

the Student's t test and Spearman's correlation coefficient, and $r > 0.3$ considered to be correlated for the Spearman's correlation coefficient.

Results

All the lambs undergoing CDH creation survived, and 12 showed intraabdominal organ prolapse into the thoracic cavity, but 1 case had minimal prolapse of the abdominal organs and was excluded from this study. The 12 lambs with complete intraabdominal organ prolapse to the thoracic cavity were used as the DH group (Group DH). The stomach was in the chest in all cases, but hepatic prolapse was rare in this model. All Group C lambs survived to term.

There was no significant difference in the body weight or the crown–rump length between Group DH and Group C. TLV was 133.3 ± 41.2 (ml) in Group DH and 326 ± 22.5 (ml) in Group C (p = 0. 0000001). TLA was 78.8 ± 17.4 (cm^2) in Group DH and 107.1 ± 10.3 (cm^2) in Group C (p = 0.006). BA was 39.6 ± 11.9 (cm^2) in Group DH and 52.2 ± 7.7 (cm^2) in Group C (p = 0.019). There was no significant difference in B/L ratio between Group DH and Group C (p = 0.28) (Table 1).

In Group DH, there was a strong correlation between TLV and TLA (r = 0.79, 95%confidence interval 0.71–0.85, p = 0.002) and TLV and BA (r = 0.73, 95% confidence interval 0.63–0.81, p = 0.007) (Table 2). In Group C, there was also a strong correlation between TLV and TLA (r = 0.97, 95% confidence interval 0.96–0.98, p = 0.005). There was only a weak correlation between TLV and BA (r = 0.67, 95% confidence interval 0.55–0.76, p = 0.22). The TLV and B/L

Table 1 Results of comparison between DH and C

	DH ($n=12$)	Control ($n=5$)	p value
Total lung volume(ml)	133.3 ± 41.2	326 ± 22.5	0.0000001
Total lung area(cm^2)	78.8 ± 17.4	107.1 ± 10.3	0.006
Bronchial area(cm^2)	39.6 ± 11.9	52.2 ± 7.7	0.019
B/L ratio	0.45 ± 0.06	0.49 ± 0.05	0.28

*$p<0.05$ was considered to be statistically significant

ratios were correlated only in the Group DH ($r=0.62$, 95% confidence interval 0.49–0.72, $p=0.03$) (Table 2).

Discussion

The prognosis in CDH is determined by the severity of the pulmonary hypoplasia in a broad sense and by the severity of the neonatal persistent pulmonary hypertension (PPHN) after birth [17–19]. In addition to the known reduction of bronchial generations in pulmonary hypoplasia [2, 3], abnormal bronchial development per se is often observed in patients with pulmonary hypoplasia. These developmental abnormalities may contribute to the poor respiratory function. Nose et al. performed bronchoscopy on patients with CDH to assess the incidence and associated effects of bronchial tree abnormalities in pulmonary hypoplasia [4]. They reported that the percentage of patients with CDH with bronchial malformations and hypoplasia was 43.6%, and their long-term survival rate was poor at 23.6% [4]. From these results, it can be seen that there is a correlation between pulmonary hypoplasia and abnormal bronchial development, and that patients with pulmonary hypoplasia in CDH have a poor prognosis.

Our hypothesis is that assessment of the severity of the abnormality of bronchial development and, by implication, the abnormality of vascular development, may allow us to assess the degree of lung hypoplasia.

Conventionally, lung area to head circumference ratio (LHR) and liver hernia into thoracic cavity as assessed using ultrasound or MRI have been used as the mainstays in the assessment of prenatal lung development and prognosis in CDH. In addition, various predictions of severity such as prenatal lung volume measurement using MRI [20] and liver/thorax ratio (LiTR) [21] have been reported.

Nishie et al. reported that the lung maturity and postnatal outcome can be predicted using lung/liver signal ratio (LLSIR) on fetal MRI [22]. Gabriel et al. reported a virtual pre-FETO (fetal endoscopic tracheal occlusion) bronchial assessment by creating 3D bronchoscopic images using MRI T2 images [23]. However, this was not accurate enough to evaluate bronchial development. Our view is that there are no consistently reliable methods for evaluating prenatal bronchial development.

In this study, we used imaging analysis software of bronchography images as a quantitative evaluation of lung and bronchial area to evaluate the degree of bronchial development. We then correlated these findings with the total lung volume which we had measured at the time of killing. From these results, lung development and bronchial development can be correlated from the area on X-ray, and can be used to easily estimate bronchial development, as a tool for evaluating the severity of prenatal lung hypoplasia. There were significant differences in total lung volume, total lung area and bronchial area between DH lambs and controls. From these results, it is clearly seen that the lungs are hypoplastic in our fetal lamb DH model.

There was no significant difference between the DH lambs and controls for the B/L ratio, which simply confirms that the poor bronchial development is reflected in a similar reduction of pulmonary parenchyma. In DH lambs, there are correlations and significant differences between TLV and TLA, TLV and BA, and TLV and B/L ratio. This suggests that evaluation by area measurement of lung or bronchus may be an accurate proxy for total lung volume at least in DH lambs. In controls, the correlation between TLV and BA did not achieve statistical significance and there was no significant difference or correlation between TLV and B/L ratio. This is likely to be due to the small number of controls and individual differences among healthy lambs. It might also be due to there being some areas of one or more of the control lamb's lungs not being fully inflated.

Schopper et al. reported that TLV can be measured by fetal MRI [20]. Given the recent significant development

Table 2 Spearman's correlation coefficient of each measured value

	Group DH ($n=12$)		Group C ($n=5$)	
	correlation coefficient** (95% confidence interval)	p value*	correlation coefficient (95% confidence interval)	p value
TLV and TLA	0.79 (0.71–0.85)	0.002	0.97 (0.96–0.98)	0.006
TLV and BA	0.73 (0.63–0.81)	0.007	0.67 (0.55–0.76)	0.2
TLV and B/L ratio	0.62 (0.49–0.72)	0.03	0.1 (– 0.08 to 0.28)	0.8

*$p<0.05$ considered to be statistically significant

**$r>0.3$ considered to be correlated

of MRI techniques which now allow the assessment of vascular detail in adult MRIs [24], there is a possibility that similar developments in fetal MRI techniques will soon allow the measurement of pulmonary vascular area. Clearly, at this point, this is not possible, but, given the rapid advances in MRI technology, it is quite likely to be possible in the near future. As noted above, Reid [2, 3] has pointed out that there is a strong correlation between the development of the bronchi and the pulmonary vasculature. While this study has focused on the bronchial area, we hypothesize that this is an adequate proxy estimate of the vascular area, which suggests that measuring the vascular area on a fetal MRI in a fetus with DH may well provide an accurate indication of prognosis.

The major limitation of this study is that it is difficult to follow the terminal bronchi on our X-ray images using RGB image analysis using AI, and it is possible that a complete bronchial development evaluation has not been performed. In a future study, we plan to assess the details of the bronchial tree manually for each lamb. It may be that such a detailed point analysis method is no more accurate than our present method, given the strong correlation with TLV. Another limitation of this study is that the lamb's survival and its actual respiratory function were not tested. However, in the initial studies using this model carried out by the senior author [13], assessing the survival of lambs with a non-repaired DH showed that survival with maximum support was measured in hours at the most, and in some of the lambs, in minutes. It would now be difficult to obtain ethical approval to repeat such a study.

Conclusion

Using a fetal lamb model of CDH, image analysis of the lung was performed at term. In the DH lambs, strong correlation was found between TLV and TLA, TLV and BA, and TLV and B/L ratio, suggesting a possible application to evaluation of bronchial and, by implication, pulmonary vascular development on fetal MRI.

Acknowledgements Management of the animals in the laboratory was supervised by Dr. Rebecca Dyson and Taylor Wilson, animal technicians at the BRU at University of Otago, Wellington. The ewes were bred by Mr. Denis Taylor, who also managed the flock on the farm between procedures. The authors thank Shigeko Ohnuma and the other staff in the Department of Pathology St. Marianna University School of Medicine for assistance in processing the histology.

Funding This study was supported by the Japanese Society for Grant-in-aid of Scientific Research.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

References

1. Leeuwen L, Fitzgerald DA (2014) Congenital diaphragmatic hernia. J Paediatr Child Health 50:667–673. https://doi.org/10.1111/jpc.12508
2. Kitagawa M, Hislop A, Boyden EA, Reid L (1971) Lung hypoplasia in congenital diaphragmatic hernia. A quantitative study of airway, artery, and alveolar development. Br J Surg 58:342–346
3. Reid L (1977) 1976 Edward B.D. Neuhauser lecture: The lung: growth and remodeling in health and disease. AJR Am J Roentgenol 129:777–788. https://doi.org/10.2214/ajr.129.5.777
4. Nose K, Kamata S, Sawai T, Tazuke Y, Usui N, Kawahara H, Okada A (2000) Airway anomalies in patients with congenital diaphragmatic hernia. J Pediatr Surg 35:1562–1565. https://doi.org/10.1053/jpsu.2000.18310
5. Shehata SM, Tibboel D, Sharma HS, Mooi WJ (1999) Impaired structural remodelling of pulmonary arteries in newborns with congenital diaphragmatic hernia: a histological study of 29 cases. J Pathol 189:112–118. https://doi.org/10.1002/(SICI)1096-9896(199909)189:1%3c112:AID-PATH395%3e3.0.CO;2-8
6. Harrison MR, Keller RL, Hawgood SB, Kitterman JA, Sandberg PL, Farmer DL, Lee H, Filly RA, Farrell JA, Albanese CT (2003) A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. N Engl J Med 349:1916–1924. https://doi.org/10.1056/NEJMoa035005
7. Deprest J, Brady P, Nicolaides K, Benachi A, Berg C, Vermeesch J, Gardener G, Gratacos E (2014) Prenatal management of the fetus with isolated congenital diaphragmatic hernia in the era of the TOTAL trial. Semin Fetal Neonatal Med 19:338–348. https://doi.org/10.1016/j.siny.2014.09.006
8. Torrents-Barrena J, Piella G, Masoller N, Gratacos E, Eixarch E, Ceresa M, Ballester MAG (2019) Segmentation and classification in MRI and US fetal imaging: Recent trends and future prospects. Med Image Anal 51:61–88. https://doi.org/10.1016/j.media.2018.10.003
9. Weis M, Hoffmann S, Henzler C, Weiss C, Schoenberg SO, Schaffelder R, Schaible T, Neff KW (2018) Isolated impact of liver herniation on outcome in fetuses with congenital diaphragmatic hernia—a matched-pair analysis based on fetal MRI relative lung volume. Eur J Radiol 105:148–152. https://doi.org/10.1016/j.ejrad.2018.05.024
10. Metkus AP, Filly RA, Stringer MD, Harrison MR, Adzick NS (1996) Sonographic predictors of survival in fetal diaphragmatic hernia. J Pediatr Surg 31:148–151 **(discussion 151–2)**
11. Yokoi A, Ohfuji S, Yoshimoto S, Sugioka Y, Akasaka Y, Funakoshi T (2018) A new approach to risk stratification using fetal MRI to predict outcomes in congenital diaphragmatic hernia: the preliminary retrospective single institutional study. Transl Pediatr 7:356–361. https://doi.org/10.21037/tp.2018.09.01
12. Obayashi J, Kawaguchi K, Koike J, Tanaka K, Seki Y, Nagae H, Manabe S, Ohyama K, Takagi M, Kitagawa H et al (2017) Evaluation of alveolar epithelial cells in the sheep model of congenital diaphragmatic hernia: type 1 alveolar epithelial cells and histopathological image analysis. J Pediatr Surg 52:2074–2077. https://doi.org/10.1016/j.jpedsurg.2017.08.034
13. Soper Robert T, Pringle Kevin C, Scofield John C (1984) Creation and repair of diaphragmatic hernia in the fetal lamb: techniques and survival. J Pediatr Surg 19:33–40

14. Tanaka K, Koike J, Obayashi J, Seki Y, Nagae H, Manabe S, Ohyama K, Sasaki C, Takagi M, Zuccollo J et al (2015) Pressure limited vesico-amniotic shunt allows normal lung growth in a fetal lamb model of obstructive uropathy. J Pediatr Surg 50:2063–2067. https://doi.org/10.1016/j.jpedsurg.2015.08.029
15. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675
16. Daly AB, Wallis JM, Borg ZD, Bonvillain RW, Deng B, Ballif BA, Jaworski DM, Allen GB, Weiss DJ (2012) Initial binding and recellularization of decellularized mouse lung scaffolds with bone marrow-derived mesenchymal stromal cells. Tissue Eng Part A 18:1–16. https://doi.org/10.1089/ten.TEA.2011.0301
17. George DK, Cooney TP, Chiu BK et al (1987) Hypoplasia and immaturity of the terminal lung unit in congenital diaphragmatic hernia. Am J Respir Crit Care Med 136:947–950
18. O'Rourke PP, Vacanti JP, Crone RK et al (1988) Use of the post-ductal PaO2 as a predictor of pulmonary vascular hypoplasia in infants with congenital diaphragmatic hernia. J Pediatr Surg 23:904–907
19. Wilson JM, Lund DP, Lillehei CW et al (1991) Congenital diaphragmatic hernia. Predictor of severity in the ECMO era. J Pediatr Surg 26:1028–1034
20. Schopper MA, Walkup LL, Tkach JA, Higano NS, Lim FY, Haberman B, Woods JC, Kingma PS (2017) Evaluation of neonatal lung volume growth by pulmonary magnetic resonance imaging in patients with congenital diaphragmatic hernia. J Pediatr 188:96–102.e1. https://doi.org/10.1016/j.jpeds.2017.06.002
21. Cannie MM, Cordier AG, De Laveaucoupet J, Franchi-Abella S, Cagneaux M, Prodhomme O, Senat MV, Mokhtari M, Vlieghe V, Nowakowska D et al (2013) Liver-to-thoracic volume ratio: use at MR imaging to predict postnatal survival in fetuses with isolated congenital diaphragmatic hernia with or without prenatal tracheal occlusion. Eur Radiol 23:1299–1305. https://doi.org/10.1007/s00330-012-2709-6
22. Nishie A, Tajima T, Asayama Y, Ishigami K, Hirakawa M, Nakayama T, Ushijima Y, Kakihara D, Okamoto D, Yoshiura T et al (2009) MR prediction of postnatal outcomes in left-sided congenital diaphragmatic hernia using right lung signal intensity: comparison with that using right lung volume. J Magn Reson Imaging 30:112–120. https://doi.org/10.1002/jmri.21829
23. Tonni G, Ruano R, Sa R, Peixoto Filho FM, Lopes J, Werner H (2018) 3D virtual bronchoscopy before FETO procedure in a fetus with severe, isolated left congenital diaphragmatic hernia. Fetal Pediatr Pathol 37:134–139. https://doi.org/10.1080/15513815.2018.1445148
24. Johns CS, Swift AJ, Hughes PJC, Ohno Y, Schiebler M, Wild JM (2017) Pulmonary MR angiography and perfusion imaging—a review of methods and applications. Eur J Radiol 86:361–370. https://doi.org/10.1016/j.ejrad.2016.10.003