Seminars in Fetal & Neonatal Medicine 19 (2014) 338–348

Contents lists available at ScienceDirect

Seminars in Fetal & Neonatal Medicine

journal homepage: www.elsevier.com/locate/siny

Review

Prenatal management of the fetus with isolated congenital diaphragmatic hernia in the era of the TOTAL trial

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Keywords:
Congenital diaphragmatic hernia
Pulmonary hypoplasia
Fetal surgery
Tracheal occlusion
Chromosomal microarrays
Exome sequencing

SUMMARY

Congenital diaphragmatic hernia (CDH) may be isolated or associated with other structural anomalies, the latter with poor prognosis. The defect allows viscera to herniate through the defect into the chest, competing for space with the developing lungs. At birth, pulmonary hypoplasia leads to respiratory insufficiency and persistent pulmonary hypertension that is lethal in up to 30% of patients. When isolated, survival chances can be predicted by antenatal measurement of lung size and liver herniation. Chromosomal microarrays and exome sequencing contribute to understanding genetic factors underlying isolated CDH. Prenatal intervention aims at stimulating lung development, clinically achieved by percutaneous fetal endoscopic tracheal occlusion (FETO) under local anesthesia. The Tracheal Occlusion To Accelerate Lung growth trial (www.totaltrial.eu) is an international randomized trial investigating the role of fetal therapy for severe and moderate pulmonary hypoplasia. Despite an apparent increase in survival following FETO, the search for lesser invasive and more potent prenatal interventions must continue.

1. Introduction

The prevalence of congenital diaphragmatic hernia (CDH) ranges between one and four per 10,000 births, which makes this condition officially a rare disease. Based on 2008 birth rates in the EU-27, this would mean between 542 and 2168 children in EU-27 [1]. CDH is the denominator for posterior lateral (Bochdalek 95%) and anterior (Morgagni defects). Eighty-six percent of Bochdalek defects are left-sided, 13% right-sided, and up to 2% bilateral. CDH is further classified as either isolated, syndromic or associated with other anomalies. In its isolated form, CDH leads to two neonatal problems: (1) the anatomical yet surgically correctable diaphragmatic defect; and (2) the coinciding pulmonary hypoplasia. The latter is the result of disturbed lung development, which starts in the embryonic period. As pregnancy continues, viscera herniate into the chest, and compete for space with the developing lungs. CDH lungs have fewer airway branches, smaller cross-sectional area of pulmonary vessels, structural vascular remodelling, and vasoconstriction with altered vasoreactivity [2]. The degree of lung development is individually quite variable; whether this is related to the size of the defect, or primarily determined by an initial underlying problem or a combination of both, remains uncertain. The in-utero death rate is around 2%, usually without demonstrable direct cause. At birth, the immediate problems are respiratory insufficiency and persistent pulmonary hypertension (PHT). Once the newborn is stabilized, the defect is corrected, which may for

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http://dx.doi.org/10.1016/j.siny.2014.09.006
1744-165X/

large defects require the use of a patch (reviewed in [3]). When the condition is not isolated, the prognosis is usually poor, as the above problems are combined with those caused by the other associated anomalies. In isolated cases, the condition is lethal in up to 30%, despite prenatal referral to a high volume center offering standardized neonatal care [4–6]. Survivors may suffer from additional morbidities, mainly chronic lung disease and/or persistent PHT, gastroesophageal reflux and feeding problems, and thoracic deformations [7]. Most babies eventually lead a life very close to normal provided they are managed in a multidisciplinary follow-up program [8,9].

In the western world, >60% of cases are diagnosed at the latest by the second trimester screening ultrasound. As for any suspected congenital birth defect, its diagnosis should prompt referral to a tertiary center for confirmation of, or differential diagnosis, further documentation of severity and exclusion of associated problems (Box 1). This will be followed by multidisciplinary counseling of parents by specialists with experience in managing the condition. Additional diagnostic steps include genetic testing, and where applicable advanced imaging, which today will include magnetic resonance. The overall goal is to define the individual prognosis, so that parents, based on factual information regarding their fetus, can choose further management, which includes expectant management with prenatal referral to a high volume center for carefully timed delivery, termination of pregnancy, or fetal intervention for selected patients.

2. The genetics of (isolated) CDH

2.1. Microarray analysis

In recent years, genetic testing for prenatal diagnosis has moved beyond conventional karyotyping. The Leuven Centre for Human Genetics moved early to introduce chromosomal microarray analysis for prenatal diagnosis in the presence of ultrasound anomalies [12], which by now is the first-tier diagnostic test for routine prenatal diagnosis in Belgium [13]. We demonstrated the added value of chromosomal microarrays for the investigation of apparently isolated CDH, both retrospectively using a custom-design targeted platform [14], as well as prospectively using a high-resolution genome-wide platform [15]. Our retrospective analysis revealed a novel duplication of Xq13.1 containing the *EFNB1* gene in a male with isolated CDH, later described in another case [16]. Our prospective analysis revealed a 9% yield of pathogenic copy number variants (CNVs) and a further 4% of cases with rare inherited CNVs. This allowed us to refine the minimal deleted region at 15q26 to a region containing only two genes, one of which is the *NR2F2* gene. We also identified additional cases of 15q25.2 deletions, a 16p11.2 deletion and a novel duplication of 4p15.2–p14. Using a gene prioritization and network analysis approach we proposed candidate CDH genes within the loci identified including *BNC1*, *BTBD1*, *TBX6*, *RBPJ* and *TGFBR3*.

An enrichment analysis was undertaken on a curated network of known CDH genes and interacting genes within CNVs identified. This analysis revealed significant enrichment for biological networks and genes known to be involved in respiratory, cardiovascular, skeletal and muscular system development and function, which is concordant with observations in humans and animal models [17]. Significant enrichment for genes involved in canonical biochemical pathways included regulation of the epithelial–mesenchymal transition (EMT) pathway, fibroblast growth factor (FGF) signaling; axonal guidance signaling, human embryonic stem cell pluripotency, Wnt/β-catenin signaling and retinoic acid receptor (RAR) activation. These findings support candidate pathways as being involved in CDH (such as the retinoic acid pathway), but also pathways and genes involved with the EMT pathway, FGF signaling and Wnt/β-catenin signaling as potential candidates for involvement in human CDH.

Importantly, our findings reveal that many of those CNVs are submicroscopic and would therefore not be identified by conventional karyotyping. This highlights that chromosomal microarray analysis is a valuable tool when diagnosing isolated CDH. Similar microarray findings have been reported in mixed cohorts of non-isolated and isolated CDH patients by others [18,19].

2.2. Whole exome or genome sequencing

Whereas microarrays provide an increase in diagnostic yield, for the majority of CDH patients no cause can be identified. Whole exome (or genome) sequencing is therefore an attractive approach to the study of both isolated and non-isolated CDH. We have applied exome sequencing to a small number of cases where a familial cause was suspected. We identified a nonsense variant in *ZFPM2* in one family with two fetuses affected with isolated CDH [20]. The *ZFPM2* p.Arg112* mutation we identified was identical to the de-novo variant previously reported by Ackerman et al. in a fetus with diaphragmatic eventration, suggesting pathogenicity [21]. Interestingly, this variant was inherited from the mother, who in turn had inherited this from her father, and was also carried by a maternal sister. All three are asymptomatic. A sibling with a total anomalous pulmonary venous return (TAPVR) was subsequently shown to carry the same variant and interestingly a mild anterior diaphragm eventration was incidentally observed upon X-ray after corrective surgery for this cardiovascular malformation. Heterozygous loss-of-function variants in *ZFPM2* thus pose a risk for diaphragmatic and possibly cardiovascular malformations, yet those variants can be apparently asymptomatic, and are therefore associated with both variable phenotypic expression and reduced penetrance. This creates challenges for genetic counselling of recurrence risk in future pregnancies. Reduced penetrance for deletions of *ZFPM2* identified by array CGH have been reported recently [18]. A similar finding of reduced penetrance and

Box 1
Non-limitative list of frequent anomalies often associated with CDH [10,11].

Identified genetic defects and syndromes

Trisomy 13, 18, 21, XO, partial trisomy 5, 20, tetraploidy 21, tetrasomy 12p

Syndromes: Fryns, Fraser, Stickler, Pierre Robin, Goldenhar, Beckwith Wiedemann, Apert, Klippel–Fiel, Rubenstein Taybi, Brachman de Lange, Coffin–Siris, Pentalogy of Cantrell

VACTERL or CHARGE association

Structural defects (in descending order of occurrence according to Dott et al. [11]): cardiovascular, gastrointestinal, urogenital, musculoskeletal, respiratory, central nervous system, craniofacial

VACTERL, Vertebral anomalies, Anal atresia, Cardiac defects, Tracheoesophageal fistula and/or Esophageal atresia, Renal and Radial anomalies, and Limb defects; CHARGE, Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital and/or urinary abnormalities, and Ear abnormalities and deafness.

variability in phenotypic expression was recently reported for a *GATA4* variant identified by exome sequencing in a familial case of isolated CDH [22]. The identification and determination of variant pathogenicity will prove challenging in sporadic cases of isolated CDH. Large cohorts of isolated CDH cases are necessary for demonstration of an excess burden of rare damaging variants. One recent report has used exome sequencing in a large cohort of 275 CDH patients, estimating the prevalence of rare damaging *ZFPM2* mutations to be almost 5% [23]. Further studies of a similar nature will be necessary to fully determine which genes harbor an excess burden of rare variants and to unravel those additional genetic factors influencing penetrance. Targeted epigenetic studies in familial and sporadic cases of CDH for genes such as *ZFPM2* and *GATA4* may reveal factors contributing to penetrance, and perhaps explain why variants in *NR2F2* have thus far not been observed in CDH patients.

Using exome sequencing, we further identified a homozygous splice-site variant in *PIGN* in a case of syndromic CDH which was inherited from the heterozygous consanguineous parents [24]. We also identified a missense variant in *PORCN* in two male fetuses with syndromic microphthalmia, one of whom had CDH. This represents the first finding of non-mosaic males affected with focal dermal hypoplasia or Goltz–Gorlin syndrome. This X-linked variant was inherited from an asymptomatic mother who demonstrated extreme skewing of X-inactivation [25]. Exome sequencing has also identified variants in *GATA6* in association with non-isolated CDH [26].

3. Prenatal imaging for assessment of severity

Next to ruling out additional anomalies, ultrasound and magnetic resonance imaging are used to assess severity. This topic is reviewed in depth by Benachi et al. [27]. In essence, two-dimensional ultrasound enables assessment of lung size and identification of position of the liver. Both parameters have been named to be predictive of outcome, though it remains unknown whether they act independently. Other less studied factors are position of the stomach, polyhydramnios, gestation at diagnosis and cardiac ventricular size. The most logical way to predict neonatal outcome is to quantify the degree of pulmonary hypoplasia – which is essentially what determines neonatal outcome in isolated cases. Lung size indeed correlates to the degree of pulmonary hypoplasia as confirmed in necropsy studies [28]. At present, the widest validated method to predict individual outcome is measurement of the lung-to-head ratio (LHR), first described by Metkus et al. [29]. It consists of measuring the lung size contralateral to the defect side at the level of the four-chamber view. This can best be done by tracing the lung contours, though the longest axes can also be used [30]. That measurement is then divided by the fetal head circumference. To correct for gestational age, the LHR of the index case is expressed as a percentage of the normal [observed/expected (O/E) l h]. An easy calculator is available to clinicians at www.totaltrial.eu. In the so-called antenatal CDH registry, observations on 354 expectantly managed yet live-born fetuses with unilateral isolated CDH were pooled. Low O/E LHR values, combined with liver herniation, correspond to increased mortality and early neonatal morbidity [31,32]. This algorithm is at present used in clinical programs in Europe, as well as for the fetal therapy trial (Fig. 1). When using the same inclusion criteria, comparable survival rates were reported by the group in Toronto [33].

Lung volume can also be estimated using three-dimensional ultrasound or magnetic resonance. Again, the effect of gestational age is offset by expressing the measured volume as a percentage of what one expects in a normal fetus (observed/expected volume ratio). The easiest match is a fetus of the same gestational age. This may be difficult in the case of uncertain dates or inaccurate when the fetal weight is beyond the normal range [35]. Therefore matching can be based on fetal body volume [36,37]. The clinical relevance of these different matching methods is uncertain [38]. Most tertiary centers in reality perform both two- and three-dimensional measurements. As lung volumetry is based on several slices through both lungs, and since magnetic resonance is not restricted by maternal factors, we think that ultimately magnetic resonance volumetry should be preferred and theoretically should be more accurate. Though apparently better [39], large datasets are required to demonstrate that magnetic resonance prediction is actually better [35].

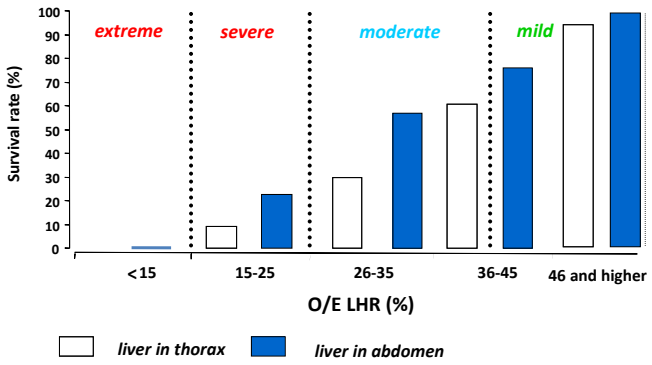

Fig. 1. Algorithm for left-sided congenital diaphragmatic hernia (CDH). Correlation of O/E LHR and survival rates for fetuses with and without liver herniation, based on data from the antenatal CDH registry. As a reference, survival rates in an independent series from Toronto, where liver position was not specified, are: 21% (O/E LHR: <25%), 50% (O/E LHR: 25–35%) and 70% (O/E LHR 35–45%) [31]. (Reprinted, with permission, from Deprest JA, et al. Semin Fetal Neonatal Med 2009;14:8–13; and from the publisher.)

Liver herniation has long been recognized as a prognostic indicator. Ultrasound usually discriminates cases as having liver "up" or "down"; magnetic resonance allows a more scaled quantification. Unfortunately there is no standardized method for calculating the amount of liver into the thorax [40–44]. Other less used predictors are position of the stomach [45,46], fetal lung vascularization and its potential for vaso-relaxation (reviewed by Claus et al. [47]). Because they are neither widely used nor formally applied in clinical trials, they are not further discussed here. Nevertheless, it would seem logical to try to improve accuracy of prediction by combining factors that may exert different effects on the clinical neonatal consequences of pulmonary hypoplasia [48,49].

The less prevalent right-sided form of CDH (RCDH) might not just be a variant of left-sided CDH (LCDH), but rather a separate entity with different outcomes, as well as treatment response [50]. Logically another prenatal prediction model would then need to be used. The currently available data are controversial, with both better and worse claimed outcomes than LCDH [51–55]. We have based antenatal prediction so far on the antenatal CDH-registry data, though it included only 25 RCDH fetuses [31]. Most have liver herniation, so that prediction is based solely on O/E LHR. In the initial report, overall survival was 44%, with no survivors when O/E LHR <45%. In a recent update of cases managed in Barcelona and Leuven, we confirmed a poorer outcome for RCDH, with survival rates for O/E LHR $\leq$45% and $\leq$30% of 17% and 0%, respectively [56] (Fig. 2).

4. Fetal therapy

The ability to prenatally identify a future non-survivor prompts the search for an intervention that can avoid that

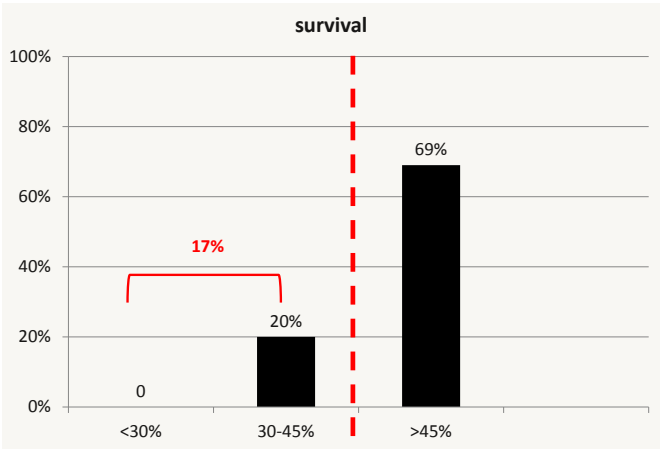

Fig. 2. Current algorithm for prenatal prediction of outcome in right-sided CDH. (Reprinted with permission from Dekoninck P, et al. Eur J Obstet Gynecol Reprod Biol 2014;178:157–62. © Wiley.)

outcome. As CDH is a developmental problem, the ideal therapeutic window of opportunity is the prenatal period. Historically this was first done by in-utero anatomical repair [57]. Today the only clinically applied intervention is fetal endoluminal tracheal occlusion (FETO; Fig. 3). TO for alleviation of pulmonary hypoplasia was to our knowledge first proposed by Jay Wilson and his team from Boston [58,59]. TO prevents egress of lung liquid, which in turn causes increased pulmonary stretch, hence accelerated lung growth as evidenced by several animal experiments [58,60–63]. Several devices have been used for occlusion, though endoscopic insertion of a balloon as well as its in-utero reversal was first described in Europe [60,64–66]. The current clinical timing of occlusion, and the European practice of removing the occlusion in utero, is based on experimental observations [60]. Sustained TO, though inducing lung growth, reduces the number of type II pneumocytes, hence surfactant expression. This can be improved by in-utero release ("plug–unplug sequence") TO. Theoretically appropriately balanced lung growth and maturation is obtained by cycles of 47 hours of occlusion and 1 hour release, but this is clinically not yet possible [67]. Perinatal steroid administration has also experimentally been shown to beneficial [68].

Since its initial clinical introduction [69,70] FETO has evolved to a percutaneous procedure under local anesthesia, with fetal pain relief and immobilization [71] (Fig. 3). Several purpose-designed fetoscopic instruments were also developed (Table 1; Fig. 4) [72]. The current diameter used is 10 Fr (3.3 mm), which is comparable to what is used for laser coagulation for twin–twin transfusion syndrome. Essentially it consists of a semi-flexible miniature fetoscope and a curved sheath allowing the catheter and balloon as well as removal instruments. The balloon is designed for endovascular occlusion; the delivery catheter was purposely shortened to 100 cm to facilitate its use in this off-label application. We are still working on smaller diameter atraumatic instruments though they are not ready for clinical use [73].

In severe cases, the FETO task force initially proposed insertion of the balloon at 26–28 weeks, and for moderate cases at 30–32 weeks. Earlier occlusion has been done but was prone to more complications. Reversal of occlusion is proposed at 34 weeks. This has been achieved by fetoscopy (50%) or ultrasound-guided puncture (19%) (Fig. 5). Removal at birth can be done on placental circulation; only in rare cases it has been done postnatally by direct laryngoscopy or percutaneous puncture.

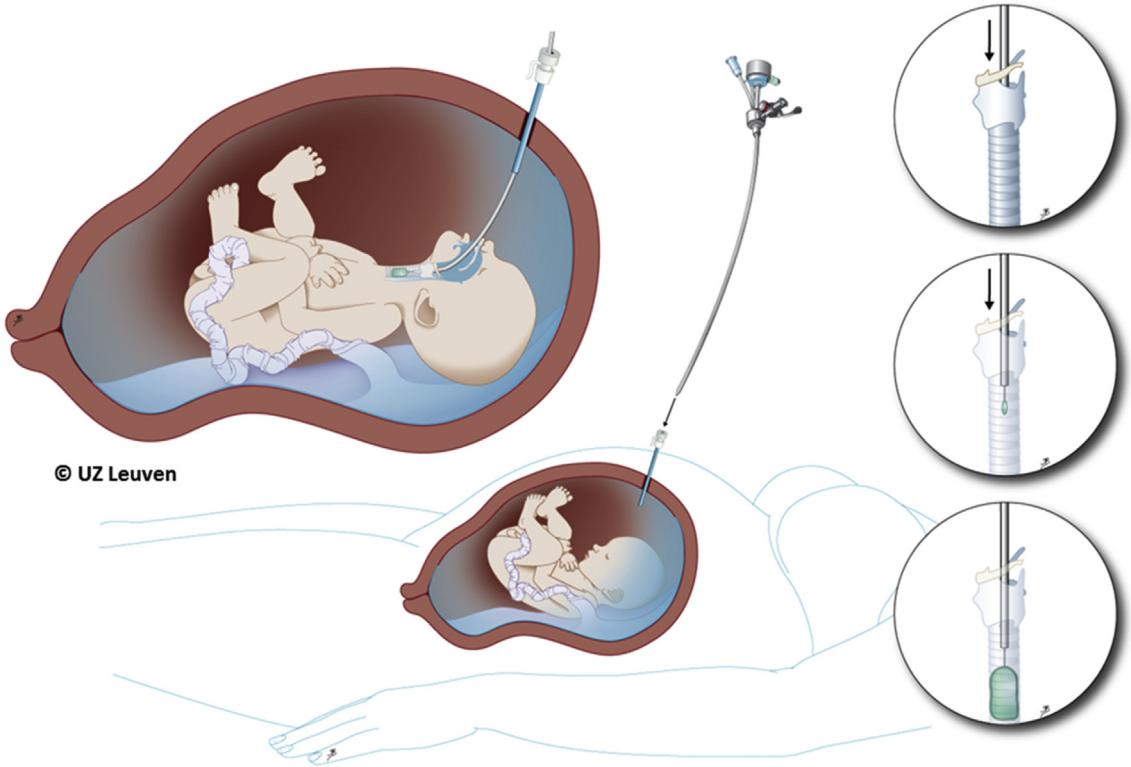

Fig. 3. Fetoscopic endoluminal tracheal occlusion (FETO). A. schematic drawing. Inserts: steps in balloon delivery. Reproduced with permission from UZ Leuven, Belgium. Video available as additional information.

Table 1
Instruments used for balloon insertion and removal.

Fetal tracheoscopy	Description	ID
1.3 mm endoscope	Miniature telescope, with remote eyepiece 0° straight forward, 30.6 cm working length	11540AA
3.3 mm sheath	Blunt curved sheath, with sand-blasted echogenic tip With stop cock for irrigation and two side openings	11540KE
1.0 mm forceps	Retrieval forceps, double action jaws, 35 cm long	11510C
0.4 mm stylet	Single use puncture stylet with adjustable torque, 50 cm long, single use	11506P
0.9 mm needle	Puncture needle to protect the catheter or for aspiration, length 35 cm, can house the stylet	11540KD
3.3 mm trocar	10 Fr pyramidal tipped trocar for use with flexible cannula RCF-10.0 (Cook, Check Flo Performer)	11650TG
0.6 mL balloon	Goldbal 2 detachable latex balloon with radio-opaque inclusion, outer diameter 1.5 mm (inflated: 7.0 mm); length 5.0 mm (inflated 20.0 mm)	Goldbal 2 (Balt)
0.9 mm microcatheter	catheter loaded with mandrel, and Touhy Boost Y-connection, max outer diameter 0.9 mm, tapered to 0.4 mm, 100 cm in length	"Baltacci" – BDPE 100 (Balt)
Direct bronchoscopy		
1.3 mm endoscope	Miniature telescope, with remote eyepiece 0° straight forward, 18.8 cm working length	10040AA
Straight bronchoscopic sheath	4.2 mm outer, 3.5 mm inner diameter 18.5 cm length (size 2.5) Is conventional neonatal "Doesel-Huzly" bronchoscope With blanking and suction plug	10339F 10924SP 10315RV
Telescope bridge	Houses telescope and has side opening for irrigation 1.5 mm outer diameter	10338LCI
1.0 mm forceps	19 cm semi-flexible forceps For balloon retrieval	10338H[a]
0.4 mm stylet	Single use puncture stylet with adjustable torque, 50 cm long, single use	11506P

Endoscopic instruments were developed by Karl Storz GmbH, supported by the European Commission in the 6th framework program (www.eurostec.eu). The balloon system is an adapted version of a commercially available vascular occlusion device. Most instruments and devices are used off label. Their use in the USA is currently the subject of an approval procedure by the Food and Drug Administration filed by the North American Fetal Therapy Network (NAFTNET).

[a] Forceps 11510C can also be used.

We have reported outcomes on 210 consecutive FETO procedures (from the first onwards) in severe cases. Early delivery is the most relevant complication. It is typically the consequence of preterm premature rupture of the membranes (PPROM), which occurs within three weeks in 16.7%. Using a collagen membrane plug at the primary procedure had no measurable effect [74]. Patients with PPROM are admitted with prophylactic antibiotics and closely watched for signs of infection, or other complications that mandate delivery. Whereas overall gestation at delivery was 35.3 weeks, current experience shows that one in three delivers prior to 34 weeks. This creates the need for emergency balloon retrieval. Especially in these emergency situations, reversal of occlusion should not be underestimated. An unprepared, unexperienced team may be unable to achieve, or have difficulties with, reversal of occlusion leading to neonatal death or tracheal damage [71,75,76]. It requires adapted instrumentation and trained personnel available 24/7. Together with the University of Toronto (Canada), we designed a model based on magnetic resonance images of 28-week fetuses, which may be a helpful training tool for this critical intervention [77].

Compared to historical controls from the antenatal CDH registry, FETO increased survival in severe LCDH from 24.1% to 49.1% ($P < 0.001$) [31]. The strongest predictors of survival were O/E LHR prior to the procedure (odds ratio: 1.490; $P = 0.019$) and gestational age at delivery (odds ratio: 1.024; $P = 0.007$). In RCDH, survival increased from 0% to 35% (12/34). In our recent update on RCDH, that trend persisted (17% expectantly managed cases vs 42% FETO cases; $P = 0.09$) [78]. Short-term (neonatal) morbidity is better than expected in same-severity expectantly managed cases. It is close to that of cases with moderate pulmonary hypoplasia [79]. The ability to remove the balloon >24 h prior to birth was, next to a better survival, also associated with lower morbidity [72,79]. The latter is the reason why we still adhere to a policy of prenatal balloon retrieval, if clinically possible.

The early clinical experience has shown few demonstrable clinical side-effects of the balloon on the developing trachea, except in very early occlusions and complications arising at the time of removal [76,80]. However, the neonates and infants do have obvious tracheomegaly (Fig. 6), which does not seem to have a clinical impact, except for a barking cough on effort [80–83]. Over time, the widening becomes less important [81]. More than 70% of newborns require surgical patching of the diaphragm, indicating the rather large size of the defect in this selected group. The use of patching has previously been shown to be a predictor of outcome [84]. High patch rates may also increase the number of later patch-related complications. Another follow-up study on a selection of patients delivering at our institution investigated the occurrence of reflux and the need for anti-reflux surgery (ARS).

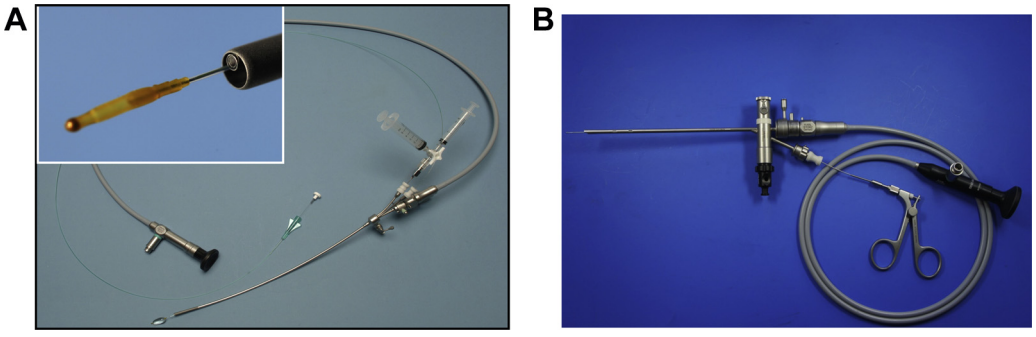

Fig. 4. Instruments for fetoscopic endoluminal tracheal occlusion (FETO). (A) Fetoscope within curved sheath (insert: catheter loaded with a balloon exiting the scope). (B) Bronchoscope loaded with retrieval forceps. Image kindly provided by Karl Storz Endoskope.

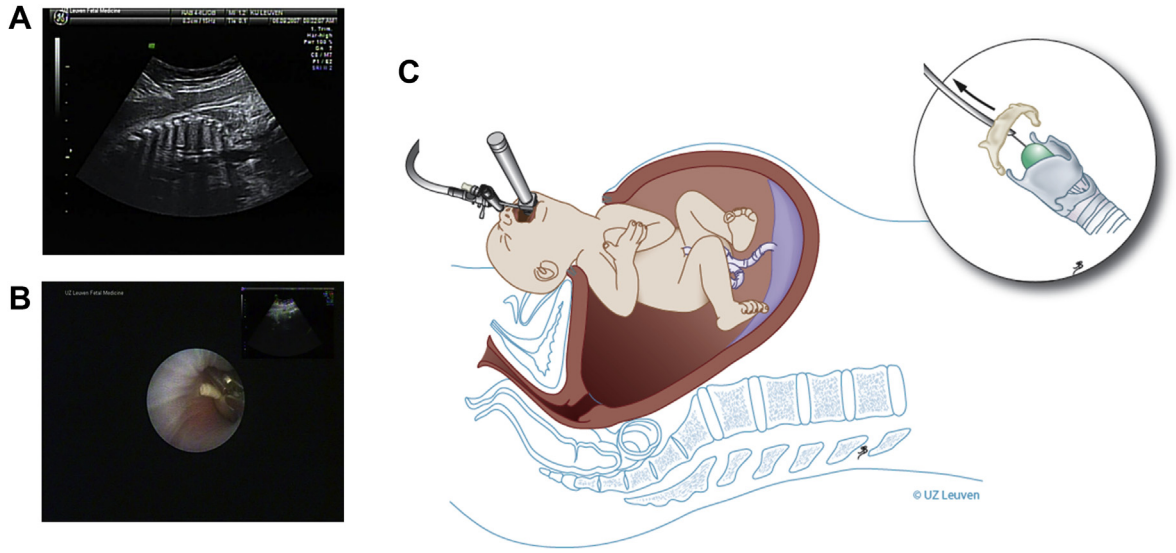

Fig. 5. Balloon removal: (A) ultrasound guided puncture of the balloon; (B) fetoscopic balloon retrieval and (C) schematic drawing of a retrieval on placental circulation (reproduced with permission of the UZ Leuven, Belgium). Videos available as additional files.

Univariate analysis for prenatal predisposing factors shows that patch repair, liver herniation, and FETO – next to postnatal pulmonary hypertension and high-frequency oscillatory ventilation – were associated with subsequent ARS. On multivariate analysis, however, liver herniated into the chest was the only independent predictor for both gastro-oesophageal reflux and need for reflux surgery [85].

The reproducibility of FETO is not at stake [86–88]. Next to smaller scale observational studies, there has been one Brazilian randomized trial showing an increased survival following FETO in severe cases [89]. The results of this study became available after the start of the randomized controlled trial (RCT) in severe CDH which was launched in Europe in 2010 (ww.clinicaltrials.gov, NCT01240057) (Table 1). The European trial was not discontinued because the investigators and the oversight committee identified limitations to the Brazilian study. First, survival rate in expectantly managed cases was very low (5%); this low rate is not representative of what can be expected at European or North American referral centers. Also, right- and left-sided cases were pooled, which does not seem appropriate, given that side has been identified as an independent predictor in several studies, including a recent meta-analysis [40].

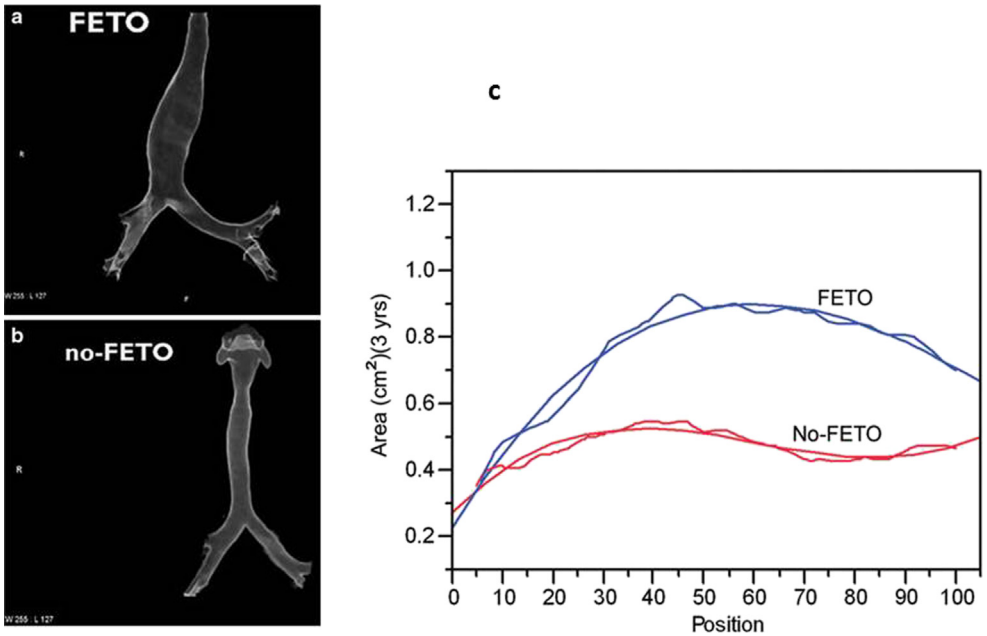

Fig. 6. Three-dimensional reconstruction of the trachea of a child between 4.5 and 5 years of age either (a) having undergone FETO or (b) expectantly managed during pregnancy, both asymptomatic. The FETO patient has distal tracheal widening as compared to the control. Images obtained by low-dose spiral CT with the child in a supine position and during quiet respiration at near functional residual lung capacity. (c) Evolution of tracheal diameter with age. Reprinted, with permission, from Breysem L, et al. Radiology 2010;257:226–32. © Radiological Society of North America.

In the TOTAL trial in severe cases, the balloon is inserted at 27–30 weeks and ideally removed at 34 weeks. The slightly later (27–30 weeks) balloon insertion point than the initial point (26–28 weeks) was chosen based on previous experience [71]. Fig. 7 displays the number of patients according to gestational weeks, and the corresponding survival rates. Low survival rates are consistently observed in babies born prior to 32 weeks. Later insertion should lessen the risk for delivery prior to 32 weeks, the latter having a negative impact on survival. This might be at the expense of achievable lung growth, because we demonstrated that later insertion yields a less vigorous lung response [90]. We continue to remove the balloon before birth, as we observed higher survival rates and less morbidity when doing so [72,79]. Elective prenatal balloon removal also avoids the need for unplanned emergency balloon retrieval procedures. Of interest is that in the two Brazilian series, there was no apparent difference in survival without prenatal balloon removal [86,87].

The FETO consortium also reconsidered fetal therapy in the moderate group. This is at present investigated within an RCT (NCT00763737; Table 7). It should be remembered that in an earlier RCT including severe and moderate cases, Harrison et al. demonstrated no benefit from fetal therapy [91]. The current technique is, however, much less invasive; it uses smaller diameter instruments and is done percutaneously and under local anesthesia. Also the initial experience in severe cases suggests that FETO reduces neonatal morbidity [32,79]. Thus we hypothesized that for moderate pulmonary hypoplasia also – which is still associated with a 40% mortality rate and significant early neonatal morbidity – FETO might improve outcome. In order not to provoke prematurity and its associated morbidity, occlusion in this group is done only at 30–32 weeks. For both trials, neonatal colleagues from all over Europe designed a standardized consensus postnatal management protocol [92] (Table 1). A comprehensive website (www.totaltrial.eu) is available, both for patients and clinicians. The information on the disease and the natural history was agreed upon by the participating centers and the oversight committee, and was then re-evaluated for its informative character and clarity by parents who have been faced with (severe) CDH [93]. It also explains why randomized trials are needed in (fetal) medicine. For clinicians, it is a didactic tool for measuring severity of pulmonary hypoplasia, and for study participants for data entry and patient randomization (Table 2).

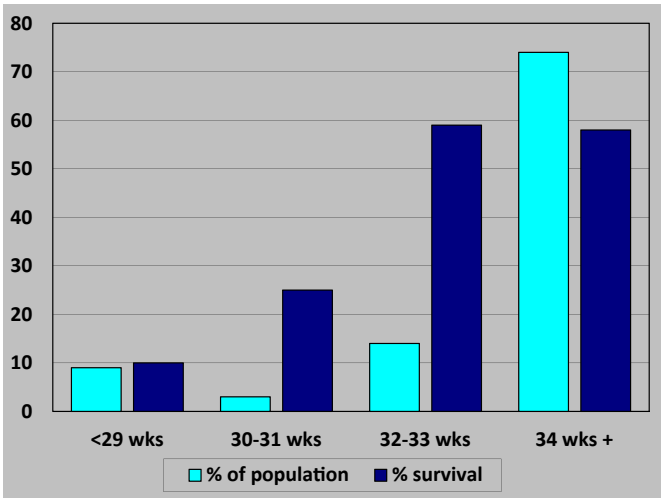

Fig. 7. Graphical display of number of patients delivering (light blue bars) and number of fetuses surviving (dark blue bars) as a function of gestational age. (Modified from Deprest J, et al. J Pediatr Surg 2011;46:22–32; with permission from the authors and the publisher.)

Table 2
Currently used criteria for TOTAL trial in left-sided CDH and the criteria used for right-sided CDH.

	Left-sided CDH		Right-sided CDH
	RCT Total trial "severe" Severe	RCT Total trial "moderate" Moderate	Observational study Severe
O/E LHR	<25%	25–45.9%	<45%
Liver	Liver "up"	If O/E LHR 25–34.9%: any position If O/E LHR 35–44.9%: liver "up"	Liver "up"
Timing FETO: between (weeks)	27^{+0} to 29^{+6} weeks	30^{+0} to 31^{+6} weeks	27^{+0} to 29^{+6} weeks

CDH, congenital diaphragmatic hernia; O/E LHR, observed/expected lung-to-head ratio; FETO, fetoscopic endoluminal tracheal occlusion.
Ranges mentioned including the limits mentioned.

We should acknowledge that these trials started off far too late, which occurred for various reasons [94–96]. Fortunately, the leading European centers are committed to this trial. Nevertheless, there are some limitations. First there is some backdoor, yet the turn over at these non-participating centers is fortunately rather limited. Other centers counsel patients with pessimistic survival rates with expectant management and therefor have some doubt there is still equipoise. Others counsel their patients with more optimistic survival rates than what was observed in the antenatal CDH registry. This is confusing for patients, though we hope that the TOTAL trial can be completed. The moderate trial has reached the first interim analysis point. The TOTAL trial may also extend to the USA and Canada, as the negotiations with the FDA on the instruments and the balloon may be reaching a conclusion (Dr Hedrick, personal communication). In Brisbane, Australia, the minimum experience of 15 cases prior to trial participation has been obtained, making it possible for them to start the trial. Also Japanese centers have agreed to concentrate their initial experience in few hands (H. Sago, personal communication). Concentration in few centers is not only a matter of common sense. The disease is rare, certainly for cases with severe hypoplasia [34]. Additionally, experience has been shown to relate to appropriate preoperative assessment [97], operation time and PPROM rates [71,72], and the ability to safely and effectively remove the balloon [71]. Also it will preclude that this technique spreads without having been shown to be of benefit [94,95].

5. Future perspectives in genetics and non-surgical strategies

The current clinical experience with fetal surgery yields less than desirable survival rates. Nor can the side-effects of FETO be ignored (up to 25% membrane rupture and preterm delivery rate) (Fig. 6). Due to its technical complexity and logistic demands, fetal surgery is neither universally applicable nor available. Therefore alternative, less invasive and/or more potent and/or easy methods for forced lung growth should be explored. Pharmacological or cell-based strategies are attractive because they should be less invasive than surgery. The most acceptable approach would be transplacental medical therapy. Experimentally induced CDH can be treated medically: transplacental retinoic acid rescues nitrofen-induced pulmonary hypoplasia by stimulated alveologenesis and alveolar cell proliferation [98,99]. However, retinoic acid is a teratogen, hence cannot be clinically used [100]. A more realistic treatment is the maternal administration of sildenafil, a generic (hence affordable) drug already used postnatally in neonates with PHT, including hypertension due to CDH. This drug seems to work in nitrofen rats, and larger animal studies are underway [101].

Delivery of stem cells is another avenue researched to stimulate (prenatal) lung development. This can be accomplished by two non-exclusive mechanisms: cells may integrate and differentiate or they may activate resident stem cells by paracrine mechanisms [102]. De Coppi et al. reviewed candidate cell types [103]. However, difficulties with isolation and expansion of lung progenitors make it hard to believe that autologous fetal lung stem cells can soon be used therapeutically. Therefore most research focuses on the use of exogenous cells, including (e.g. amniotic fluid-, bone marrow- or umbilical cord blood-derived) mesenchymal stem cells. Pederiva et al. described the rescue of nitrofen-induced pulmonary hypoplasia in rat lung explants. Exposure to AFS increased lung growth and bronchial motility, most likely via paracrine effects [104]. These effects are now being tested in larger animal models [56]. Stem cells may also be used for tissue engineering purposes, e.g. to engineer a patch for a more functional neonatal repair, as first proposed by Fauza [105]. Most probably, autologous amniotic fluid-derived stem cells will be the first choice, as they become available at routine amniocentesis for this condition. Also they can be routinely expanded, including for this condition, as earlier demonstrated (Fig. 8) [56,106]. Our group has reviewed advances in this field [107].

Recent advances in genomics technologies have increased the understanding of genetic factors underlying isolated CDH as well as syndromic CDH. Chromosomal microarrays have identified novel submicroscopic CNVs revealing new candidate genes and refined known genomic loci pinpointing others. The use of microarrays for prenatal diagnosis will continue to reveal CNVs associated with CDH and assist in determination of the penetrance risk for CDH as well as for other associated malformations. Exome sequencing has also revealed the involvement of specific genes in individual families, including *ZFPM2* and *GATA4* in association with isolated CDH. However, these variants are typically associated with reduced penetrance and/or variable phenotypic expression, creating challenges for genetic counselling of these patients. For exome sequencing to impact positively upon pregnancy management, a rapid turnaround is essential. Advancements in sequencing technologies continue to reduce costs, and it is already possible to go from sample receipt to variant calls in 3–5 days. The major challenge remaining for prenatal diagnosis is rapid causal variant identification. Analysis platforms such as annotate-it (www.annotate-it-org) and Cartagenia NGS (www.cartagenia.com) are aiding this process, and gene prioritization tools are being adapted to variant prioritization with tools such as eXtasy (http://homes.esat.kuleuven.be/~bioiuser/eXtasy/).

Transcriptome analysis by RNA sequencing will complement future studies of prenatal medical therapies in animal and cellular models, providing knowledge at the molecular level of the changes induced in gene expression. These studies will be complemented by downstream network or pathway analysis to understand the underlying biological processes and functions that are affected in CDH and modified by therapeutic interventions. This combination of identification of the genetic factors contributing to CDH in

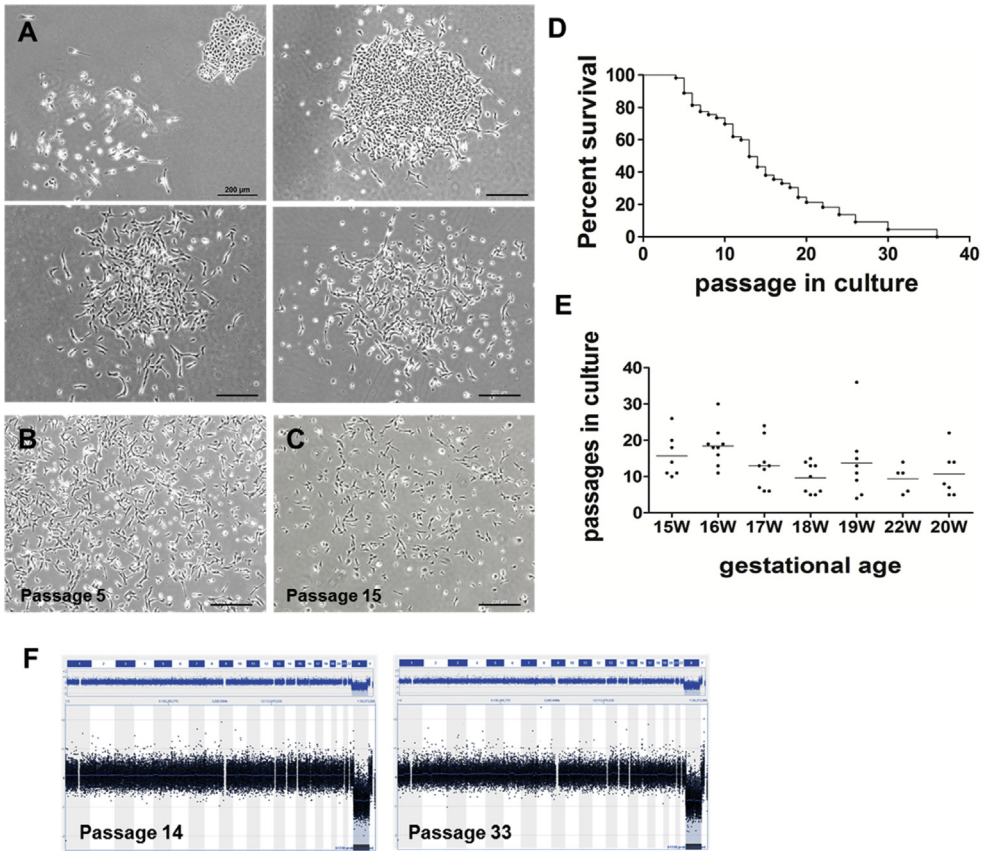

Fig. 8. Derivation of amniotic fluid derived stem cells by mechanical selection. (A) Representative image of AFSC colonies derived from a fresh amniotic fluid sample with an example of spindle and round shaped cells forming a colony. Morphology of AFSC at passage 5 (B) and passage 15 (C) in culture. Proliferative ability of AFSC. Cell Survival curve (D) for all clones derived from different gestational time point. (E) Passages in culture reached by every single clone grouped for gestational age. (F) Normal karyotype assay on one clone of AFSC at early passage (passage 14) in culture and at later (passage 33). Parts of this figure were earlier published in Zia S, et al. Prenat Diagn 2013;33:921–8; and are reproduced with permission of the authors and the publisher.)

individual patients and how specific biological pathways are influenced by novel therapies will lead to a more personalized approach to prenatal management.

Practice points

- Prenatal diagnosis and counseling is based on comprehensive assessment at a tertiary center using advanced genetic testing, modern imaging methods, individualized prognosis and familiarity with the multidisciplinary pre- and postnatal management of CDH.
- In isolated cases with normal genetic tests, individualized prognosis is based on the side of the lesion, lung size, liver herniation, and possibly position of the stomach.
- The benefit of fetal therapy has not yet been demonstrated in the setting of conditions available at tertiary centers in North America or Europe. Therefore, FETO should be offered within the framework of one of the ongoing clinical trials.
- Chromosomal microarray analysis has been demonstrated to reveal (novel) pathogenic CNVs and risk factors associated with isolated CDH, increasing the diagnostic yield.
- The clinical utility of exome sequencing needs to demonstrated on larger cohorts of sporadic cases of isolated CDH before introduction into the clinical setting of prenatal diagnosis.

Research directions

- There is still a need for large studies on prenatal prediction of mortality and morbidity, defining which predictors of outcome act independently.
- Alternatives to surgical fetal therapy should be explored, because the side-effects of FETO are clinically relevant, and, despite increasing experience, these side-effects are probably here to stay.
- Chromosomal microarray analysis should be the first-tier test for prenatal diagnosis of isolated CDH fetuses, complemented with conventional cytogenetic techniques.
- Large scale exome (or genome) sequencing studies are necessary to understand the full spectrum of genes involved in pathogenesis of isolated CDH as well as those genetic factors affecting penetrance.

Acknowledgements

J.D.P. is a fundamental clinical researcher supported by the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO; 1.801207). E.G. and J.D. steer an Erasmus Joint Doctoral program of the Universities of Barcelona, Leuven and Lund, which is sponsored by the European Commission (2013-0040). The TOTAL trial was initially set up within the framework of the "Eurostec" FP-6 program (EuroSTEC, LSHC-CT-2006-037409) on perinatal tissue engineering for congenital birth defects, sponsored by the European Commission.

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