Current and future antenatal management of isolated Congenital Diaphragmatic Hernia

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Abstract

Congenital Diaphragmatic Hernia (CDH) is surgically correctable, yet the poor lung development determines mortality and morbidity. In isolated cases the outcome can be predicted prenatally by medical imaging. Cases with a poor prognosis could be treated before birth. Prenatal modulation of lung development however remains experimental. Fetoscopic Endoluminal Tracheal Occlusion triggers lung growth and is currently being evaluated in a global clinical trial (www.totaltrial.eu). Prenatal transplacental sildenafil administration may in due course be a therapeutic approach, reducing the occurrence of persistent pulmonary hypertension, either alone or in combination with fetal surgery.

Keywords: congenital diaphragmatic hernia – prenatal therapy - pulmonary hypoplasia - pulmonary hypertension - sildenafil

Introduction

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 in 2500 births and therefore, in the EU-27, approximately 2,100 babies with are born annually. The defect typically is left sided in 85% (LCDH), yet 13% are right sided (RCDH), while the reminder minority is bilateral or there is complete agenesis [1, 2]. From the embryonic phase, abdominal organs herniate through the defect interfering with lung growth, resulting in developmental arrest of both airways and vasculature. Hypoplastic lungs have fewer and less mature airway branches and impaired vascular development, including a smaller cross-sectional area of pulmonary vessels, structural vascular remodeling and altered vasoreactivity [3]. At birth, this results in respiratory insufficiency and persistent pulmonary hypertension (PHT), which cannot be solved simply by the surgical repair of the diaphragm. Despite improved and more standardized neonatal management, overall survival of neonates with CDH remains at around 70% [4]. PHT is a major cause of neonatal death and morbidity.

Antenatal assessment.

Prenatal ultrasound identifies two out of three cases of CDH [5], providing the opportunity for in utero referral to a tertiary care center for expert assessment and perinatal management. Additional genetic and morphologic assessment using ultrasound or magnetic resonance imaging can be used to rule out associated malformations [6]. For *isolated* cases clinicians can individualize prognosis to counsel parents about prenatal options. A detailed description of potential prognostic indices goes beyond the purpose of this review. Briefly, most of the prediction methods are based on lung size, liver herniation and pulmonary circulation, and more recently stomach position [7-12]. Ultrasound measurement of the lung-to-head-ratio (LHR) is

most widely practiced. It involves a standardized 2D-ultrasound measurement of the lung contralateral to the defect at the level of the four chamber view of the heart. The *observed* LHR is expressed as a proportion of what is *expected* in a normal fetus of the same gestational age (o/eLHR), allowing prediction of prognosis independently of gestational age [7, 13]. The combination of liver herniation and o/eLHR is currently used by prenatal management centers to stratify fetuses with LCDH and RCDH into groups with different increasing pulmonary hypoplasia and corresponding mortality rates (Figure 1). Those with the worst prognosis may be offered intrauterine treatment. Severity assessment by Magnetic Resonance Imaging (MRI) theoretically has several advantages over ultrasound. Visualization is not limited by maternal habitus, amniotic fluid volume or fetal position. Furthermore, MRI volumetric measurement of both lungs may better predict postnatal lung function. Volumetry may also accurately quantify liver and stomach herniation [14, 15]. Though one may claim that lung volume and liver herniation MR better predict outcome than ultrasound [16], in clinical practice, it may be difficult to prove [17].

Lung size and liver herniation also predict neonatal morbidity, such as the duration of assisted ventilation, the need for supplemental oxygen, the need for patch repair and the time it takes to full enteral feeding [18, 19]. The literature on prediction of PHT is more limited (systematically reviewed in [20]). Several candidate parameters have been suggested in single case series, including lung size, presence of visceral herniation, and also direct assessment of the pulmonary vasculature, which may provide additional information.

Fetal Therapy for CDH today

The ability to identify a future non-survivor *prenatally* prompts the quest for a **prenatal intervention** that can improve outcome. In fetuses with poor prognosis, fetal lung growth can be stimulated by fetoscopic endoluminal tracheal occlusion (FETO) with a balloon [9, 21-23]. The concept "*plug the lung until it grows*" is inspired by clinical observations in fetuses with laryngeal atresia who have a marked increased lung volume and alveolar number. Airway obstruction prevents egress of pulmonary fluid which experimentally has been shown to prompt lung growth by a mechanism of stretch of lung parenchymal cells [24]. Those experimental findings have been clinically translated into a percutaneous procedure without serious maternal morbidity (Figure 2).

Technique for balloon insertion.

We perform FETO under local anesthesia and sono-endoscopic guidance. First the fetal and placental position are determined for optimal trocar insertion. When necessary, the fetus is externally manipulated to enable a safe and effective access to the mouth. A neuromuscular blocking agent, fentanyl and atropine are administered to the fetus for immobilization, anesthesia, and prevention of fetal bradycardia respectively. A disposable, thin-walled flexible cannula loaded with a pyramidal trocar or using the Seldinger technique, is inserted through the skin and myometrium avoiding the placenta. Under ultrasound guidance the cannula is targeted to or above the fetal nose tip. Fetoscopic instruments specifically designed for FETO include a slightly bent 3.3 mm sheath loaded with a fiber optic endoscope (1.3 mm; Karl Storz) and the balloon occlusion system (catheter loaded with a detachable inflatable latex balloon with integrated one-way valve Goldbal 2, Balt Extrusion, France). This balloon can accommodate an increasing diameter as the fetal trachea grows during pregnancy. It appears on ultrasound examination as a fluid-filled structure. Through the sheath we

also pass a stylet and/or forceps to remove the balloon if wrongly positioned. Irrigation for clearing the operative field and improving visualization can be connected to the side port. Fetoscopic landmarks are the philtrum and upper lip, the tongue and raphe of the palate, uvula, epiglottis, and eventually the vocal cords. The endoscope is advanced into the trachea until identification of the carina, above which the balloon is positioned by inflation and detachment from the catheter. The median duration of FETO is 10 (range, 3–93) minutes, dependent on both the experience of the operator and the position of the fetus [25]. A longer operation time is the main risk factor for membrane rupture.

Technique for balloon removal

Experimental data suggest benefit of *temporary* tracheal occlusion ("plug-unplug" sequence) by stimulating lung maturation [26, 27], which prompted clinicians to attempt timely *in utero* reversal as much as possible. Also clinical data suggest that prenatal balloon removal increases neonatal survival [21] and reduces neonatal morbidity [8]. Leaving the tracheal occlusion until delivery may provide additional lung growth and theoretically avoid the risk for preterm delivery from the second fetoscopic intervention of balloon removal [23, 28]. Conversely, it may lead to more emergency removals at the time of birth which are challenging and risky [29].

We therefore schedule elective intrauterine occlusion reversal at 34 weeks in patients with an uneventful post-operative course. We have used ultrasound-guided puncture, fetoscopic removal, tracheoscopic removal on placental circulation and postnatal puncture. Ultrasound-guided in utero balloon puncture is done after fetal immobilization and fetal analgesia. The lung fluid which is at high pressure under the occlusion pushes

the punctured balloon into the pharynx, from where it is either swallowed or falls into the amniotic cavity. Tracheal patency may be confirmed by a change in tracheal diameter and flushing under ultrasound Power Doppler examination. Fetoscopic removal is done with similar instruments as for insertion. Initially we used a forceps (11510C Karl Storz) only, yet fetoscopic puncture with a stylet (11506P Karl Storz) dramatically reduced the operation time. The forceps is still used to grasp the tail of the balloon and retrieve it [29].

In 20% of cases, patients present earlier than planned with threatened preterm delivery, with or without ruptured membranes. Whenever clinically possible, we try to remove the balloon in utero, using the same techniques. This will be delayed as long as possible and may need to be done in an emergency. If considered not possible or safe, removal will be done at delivery. We have performed in utero balloon removal in the presence of ruptured membranes, in early labour or with early signs of chorio-amnionitis. If in utero retrieval does not seem safe or possible, we remove the balloon by laryngo-tracheoscopy during a modified cesarean section under loco-regional anesthesia, with the fetal head and shoulders delivered, while the fetus remains on placental circulation (Figure 3A) [30]. Post-delivery removal is a last resort, which we do by video laryngo-tracheoscopy, although blind or ultrasound-guided ex utero puncture have also been reported [31].

We recently reported outcomes on 302 balloon insertions at three FETO-centers [29]. Balloon removal was elective in 72% of cases and as an emergency in 28%. The primary method was by fetoscopy in the majority of cases (67%), by ultrasound guidance in 21%, by tracheoscopy on placental circulation in 10%, and postnatal tracheoscopy in 1%. In 3%, a second removal attempt was required. Each surgeon had a different preferred primary technique. There was no difference in the interval to

delivery between fetoscopic and ultrasound-guided removal in elective cases. There were nine balloon removals attempted outside the FETO centers. Retrieval was impossible in three cases, leading to iatrogenic neonatal death (Figure 3B).

Outcomes of FETO

Compared to historical controls of similar severity, FETO increases survival rate from 24% to 49% in LCDH with o/eLHR<25%, and from 17% to 42% in RCDH with o/eLHR<45% [25, 32]. FETO also seems to reduce early neonatal respiratory morbidity [8, 19]. This potential benefit is now being investigated in two parallel randomized clinical trials (RCT) “Tracheal Occlusion To Accelerate Lung growth” (www.TOTALtrial.eu), in fetuses with LCDH and severe or moderate lung hypoplasia (NCT01240057 and NCT00763737) [33]. Current participating fetal therapy centers are from Europe (Leuven, Belgium; Paris, France; London, United Kingdom; Barcelona, Spain; Milano & Rome, Italy; Bonn, Germany); from Toronto, Canada; Brisbane, Australia and most recently Houston, Texas. The second interim analysis (n= 147) of the moderate trial did not identify safety issues. With an increase in recruitment, as additional centers from the United States and Japan will join, it is hoped to finish the moderate lung hypoplasia trial within 2-3 years. The trial on severe lung hypoplasia cases has reached recruitment for the first interim analysis time point.

Whatever the outcome of the TOTAL-trial, FETO is invasive and has an increased risk for preterm delivery, partly offsetting the beneficial effects of fetal therapy. In our pre-trial experience (>200 cases), preterm rupture of membranes <34 weeks occurred in 25% of patients. Delivery took place at a median of 35 weeks, but 30% delivered before 34 weeks, requiring urgent balloon retrieval. Gestational age at delivery logically is an important predictor of survival and morbidity in CDH fetuses, also when undergoing fetal therapy [8] (Figure 4). Based on our available data, TO does lead to tracheal

widening, yet without impact on survival or the requirement for early respiratory support [34-36].

The logistics and skills for FETO are not a given, therefore FETO is difficult to widely implement. Finally, the maximum post-FETO survival reported in severe cases is 50-60%, which in part is caused by insufficient airway growth and, above all, limited improvement of vascular development. Indeed, while FETO increases lung size, it does not seem to solve the problem of PHT. Alternative prenatal strategies are therefore required, ideally also addressing the problem of PHT and preferentially medical rather than surgical, to overcome the risks and limitations of fetal procedures.

Non-surgical experimental antenatal solutions

We, as well as others, are therefore exploring transplacental pharmacologic modulation of lung development. We have focused on methods also affecting vascular development. Though new ventilation strategies and medical treatments may have improved the outcome of infants with respiratory insufficiency, there is no post-natal therapy with proven efficacy for CDH-related PHT. PHT has become the major cause of mortality and morbidity in CDH [37]. Infants with CDH, unlike for other causes of neonatal respiratory failure, often present with PHT refractory to inhaled nitric oxide (NO) [38]. The presence and severity of PHT predicts pulmonary morbidity and death [39, 40]. Beyond PHT in the neonatal period, newly emerging patterns of late (months after birth) and chronic (years after birth) PHT also affect the long-term quality of life in CDH survivors [41]. PHT is also a serious economic burden. Postnatal management costs of a CDH-newborn are higher when PHT is present, mainly due to the increased use of extra-corporeal membrane oxygenation [42]. To this, the burden of chronic PHT must be added. Chronic PHT is associated with an increased risk of death, cardiac and pulmonary complications and re-hospitalization, and requires chronic medical

treatment and follow-up [43]. For all these reasons, an effective strategy to treat or prevent PHT would be welcomed. As for airway development, this ideally would already start in utero, in order to *prevent* the structural changes that lead to PHT after birth.

The best candidate would be a treatment for PHT already proven safe and effective postnatally. Ideally the drug should be effective after maternal intake, and should have no significant adverse effects on the mother or fetus. This strategy would also allow a wider implementation than fetal surgery, including in low-income countries. Such an approach would be the first universally affordable therapy for CDH ever.

Sildenafil

Sildenafil is a selective and potent inhibitor of phosphodiesterase 5 (PDE5), which specifically degrades cyclic guanosine monophosphate (cGMP). It is found in high concentrations in pulmonary arteries and the corpora cavernosa. PDE5 is abundantly expressed during fetal life, where it acts as a key regulator of the perinatal pulmonary circulation [44]. Experimental studies on chronic PHT in newborn animals have demonstrated impaired endothelial release of NO and increased production of vasoconstrictors [45]. Increased PDE5 activity may contribute to this phenomenon [46]. The mechanism of action of sildenafil on the pulmonary vasculature is depicted in Figure 5. Apart from having a vasodilatory effect, sildenafil promotes pulmonary angiogenesis and inhibits pulmonary artery remodelling [47]. These properties make sildenafil a potential candidate to prevent the vascular changes leading to PHT in CDH newborns.

Sildenafil is approved by the European Medical Agency and the Food & Drug Agency for use in PHT in *adults* [48]. It is also effective and well tolerated in children affected by PHT, as shown in the STARTS RCTs [49, 50]. There is a growing interest for the

use of sildenafil in the newborn for the treatment of PHT of various etiologies [51], including CDH [52]. Sildenafil has also already been widely used in pregnant women. There were initial case reports on its chronic use in mothers with PHT, showing maternal improvement without apparent side effects on the fetus [53-55]. Sildenafil has also been evaluated in two small case series for the treatment of early onset preeclampsia [56] and intrauterine growth restriction (IUGR) [57], and one RCT in pregnancies complicated by idiopathic oligohydramnios [58]. No fetal, neonatal or maternal side effects have yet been shown. Finally, there are to our knowledge currently four ongoing phase III trials with sildenafil treatment during pregnancy, three for severe early onset IUGR [59] and one in normal pregnancies to evaluate safety to reduce intrapartum fetal compromise [60]. All this suggests that sildenafil is a reasonable option to be evaluated in clinical trials with CDH cases from mid-gestation onwards.

Thébaud was the first to show the benefit of transplacental sildenafil in CDH using the nitrofen induced model of CDH [61]. Sildenafil was administered subcutaneously to the dams from E11.5 to E20.5 (term=21d) and was detected in fetal blood. It induced a marked increase in lung cGMP in CDH fetuses, and a significant attenuation in active PDE5 expression, indicating biological activity in the fetal lung. Sildenafil improved lung structure, increased pulmonary vessel density, reduced right ventricular hypertrophy and improved postnatal pulmonary artery relaxation. It had no demonstrable adverse effects on the fetal retina and brain, neither adverse maternal effects. Since then, the efficacy of antenatal sildenafil in that nitrofen CDH rat-model has been confirmed [62-65], also when given at a more clinically relevant timing [66].

In a recent placebo-controlled trial with transplacental sildenafil given to fetal rabbits with CDH, we confirmed both the safety and efficacy of the drug (Figure 6) [67]. The

rabbit model is clinically more relevant than the nitrofen-CDH rodent as it is a larger species. We first performed a dose-finding study demonstrating that subcutaneous administration of 10 mg/Kg/d sildenafil resulted in therapeutic levels (target 47 ng/mL, proven therapeutic in the STARTS-1 trial for PHT in children [49]) for at least 22 h/day. There were no differences in maternal weight change, heart rate change, behavior, fetal loss rate and fetal body weight in animals treated with sildenafil or placebo. Then efficacy was shown in 38 rabbit fetuses with CDH, randomized to either daily placebo or sildenafil from gestational day 24 until term (31d). The wall thickness of peripheral pulmonary vessels was increased in placebo-exposed CDH-fetuses as compared to normal controls, yet fell in the normal range when CDH-fetuses were exposed to sildenafil. CDH fetuses had also proportionally more muscularized peripheral vessels than control pups, whereas sildenafil treatment was associated with peripheral muscularization in the normal range. Three-dimensional blood vessel architecture was assessed using micro-CT showing fewer vessels of the fifth order or higher (thus distal) in placebo exposed CDH-pups compared to controls. Again, the number of `distal` vessels of sildenafil-exposed CDH fetuses was in the normal range. Small diameter arteries are the main determinants of pulmonary vascular resistance. An increase in their number will reduce resistance. This, combined with a change in their compliance and thinner and less muscularized walls, should better accommodate post-natal transition and help prevent PHT. We also studied the functional effects of sildenafil, i.e. reduced in utero pulmonary vascular resistance evidenced at term by micro-ultrasound Doppler studies. Besides the effect on vessels, the drug also improved the morphology of the airways, and improved postnatal lung mechanics as demonstrated by Flexivent-ventilation. In summary, sildenafil-induced changes in the vascular compartment were paralleled by changes in airway development. Of interest, sildenafil

seemed to have a detrimental effect on vascular branching in non-hypoplastic lungs of normal rabbit fetuses.

We later investigated the effect of combining maternal sildenafil and fetal TO, again in fetal rabbits with CDH [68]. In line with earlier experiments, fetal TO alone restored normal lung size and airway structure, yet did not reverse pathologic changes in peripheral pulmonary vessels. Conversely, sildenafil alone reversed these vascular abnormalities to normal values, yet also *partly* rescued airway development, again without increasing lung size. CDH fetuses treated with sildenafil *and* TO had both a normal lung vascular structure and normal lung parenchyma and size. These results suggest that maternal sildenafil combined with fetal TO have a synergistic effect on vascular and parenchymal lung development.

As a next step to clinical translation, we evaluated transfer of sildenafil through the human placenta, by using an *ex-vivo* placenta perfusion model [69]. Our preliminary data demonstrate that sildenafil crosses the placenta at increasing rates according to maternal concentration [70]. The relatively high transfer rate, with a feto-maternal concentration ratio between 1 and 1.2 at steady state, suggests that there is sufficient placental transfer to reach target fetal drug levels at non-toxic maternal doses. All these results have prompted us to apply to the European Medical Agency for orphan designation of this drug for the prenatal prevention of PHT in CDH.

Practice points

- Patients with an antenatal diagnosis of CDH should receive individualized counseling on expected outcome
- In severe to moderate lung hypoplasia cases, prenatal interventions may be considered.
- FETO is the only fetal treatment currently available, yet it is investigational and should be offered within the framework of the TOTAL randomized clinical trial.
- FETO may fail to induce sufficient parenchymal and vascular lung growth and/or may cause prematurity.

Research directions

- Investigating alternative, preferentially non-surgical, antenatal strategies
- Defining effective interventions to prevent both respiratory insufficiency and PHT
- Transplacental sildenafil, above all, seems close to clinical application.

Acknowledgements

JDP is a Clinical Researcher of the Flanders Research Foundation (FWO Vlaanderen; 1.8.012.07) and is partly funded by GOSH Children's Charity. PDC is a NIHR Professor and supported by the Catapult Cell Therapy, the GOSH Children's Charity and GOSH NIHR Biomedical Research Centre's funding scheme. Additionally, PDC and JDP have been jointly funded by GOSHCC, Sparks and CDHUK. MPE and LvV are supported by the Erasmus+ Programme of the European Commission (2013-0040). ALD is supported by the UCL/UCLH NIHR Biomedical Research Centre. Our research is in part supported by an Innovative Engineering for Health award by the Wellcome Trust (WT101957), the Engineering and Physical Sciences Research Council (EPSRC) (NS/A000027/1) and the Fetal Health Foundation (USA). We thank our co-workers R Devlieger, L De Catte, L Lewi (UZ Leuven) and R Wimalasundera (UCLH) for their help in the clinical program. TOTAL co-investigators are thanked for their endurance in this difficult clinical trial, yet already including over 180 patients.

Conflict of interest

The authors declare no conflict of interest. The KU Leuven has deposited a request for orphan designation for sildenafil to the EMA.

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Captions

Figure 1: Patient stratification and selection of candidates for intrauterine therapy according to the o/e LHR for LCDH (A) and RCDH (B). Adapted from Jani et al [13] and DeKoninck et al [32].

Figure 2: Schematic drawing of percutaneous fetoscopic endoluminal tracheal occlusion. Inset: a detachable balloon, normally used for endovascular occlusion, is positioned in the trachea. Reproduced with permission from UZ Leuven, Leuven, Belgium. Drawing Myrthe Boymans.

Figure 3: A Schematic drawing of balloon removal on placental circulation by laryngeo-tracheoscopy. Reproduced with permission from UZ Leuven, Leuven, Belgium. Drawing Myrthe Boymans. **B** Flow chart of balloon removal technique. Numbers are from a multicenter study, with both % of primary attempts and the success rate of the technique. Adapted from Jimenez et al [29].

Figure 4:Survival rate in LCDH cases with o/eLHR <25% after FETO, according to gestational age at delivery [71].

Figure 5: Schematic representation of the mechanism of action of sildenafil on the pulmonary vasculature. eNOS: endothelial nitric oxide synthase; NO: nitric oxide; GC: guanylate cyclase; GTP: guanosine triphosphate; GMP: guanosine triphosphate; cGMP: cyclic GMP; PDE: phosphodiesterase; ATP: adenosine triphosphate; AMP: adenosine monophosphate; cAMP: cyclic AMP; PKG: cGMP dependent protein kinase; PKA: cAMP dependent protein kinase. Reproduced with permission from UZ Leuven, Leuven, Belgium. Drawing Myrthe Boymans.

Figure 6: Effect of sildenafil on lung vasculature in the CDH rabbit model. (A) Representative and Miller's stained lung sections showing differences in lung in medial and adventitial thickness of peripheral vessels in the four study groups. (B)

Representative 3D surface rendered images showing impaired vascular branching in CDH-fetuses, which is restored by exposure to sildenafil. Reproduced, with permission from the publisher, and from Russo et al [67].