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Review article

Pulmonary hypertension associated with bronchopulmonary dysplasia in preterm infants

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ABSTRACT

Bronchopulmonary dysplasia (BPD) and BPD-associated pulmonary hypertension (BPD-PH) are chronic inflammatory cardiopulmonary diseases with devastating short- and long-term consequences for infants born prematurely. The immature lungs of preterm infants are ill-prepared to achieve sufficient gas exchange, thus usually necessitating immediate commencement of respiratory support and oxygen supplementation. These therapies are life-saving, but they exacerbate the tissue damage that is inevitably inflicted on a preterm lung forced to perform gas exchange. Together, air-breathing and necessary therapeutic interventions disrupt normal lung development by aggravating pulmonary inflammation and vascular remodelling, thus frequently precipitating BPD and PH via an incompletely understood pathogenic cascade. BPD and BPD-PH share common risk factors, such as low gestational age at birth, fetal growth restriction and perinatal maternal inflammation; however, these risk factors are not unique to BPD or BPD-PH. Occurring in 17–24% of BPD patients, BPD-PH substantially worsens the morbidity and mortality attributable to BPD alone, thus darkening their outlook; for example, BPD-PH entails a mortality of up to 50%. The absence of a safe and effective therapy for BPD and BPD-PH renders neonatal cardiopulmonary disease an area of urgent unmet medical need. Besides the need to develop new therapeutic strategies, a major challenge for clinicians is the lack of a reliable method for identifying babies at risk of developing BPD and BPD-PH. In addition to discussing current knowledge on pathophysiology, diagnosis and treatment of BPD-PH, we highlight emerging biomarkers that could enable clinicians to predict disease-risk and also optimise treatment of BPD-PH in our tiniest patients.

1. Introduction

Once placental function is lost at birth, the preterm infant is suddenly confronted with the challenging task of oxygenating the blood and removing metabolically-produced carbon dioxide (CO_2) using a respiratory system that is inadequately developed for the task. The rate of transfer of gas across the lung is directly proportional to the surface area for exchange and inversely proportional to the diffusion distance between blood and air, according to Fick's Law of diffusion. The lungs of infants born very prematurely, i.e. infants born at 28 weeks of gestation or less, are yet to reach the alveolar stage of lung development, when lung maturation becomes adequate for efficient gas exchange (Madurga et al., 2013; Mullassery and Smith 2015). Therefore, as gestational age falls towards 23 weeks (the gestational age currently widely regarded as the threshold of viability), gas exchange occurs across an ever-smaller surface area and increasing diffusion distance. To bolster oxygen (O_2) transfer to the baby, the clinician is forced to adjust a third variable in Fick's Law, namely the concentration of O_2 in the lung, which can readily be increased by adding O_2 to the inspired air. In the face of a preterm infant's immaturity, both at the level of the lung and the mechanics of the thorax, it is often necessary to use a mechanical ventilator to maintain lung volume, adequate ventilation and arterial oxygenation. Although necessary to sustain the preterm infant's life, high concentrations of inspired oxygen and mechanical ventilation damage the lung by precipitating pulmonary inflammatory pathways and by disrupting lung angiogenesis and vasculogenesis (Gien and Kinsella 2011). At present, with no effective therapy to counter inflammation and vascular dysgenesis, many preterm infants develop two

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diseases that severely compromise lung function and can lead to death, namely bronchopulmonary dysplasia (BPD) and its gravest complication, pulmonary hypertension associated with BPD (BPD-PH) (Abman 2011).

Although this review briefly introduces BPD, its main focus is BPD-PH, which we discuss from the perspectives of classification, epidemiology, pathogenesis, diagnosis and current therapeutic options. In light of emerging evidence for the need of early intervention in the prevention or treatment of BPD-PH, another focus of this review lies on potential physiological, biochemical and genetic predictors of BPD-PH.

2. Bronchopulmonary dysplasia (BPD)

BPD is a severe inflammatory chronic lung disease that affects 10,000–15,000 preterm babies each year in the USA (Van Marter 2009; Stoll et al., 2010). BPD is a major contributor to neonatal morbidity with an incidence that is inversely related to gestational age and birth weight. In one study in the USA, approximately 50% of infants weighing less than 1000 g developed the disease (Martin et al., 2013), while in Australia and New Zealand, the incidence of BPD is 61% in infants born at ≤25 weeks gestational age (GA) (Chow 2014). As no safe and effective treatment for BPD is available, the health care costs associated with this disease are staggering, exceeding $2.4 billion annually in the USA alone (McAleese et al., 1993).

2.1. Definition and classification of BPD

The current definition of BPD is based upon a patient's need for respiratory support and supplemental oxygen therapy at 36 weeks corrected GA (cGA). With such a subjective basis for categorising BPD, it is unsurprising that BPD diagnosis and classification of its severity vary widely between hospitals– and despite the efforts described below, defining BPD frequently continues to pose a challenge (Jobe and Steinhorn 2017).

In order to overcome inter-hospital variability, a more objective 'physiological' definition of BPD was established based upon the degree of respiratory support at 36 weeks cGA and a "room air challenge" if required (Jobe and Bancalari 2001; Walsh et al., 2003; Walsh et al., 2006). In accordance with these guidelines, the Australia and New Zealand Neonatal Network (ANZNN) defines BPD as lung disease with ongoing requirement for supplemental oxygen therapy or respiratory support (continuous positive airway pressure or mechanical ventilation) at 36 weeks cGA (Chow 2014). In addition to this physiological classification, BPD severity can be classified into one of three categories as per the NICHD (United States National Institute of Child Health and Human Development) and the NHLBI (United States National Heart, Lung, and Blood Institute), namely mild, moderate and severe, according to the criteria shown in Table 1 (Jobe and Bancalari 2001; Short et al., 2007; Hayes et al., 2010).

While the diagnosis of BPD is assigned at 36 weeks cGA, the presence of the underlying pulmonary pathology can be suspected earlier; the term used in this case is 'evolving BPD'. In this review, "BPD" will refer to the disease as defined by ANZNN and "evolving BPD" will refer to suspected BPD before a diagnosis can formally be made.

Table 1
NICHD/NHLBI diagnostic criteria for severity of BPD. The three categories of severity are mild, moderate and severe BPD. Modified from Jobe and Bancalari (2001), Short et al. (2007) and Hayes et al. (2010).

BPD Severity	Criteria
Mild	Supplemental oxygen required ≥28 days, termination of supplemental oxygen by 36 weeks cGA or discharge
Moderate	Supplemental oxygen required ≥28 days, requirement of < 30% O_2 at 36 weeks cGA or discharge
Severe	Supplemental oxygen required ≥28 days, requirement of ≥30% O_2 and/or continuous positive airway pressure or mechanical ventilation at 36 weeks cGA or discharge

2.2. Sequelae of BPD

Clinical management of BPD requires substantial intensive care interventions, including mechanical ventilation and oxygen supplementation. These necessary interventions frequently have adverse effects, including aggravation of the disease process, whereby they contribute to the development of short- and long-term sequelae. Examples of such complications include increased likelihood of neurodevelopmental impairments (such as lower IQ and delayed cognitive, motor and language development at 3 years of age (Short et al., 2007)), reduced lung function in adolescence and adulthood (Kotecha et al., 2013; Gough et al., 2014; Um-Bergstrom et al., 2017), and increased airway obstruction at 18 years of age (Doyle et al., 2017). Another serious complication of BPD is pulmonary hypertension (PH), which can significantly add to the disease burden of extremely preterm infants (Khemani et al., 2007; Bhat et al., 2012) as discussed below.

3. Pulmonary hypertension (PH)

The paediatric PH guidelines released in 2015 by the American Heart Association and American Thoracic Society define PH as a resting *mean* pulmonary artery pressure of ≥ 25 mmHg at sea level in children at 3 months of age or older (Abman et al., 2015). In younger infants, 36 mmHg of *systolic* pulmonary artery pressure is commonly considered the upper limit of normal (Lau et al., 2011; Parasuraman et al., 2016). There is a lack of consensus on how to classify PH severity, possibly due to the complexity and heterogeneity of the disease and its clinical outcomes.

Two types of PH occur in the neonatal period, namely persistent pulmonary hypertension of the newborn (PPHN) and BPD-PH. PPHN is most readily understood with reference to the normal haemodynamic transition at birth (Steinhorn 2010), which involves the first breaths filling the lung with air, and a subsequent rapid rise in pulmonary and systemic oxygen partial pressure and saturation. As oxygen partial pressure and saturation rise, the hypoxic vasoconstriction that characterises the fetal pulmonary vascular bed begins to abate (Abman 1999; Polglase et al., 2012), pulmonary blood flow increases, and flow across the ductus arteriosus and foramen ovale falls. The ductus arteriosus and foramen ovale eventually close, and the pulmonary circulation transforms into its mature condition of high flow, low resistance and low pressure (Polglase and Hooper 2006). A failure to decrease pulmonary vascular resistance immediately after delivery results in continued right-to-left shunting across the ductus arteriosus and foramen ovale, and persistence of high resistance and pressure in the pulmonary circulation (Abman 1999), resulting in the condition termed PPHN. The causes and consequences of PPHN have been extensively reviewed recently (Teng and Wu 2013; Sharma et al., 2015) and thus will not be discussed further here. Instead, in view of its high morbidity and mortality, we focus on BPD-PH.

Of note, PH is currently classified by the World Health Organisation into 5 subtypes according to shared pathologic and clinical features and treatment strategies (Simonneau et al., 2014). However, this classification is based on adult PH and does not take into account the heterogeneity of neonatal and paediatric PH and the multifactorial mechanisms that contribute to its development. The Pediatric Taskforce of the Pulmonary Vascular Research Institute (PVRI) has proposed an alternative classification of PH encompassing 10 categories that may be more relevant to PH in neonates and children (Cerro et al., 2011); its use is currently being evaluated.

3.1. Pulmonary hypertension secondary to BPD (BPD-PH)

PH can be considered the gravest complication of BPD as it

substantially worsens the prognosis of afflicted infants. PH occurs in 17–24% of BPD patients (Bhat et al., 2012; Kim et al., 2012; Ali et al., 2013; Al-Ghanem et al., 2017), and as with BPD, the incidence of PH increases as gestational age decreases, rising to 59% in infants born at or prior to 25 weeks cGA (Khemani et al., 2007). Mortality in infants with BPD-PH was reported to be between 14% and 38% (An et al., 2010; Slaughter et al., 2011; Kim et al., 2012); however, in another study, approximately half of the babies with severe PH succumbed to heart failure within 2 years of diagnosis (with severe PH defined as an estimated right ventricular (RV) pressure ≥100% of systemic pressure) (Khemani et al., 2007).

Many of the risk factors for PH overlap with those for BPD, such as low gestational age, fetal growth restriction, oligohydramnios, prolonged mechanical ventilation as well as oxygen dependency (Khemani et al., 2007); multivariate analysis is yet to reveal any risk factors that are unique to PH. In fact, the factors that contribute to the development of PH are poorly understood, and there is controversy regarding the correlation between BPD and PH: Some studies conclude that increasing BPD severity is not always associated with a higher incidence of PH (Khemani et al., 2007), whereas other papers report that infants with severe BPD are more likely to also develop PH (An et al., 2010; Bhat et al., 2012). Thus, the association between the severity of BPD and the development of PH warrants further research.

The long-term outcomes for survivors of BPD-PH are largely unknown, which has prompted an urgent call for research to better understand these outcomes, in particular the pulmonary and neurodevelopmental consequences of the disease (Al-Ghanem et al., 2017). The few existing reports suggest that structural pulmonary vascular disease and increased vascular tone can persist into adolescence in BPD-PH patients (Mourani et al., 2004). Despite normalisation of pulmonary arterial pressures in childhood, hyper-reactivity to hypoxic stress was observed in BPD-PH children at 5 years of age (Mourani et al., 2004; Poon et al., 2013). Moreover, even in the absence of other signs of PH, survivors of BPD-PH exhibited decreased right ventricular systolic function, which is an independent predictor of future RV failure (Sachdev et al., 2011; Kwon et al., 2016). In the shorter term, elevated pulmonary arterial pressures have been shown to persist until discharge in survivors of BPD-PH (Bhat et al., 2012).

3.2. Pathogenesis of BPD-PH

The patho-mechanisms that underlie BPD and BPD-PH are not well understood, but it has been recognised that various insults, including disruption of fetal lung development, perinatal infection/inflammation, mechanical ventilation and hyperoxia, cause pulmonary inflammation that can have deleterious impact on both the airways and alveoli as well as the pulmonary vasculature (Hislop 2002; Speer 2006; Madurga et al., 2013; Vogel et al., 2015).

Extremely preterm infants, who are at greatest risk of developing BPD and BPD-PH, are born before 28 weeks and 0 days of gestation (28 + 0), when the rudimentary bronchioles are developing and the first stages of alveolar development can be discerned. Dysregulation of lung development during this vulnerable phase substantially compromises gas exchange, so that extensive and prolonged intensive care interventions such as mechanical ventilation and oxygen supplementation are required. These necessary and life-saving interventions frequently have adverse effects, which, similar to the pathogenetic triggers, include the induction of a pulmonary inflammatory response. The resultant inflammatory milieu has been linked to disruption of alveolar and pulmonary vascular development (Fig. 1) (Benjamin et al., 2010; De Paepe et al., 2010). Such disruption leads to a reduction in the cross-sectional area of the pulmonary vasculature, and thus to a higher than normal pulmonary vascular resistance (PVR) and to an increase in pulmonary arterial pressure (Fig. 1) (Hislop and Haworth 1990; Coalson 2003; Khemani et al., 2007).

Maturation of the pulmonary vasculature and the lung parenchyma occurs concurrently. The proximal arteries develop via vasculogenesis (de-novo synthesis of vessels from mesoderm-derived haemangioblasts), whereas the pre-acinar arteries develop via angiogenesis (the growth of new capillary blood vessels from existing vasculature) (Hislop 2002). Dysregulation of the processes underlying blood vessel growth is thought to be an early contributing factor to the development of PH (Fig. 1) (Budhiraja et al., 2004). Vascular endothelial growth factor (VEGF) is an important regulator of angiogenesis and vasculogenesis, mediating normal development of the pulmonary vasculature (Hislop 2002; Baker et al., 2014). Nitric oxide (NO) has also been implicated in normal vascularisation, playing important roles in stimulating endothelial proliferation through the VEGF-NO pathway (Hislop 2005; Baker et al., 2014). Disruption of the VEGF and/or NO pathways leads to impairment of pulmonary microvascular and alveolar formation and has been implicated in the pathogenesis of BPD-PH (Baker et al., 2014).

Such dysregulation of the growth of the pulmonary vasculature as well as the lung parenchyma results not only in a reduced surface area available for gas exchange, but also in an abnormal architecture of the lung parenchyma where gas exchange occurs. These abnormalities give rise to ventilation-perfusion mismatch, which exacerbates hypoxaemia and hypercapnia and contributes to an increase in PVR via hypoxic vasoconstriction (Fig. 1) (Aaronson et al., 2006). Moreover, hypoxia, hyperoxia and mechanical ventilation aggravate pulmonary inflammation, which in turn may precipitate bronchial smooth muscle and airway hyper-responsiveness (Hislop 2002; Royce et al., 2016). Such hyper-responsiveness in combination with increased pulmonary blood pressure promotes remodelling of the pulmonary vasculature (Fig. 1). If not prevented from progressing, such remodelling may become irreversible and lead to long-term dependence on respiratory support, right heart failure and death (Khemani et al., 2007; Farquhar and Fitzgerald 2010; Bhat et al., 2012).

3.3. Diagnosis of BPD-PH

Cardiac catheterisation is the gold standard for diagnosing PH, but its invasiveness, the difficulty of performing it in tiny patients, and its limited availability, explain why it is rarely used in preterm infants (Revanna et al., 2016). Instead, non-invasive echocardiography usually is the diagnostic tool of choice (Kim 2010; Revanna et al., 2016). Although echocardiography relies upon skilled clinicians to perform the examination and evaluate the results, a study showed that echocardiography correctly diagnosed PH in children under the age of two years in 79% of cases (Mourani et al., 2008).

Echocardiography uses ultrasound to image the heart and the large vessels and Doppler-based methods to obtain estimates of pressures (Bhat et al., 2012). In simple terms, measurement of the tricuspid regurgitant jet velocity (TRJV) can be used to estimate systolic pulmonary artery pressure. A TRJV of 2.8 m/s is equivalent to 36 mmHg, i.e. the upper limit of normal (Lau et al., 2011; Parasuraman et al., 2016). However, despite TRJV being the most commonly used echocardiography diagnostic parameter of PH (Berkelhamer et al., 2013), it is only measurable in 31–61% of infants with BPD and suspected PH, and absence of a measureable TRJV does not necessarily rule out PH (Mourani et al., 2008; Revanna et al., 2016). When the TRJV cannot be measured, additional echocardiographic variables such as right atrial enlargement, RV hypertrophy and/or dilatation, septal flattening during systole, left ventricular eccentricity index (LV sEI) and the time to peak velocity/right ventricular ejection time ratio (TPV/RVET) have been shown to be useful indicators of PH (King et al., 1983; Kim 2010; Ajami et al., 2011; Howard et al., 2012; Revanna et al., 2016).

3.4. Treatment of BPD-PH

As for BPD alone, there is currently no safe and effective treatment for BPD-PH. Besides therapeutic strategies aimed directly at BPD-PH,

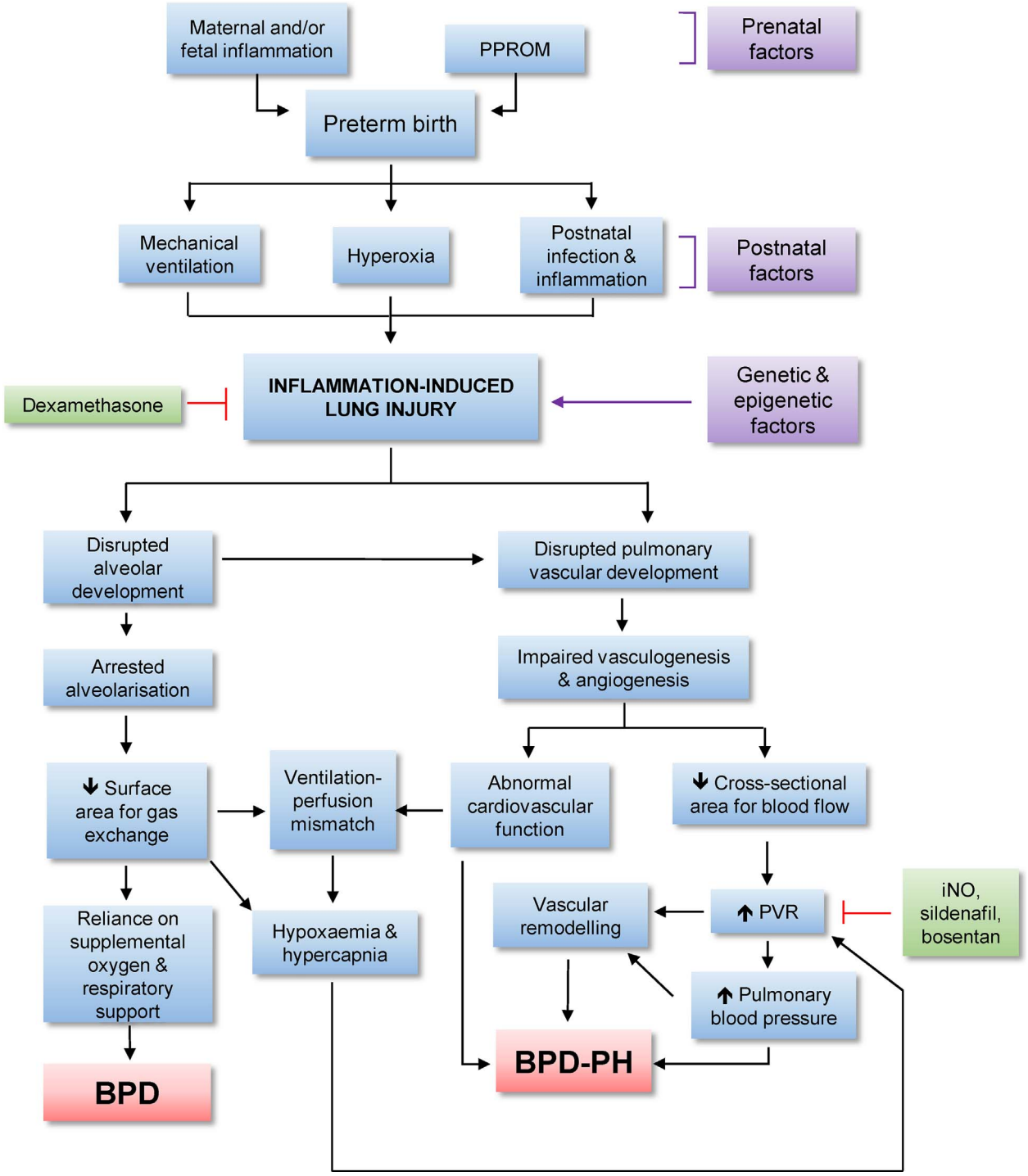

Fig. 1. Pathogenesis of BPD and BPD-PH.
Prenatal and postnatal events as well as genetic and epigenetic factors (purple headings) each precipitate injurious pulmonary inflammation in the preterm infant. Subsequently, development of the lung, i.e. of alveoli and blood vessels, is disrupted, resulting in turn in BPD and/or BPD-PH (red). Key events in the pathogenetic cascades are shown in the blue boxes. Exemplary therapeutic approaches currently used to treat BPD and PH (green) include: dexamethasone, a non-specific anti-inflammatory corticosteroid; iNO, a selective pulmonary vasodilator; sildenafil, a selective PDE_5 inhibitor; and bosentan, non-selective ET-1 receptor antagonist. BPD, bronchopulmonary dysplasia; iNO, inhaled nitric oxide; PH, pulmonary hypertension; PPROM, preterm premature rupture of membranes; PVR, pulmonary vascular resistance.

this section discusses BPD-focused therapies that may affect the course of BPD-PH.

3.4.1. *Corticosteroids*

Despite the critical role of inflammation in the pathogenesis of BPD and BPD-PH, the only anti-inflammatory drugs in clinical use to directly target BPD in preterm infants are corticosteroids. Dexamethasone, the best-studied corticosteroid, is a potent, non-specific inhibitor of inflammation that ameliorates BPD (Fig. 1) (Doyle et al., 2010a, 2010b). While dexamethasone can thus be considered effective, it has severe short-term (e.g. arterial hypertension, gastrointestinal perforation) as well as long-term adverse effects (e.g. neurodevelopmental abnormalities, in particular cerebral palsy) (Shinwell et al., 2000; Doyle et al., 2010a, 2010b). An additional drawback of glucocorticoids in preterm

infants is that they inhibit alveolar growth (Vyas and Kotecha 1997), the very pathway essential for permanent healing of BPD. Thus, dexamethasone cannot be recommended for routine therapy, but is reserved for life-threatening pulmonary failure in the setting of BPD or evolving BPD (Doyle et al., 2010a, 2010b).

3.4.2. Surfactant, caffeine and diuretics

Other drugs in clinical use to prevent or treat BPD or evolving BPD include exogenous surfactant, caffeine and diuretics. Surfactant is usually administered within the first 2 days after birth. When its use became widespread in the 1980s and 1990s, there was hope that surfactant would render BPD preventable, but unfortunately surfactant administration only moderately reduces the risk of BPD or death (RR 0.83) (Bahadue and Soll 2012). Caffeine also provides only moderate benefits, and the evidence for diuretics in BPD is conflicting at best (Schmidt et al., 2006). No effects for these drugs on BPD-PH have been described (Berkelhamer et al., 2013).

3.4.3. Inhaled nitric oxide (iNO)

Pioneered by Abman and Kinsella (Kinsella et al., 2006), today iNO is frequently used in infants to reduce acute increases in pulmonary arterial pressure (Fig. 1) (Banks et al., 1999; Mourani et al., 2004). NO is a potent vasodilator, which induces cyclic GMP (cGMP) and its second messenger cascades to promote vascular smooth muscle relaxation (Thomae et al., 1995; Dweik 2002).

Inhaled NO as a therapy was reported to be effective in reducing the incidence of BPD in infants born before 32 weeks of gestation with a birth weight < 1250 g when administered within the first 7–21 days of life (Ballard et al., 2006). However, more recent trials showed that iNO has limited or no efficacy for preventing BPD (Mourani et al., 2008; Donohue et al., 2011; Kinsella et al., 2014), and a systematic review of several randomised controlled trials found that the incidence of BPD at 36 weeks cGA or death was reduced by only 7% in preterm infants treated with iNO (Donohue et al., 2011). Thus, although iNO has a modest benefit in BPD-PH and does not adversely affect neurodevelopment (Walsh et al., 2010; Kinsella et al., 2014), the need to intubate patients and the high cost of delivering the treatment argue against its use.

3.4.4. Sildenafil

Sildenafil is a selective phosphodiesterase type 5 (PDE_5) inhibitor which induces NO-mediated vascular relaxation and suppresses smooth muscle proliferation (Fig. 1) (Samiee-Zafarghandy et al., 2014). The efficacy of sildenafil to ameliorate idiopathic PH is proven, and the drug is currently used in children and adults for this purpose; however, it is less clear whether sildenafil is safe and efficacious in infants with BPD-PH (Herbert and Tulloh 2016). In 2013, the U.S. Food and Drug Administration (FDA) cautioned against the use of sildenafil in children with PH in light of the increases in mortality observed in children receiving high doses (Barst et al., 2012; Wardle and Tulloh 2013). Despite a lack of high quality evidence and regulatory approval, sildenafil is used off-license as a treatment of BPD-PH (Wardle and Tulloh 2013). For example, a retrospective study involving sildenafil administration to 22 infants with a diagnosis of BPD, a mean GA of 25 + 4 and a birth weight of 631 g (Tan et al., 2015), reported a significant improvement in echocardiographic markers of PH 27.5 days post initiation of treatment. Indeed, two thirds of the cohort showed a ≥ 20% decline in RV systolic pressure and the FiO_2 decreased significantly from 0.57 to 0.42 ($P = 0.02$).

Animal data are also encouraging: In newborn rats, sildenafil promoted adequate lung angiogenesis, decreased PVR, RV hypertrophy and arterial medial wall thickness, and moreover reduced the pulmonary inflammatory response and attenuated airway reactivity and reduced fibrin deposition (Ladha et al., 2005; De Visser et al., 2009).

3.4.5. Bosentan

Bosentan, a non-selective antagonist of the endothelin-1 (ET-1) receptors ET-A and ET-B, has potent vasodilatory effects, thus reducing pulmonary vascular resistance (Fig. 1) (Williamson et al., 2000; Nakwan et al., 2009). ET-1 is a potent vasoconstrictor and promotes endothelial cell dysfunction, inflammation and fibrosis (Yanagisawa et al., 1988; Filep et al., 1995). Bosentan is widely and effectively used to treat PH in adult patients. However, evidence for the efficacy of bosentan therapy for BPD-PH is limited, and importantly, long-term studies are lacking (Rubin et al., 2002; Nakwan et al., 2009; Kim 2010; Berkelhamer et al., 2013).

4. Predictors of BPD-PH

As the lung injury that ultimately leads to BPD and PH commences with preterm birth, it is intuitive that interventions should be administered as early as possible so that pathogenetic cascades can be prevented before irreversible damage is done. This concept was endorsed by the NIH (National Institutes of Health) workshop on BPD (Walsh et al., 2006), and we have published evidence in mouse models that early therapeutic intervention promises success whereas late intervention is far less effective (Nold et al., 2013; Rudloff et al., 2017). Therefore, identifying biomarkers that predict an infant's risk of developing BPD-PH is one of the important unmet challenges in this field. Effective biomarkers for BPD and/or BPD-PH would be of great value to treating clinicians who could use them to ensure that potential therapies are given only to patients in need, sparing other babies a treatment from which they will not benefit (McEvoy et al., 2014). Others have reviewed potential predictive biomarkers in BPD (Bose et al., 2008; Bhandari and Bhandari 2013; Zhang et al., 2014; Rivera et al., 2016); thus, this review focuses on BPD-PH.

4.1. Predictive capacity of echocardiography

Whether there is an association between early signs of pulmonary vascular disease and the development of BPD or BPD-PH was recently assessed in 277 infants by comparing echocardiograms performed at day 7 of postnatal age and at 36 weeks corrected GA (Mourani et al., 2015). The parameters measured and used to diagnose PH in the infants included estimated RV systolic pressure by TRJV, cardiac shunt and ventricular septal wall flattening. In this cohort, early PH (day 7) was identified in 42%, and late PH (36 weeks) was found in 14% of infants. Of the 115 infants with early PH, 24 (21%) were diagnosed with PH at 36 weeks cGA and they had an increased risk of developing BPD. More importantly, infants without evidence of PH at day 7 were unlikely to have PH at 36 weeks cGA (negative predictive value 91%). The authors suggest that early echocardiograms at day 7 of life can be used to identify infants at high risk for BPD and late PH. These findings corroborate an earlier study on 145 preterm infants; in that cohort, PH was detected at 4–6 weeks of age in 9 out of the 26 babies (35%) who presented with PH at 36 weeks cGA (Bhat et al., 2012). Thus, early signs of PH can be detected by echocardiography in approximately one third of infants in at-risk populations.

In addition to the parameters described above, recognition of the incidence and magnitude of RV dysfunction, which is now possible due to advances in ultrasound technology, may contribute to BPD-PH prediction. For example, RV function was assessed in extremely preterm infants with severe BPD at 36 weeks cGA to ascertain correlation with respiratory outcomes (Sehgal et al., 2016). This prospective study measured 2D fractional area change (FAC), M-mode tricuspid annular plane systolic excursion, tissue Doppler velocities and the Tei index, all parameters that are used to assess RV function. Higher mitral tissue Doppler E/E' (which estimates left atrial filling pressure and is indicative of diastolic dysfunction) and lower FAC (which indicates decreased RV function) showed strong correlations (both $r = 0.8$, $P < 0.0001$) with the duration of subsequent respiratory support. On

comparison with infants with no BPD, tissue Doppler imaging (TDI) systolic velocities and RV FAC were significantly lower.

B-type natriuretic peptide (BNP) is a mediator released by cardiomyocytes in response to ventricular stretch and thus has been used as a surrogate marker of ventricular dysfunction (De Lemos et al., 2003). The abundance of BNP in the serum of adult PH patients was shown to correlate with PH severity and to predict survival (Fijalkowska et al., 2006). In a study involving 36 extremely low birth weight infants with BPD-PH, peak serum BNP was lower in infants that survived in comparison to those that did not (128 vs 997 pg/ml; $P < 0.004$) (Cuna et al., 2014). These findings suggest that serum BNP could predict mortality in extremely low birth weight infants with BPD and BPD-PH. Combining serum BNP measurement with echocardiography at 36 weeks cGA in 83 extremely preterm infants diagnosed with BPD, König et al. showed that serum BNP was significantly higher if a TRJV was detectable than when this was not the case (55 vs 41 pg/ml; $P = 0.043$) (König et al., 2016). In addition, serum BNP was significantly higher in babies with severe BPD than in infants without severe BPD (57 vs 39 pg/ml; $P = 0.02$). The difference in median serum BNP in infants with BPD-PH between the two studies may be related to the time point of BNP serum analysis: Cuna et al. considered the highest serum BNP abundance for each infant during the duration of its hospitalisation, whereas König and colleagues investigated serum BNP at only one time point of 36 weeks cGA.

Thus, echocardiograms performed earlier than 36 weeks cGA may be able to identify infants at risk of developing BPD-PH. Assessment of RV dysfunction by mitral tissue Doppler E/E', FAC and TDI systolic velocities may also be useful in BPD-PH prediction. Furthermore, in combination with echocardiography or alone, BNP emerges as a promising biomarker for BPD-PH, although additional studies in larger cohorts are necessary to determine the ideal timing of sampling and the threshold BNP serum abundance.

4.2. Markers of oxygen toxicity

The vital supplementation of oxygen for preterm infants comes at the price of oxygen toxicity, which has been linked to the pathogenesis of BPD and BPD-PH (Wedgwood and Steinhorn 2014). Exposure of a preterm infant to hyperoxia results in an inflammatory response in the immature lung, and the production of reactive oxygen species (ROS) by infiltrating macrophages and neutrophils leads to a marked increase in cell death as well as pulmonary airway remodelling (Bhandari and Bhandari 2013). In the preterm infant, tissue-damaging free radicals can disrupt postnatal alveolar development and exacerbate pulmonary inflammation (Madurga et al., 2013).

One such free radical is the superoxide anion, which causes oxidative injury to DNA, proteins and lipids of lung cells, and results in the formation of isoprostanes (O'Donovan and Fernandes 2004). F_2-isoprostanes in particular have been used as surrogate markers of oxidative stress in adult patients with pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and asthma, as well as in idiopathic PH (Pratico et al., 1998; Montuschi et al., 1999; Cracowski et al., 2001). In a study of 83 mechanically ventilated infants with a bodyweight of 0.5–1 kg, it was shown that plasma abundance of F_2-isoprostanes was increased by approximately 40% comparing infants that subsequently went on to develop BPD with those that did not (from 35 to 50 pg/ml on day 3 of life, and from 40 to 57 pg/ml on day 7; $P = 0.001$) (Ahola et al., 2004). In contrast, another study of 40 babies born at less than 30 weeks GA found no correlation between urinary F_2-isoprostanes measured in the first and third postnatal week and the subsequent development of BPD (Reuter et al., 2007). In a smaller study, plasma F_2-isoprostane was 25% lower in patients with BPD-PH (n = 12, 15 pg/ml) when compared to control patients (n = 18, 20 pg/ml; $P < 0.02$) (Vera et al., 2012). It is important to note that this smaller study was conducted in children between the ages of 1–16 years, with a median age of 2 for patients with BPD-PH. Due to the ages assessed in this study, no conclusion can be drawn on the predictive utility of F_2-isoprostanes in the perinatal and postnatal periods when inflammation wreaks its damage on the preterm lung, and when intervention is most likely to be successful. Of interest, however, F_2-isoprostanes were elevated in patients with paediatric idiopathic PH when compared to control and BPD-PH patients. The authors concluded that these results may highlight differences in the pathogenic mechanisms between the two diseases (Vera et al., 2012).

In summary, the three studies performed to date suggest diverging conclusions regarding the correlation between F_2-isoprostanes and BPD-PH, which may be due to differences in patient age and biological fluid tested. Further research into the potential of F_2-isoprostanes as a predictive marker for BPD-PH is thus warranted.

4.3. Markers of angiogenesis

Pulmonary dysangiogenesis as a result of BPD is likely to contribute to the development of BPD-PH. As in other organs, normal angiogenesis in the lung involves the interplay between pro- and anti-angiogenic factors, and there is evidence that inflammation disrupts this balance (Thebaud 2007). Serum abundance of angiopoietin-1 (Ang-1), a pro-angiogenic factor, and endostatin (ES), an anti-angiogenic mediator, were measured in 318 infants of less than 30 weeks GA or a birth weight of less than 1250 g (Kim and Kim 2014). Thirty-seven of these infants developed severe BPD, of whom 15 were subsequently also diagnosed with BPD-PH. There was a significant increase in the serum ES:Ang-1 ratio on postnatal day 7 in infants who went on to develop severe BPD and BPD-PH in comparison to infants who subsequently developed BPD but not PH (from a ratio of 19 in BPD without PH to 62 in BPD-PH patients; $P < 0.05$) (Kim and Kim 2014). The authors infer that this association may indicate impaired angiogenesis and that the ES:Ang-1 ratio may be a promising biomarker for early PH-risk prediction in preterm infants with severe BPD.

4.4. Genetic biomarkers

Based on twin research that suggested BPD to have a genetic basis (Bhandari et al., 2006; Lavoie et al., 2008), an increasing number of studies have since focused on the identification of genes associated with the risk to develop BPD, for example through candidate gene approaches, genome wide association studies (GWAS), identification of disease-related single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) (Hadchouel et al., 2011; Elhawary et al., 2013; Shaw and O'Brodovich 2013; Hoffmann et al., 2014; Wang et al., 2014; Carrera et al., 2015; Li et al., 2015; Poggi et al., 2015; Petersen et al., 2016; Piersigilli and Bhandari 2016). The results of these efforts are summarised in several reviews (McEvoy et al., 2014; Lal and Ambalavanan 2015; Yu et al., 2016). Whereas some GWAS failed to identify SNPs associated with BPD (Wang et al., 2013), others have identified polymorphisms that may be protective against BPD (see (Lal and Ambalavanan 2015)). An interesting postulation from these studies is that severe BPD could have a different genetic basis than mild/moderate BPD (Ambalavanan et al., 2015).

While paediatric PH not associated with BPD has known genetic associations, and genetic testing for several PH-associated genes has been recommended for children with PH of unknown cause (Hansmann et al., 2016), less is known about possible genetic contributions to the development of BPD-PH. However, polymorphisms in the *DDHA1* (dimethylarginine dimethylaminohydrolase) and *ARG1* (arginase 1) gene have been described as protective against BPD-PH (Trittmann et al., 2014; Trittmann et al., 2016). For example, one of 17 SNPs in the *ARG1* gene (rs2781666) was less common in a group of preterm infants whose odds of PH were 43% less than expected for their age (Trittmann et al., 2014); also, of 36 SNPs examined in BPD patients in the *DDHA1* and *DDHA2* genes, one SNP in the *DDHA1* gene (rs480414) was 92% sensitive and 53% specific for the presence of PH and decreased the risk of

PH (odds ratio of 0.39) (Trittmann et al., 2016). As DDHA and Arg-1 are part of arginine- and NO-related metabolic pathways, they are of marked interest. While human data are few, animal research has identified several mediators involved in the disruption of vascular remodelling associated with BPD-PH, including phosphodiesterase type 5 (PDE5), β-catenin, VEGF receptor 2 (VEGFR2), extracellular superoxide dismutase (EC-SOD), and endothelial nitric oxide synthase (eNOS) (Alapati et al., 2014; Delaney et al., 2015; Heilman et al., 2015).

Variations in genes that display such associations are of interest and recent advances in exome and genome sequencing are likely to be productive. If genetic variations associated with BPD-PH can be identified, verification of results in replication cohorts and prospective studies to establish sensitivity, specificity and predictive values of gene variations of interest would be needed to translate the knowledge gained into clinical practice.

4.5. Epigenetic biomarkers

Epigenetic mechanisms allow for modulation of gene expression in response to the environment without change in gene sequence. Many environmental stressors, such as inflammation, hypoxic and hyperoxic stress and ventilator-associated trauma, are likely to affect epigenetic gene regulation in preterm babies at risk for BPD and BPD-PH; therefore, exploration of epigenetics is highly relevant to the study of the environmental and developmental origins of BPD and BPD-PH.

The main epigenetic mechanisms are histone modification, micro-RNAs and DNA methylation. The role of epigenetic mechanisms in lung remodelling (Hagood 2014) and gene expression in the lung (Yang and Schwartz 2011) has been reviewed by others. While studies have investigated the role of epigenetics in the development of pulmonary fibrosis, asthma and chronic obstructive disease (Hagood 2014), studies in BPD and BPD-PH have been restricted to experimental models rather than humans.

Mediators of interest identified through animal models are methyl CpG-binding protein 2 (MeCP2) and histone deacetylases (HDAC1, HDAC2), which are epigenetic modifiers of gene expression; as well as peroxisome proliferator-activated receptor gamma (PPARγ), insulin like growth factor 1 (IGF-1) and its receptor (IGF-1R) (Joss-Moore et al., 2011; Hagood 2014), which are associated with cellular signalling pertinent to vascular tone and remodelling (Rabinovitch 2010; Yang et al., 2015). In fact, it has been postulated that epigenetic regulation of IGF-1/IGF-1R may be involved in neonatal PH (Madonna et al., 2015). The emerging role of micro-RNAs as epigenetic mediators of PH has also been emphasised (Gamen et al., 2016; Huston and Ryan 2016). Further investigation into the impact of histone modification, micro-RNAs and DNA methylation on the development of BDP-PH is thus of interest.

Due to relatively easy access to white blood cells, DNA methylation patterns of white blood cell-derived genomic DNA would be well suited to identify epigenetic biomarkers of potential clinical utility. Recently developed array technologies require only small amounts of DNA and have thus allowed genome wide and epigenetic studies in preterm babies. For example, methylation of blood-derived DNA of preterm babies shows differences to term infants for thousands of CpG dinucleotide sites (Cruickshank et al., 2013). Another study reported that a subgroup of DNA methylation sites of blood-derived DNA can be utilised to determine a baby's gestational age, and that DNA methylation patterns "mature" in preterm babies between preterm birth and term-equivalent age (Knight et al., 2016). Moreover, DNA methylation of thousands of markers changes in preterm babies exposed to neonatal intensive care interventions after only 7 days (Theda, personal communication). These findings indicate that developmental as well as environmental modifications occur, even in short time periods. Thus, further studies should explore potential utility of epigenetic changes as biomarkers for the development of BPD and BPD-PH.

5. Conclusion

BPD and BPD-PH are common scourges of infants born prematurely that are currently neither preventable nor treatable. Both diseases are driven by incompletely characterised pulmonary inflammation, thus anti-inflammatory treatment approaches hold promise; and in fact, this promise is illustrated by preclinical studies (not reviewed here) in which disease severity was reduced using strategies to curb inflammation. Recognising that inflammatory responses begin to wreak their damage right from birth, anti-inflammatory treatments must commence early in order to be effective. Early identification of patients at risk of developing BPD and/or BPD-PH is therefore critical; accordingly, diagnostic biomarkers that predict the risk of individual patients to develop BPD and/or BPD-PH represent an important unmet medical need. Despite attempts to identify biomarkers for prodromal signs to enable early diagnosis, efforts have not yet yielded robust biomarkers. In this review, we considered markers of oxidative stress and angiogenesis as well as physiological/echocardiographic, genetic and epigenetic markers that have demonstrated some potential to predict BPD-PH. We conclude that progress in conquering BPD and BPD-PH awaits identification of reliable and sensitive disease predictors, as well as the discovery of new anti-inflammatory treatments that ameliorate or even prevent these devastating diseases.

Conflict of interest

The authors declare no conflict of interest.

Authorship statement

All authors were involved in drafting the review or revising it critically for important intellectual content, and all authors approved the final version to be published.

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References

Aaronson, P.I., et al., 2006. Hypoxic pulmonary vasoconstriction: mechanisms and controversies. J. Physiol. 570, 53–58.
Abman, S.H., et al., 2015. Pediatric pulmonary hypertension: guidelines from the american heart association and american thoracic society. Circulation 132, 2037–2099.
Abman, S.H., 1999. New developments in the pathogenesis and treatment of neonatal pulmonary hypertension. Pediatr. Pulmonol. Suppl. 18, 201–204.
Abman, S.H., 2011. Pulmonary vascular disease and bronchopulmonary dysplasia: evaluation and treatment of pulmonary hypertension. Neoreviews 12, 645–651.
Ahola, T., et al., 2004. Plasma 8-isoprostane is increased in preterm infants who develop bronchopulmonary dysplasia or periventricular leukomalacia. Pediatr. Res. 56, 88–93.
Ajami, G.H., et al., 2011. Accuracy of doppler-derived estimation of pulmonary vascular resistance in congenital heart disease: an index of operability. Pediatr. Cardiol. 32, 1168–1174.
Al-Ghanem, G., et al., 2017. Bronchopulmonary dysplasia and pulmonary hypertension: a meta-analysis. J. Perinatol. 37, 414–419.
Alapati, D., et al., 2014. Inhibition of beta-catenin signaling improves alveolarization and reduces pulmonary hypertension in experimental bronchopulmonary dysplasia. Am. J. Respir. Cell Mol. Biol. 51, 104–113.
Ali, Z., et al., 2013. Predictors of bronchopulmonary dysplasia and pulmonary hypertension in newborn children. Dan. Med. J. 60, A4688.
Ambalavanan, N., et al., 2015. Integrated genomic analyses in bronchopulmonary dysplasia. J. Pediatr. 166, 531–537 (e13).
An, H.S., et al., 2010. Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. Korean Circ. J. 40, 131–136.

Bahadue, F.L., Soll, R., 2012. Early versus delayed selective surfactant treatment for neonatal respiratory distress syndrome. Cochrane Database Syst. Rev. 11, CD001456.
Baker, C.D., et al., 2014. Pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. Pediatr. Allergy Immunol. Pulmonol. 27, 8–16.
Ballard, R.A., et al., 2006. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. N. Engl. J. Med. 355, 343–353.
Banks, B.A., et al., 1999. Changes in oxygenation with inhaled nitric oxide in severe bronchopulmonary dysplasia. Pediatrics 103, 610–618.
Barst, R.J., et al., 2012. A randomized, double-blind, placebo-controlled, dose-ranging study of oral sildenafil citrate in treatment-naive children with pulmonary arterial hypertension. Circulation 125, 324–334.
Benjamin, J.T., et al., 2010. Nf-kappab activation limits airway branching through inhibition of sp1-mediated fibroblast growth factor-10 expression. J. Immunol. 185, 4896–4903.
Berkelhamer, S.K., et al., 2013. Pulmonary hypertension in bronchopulmonary dysplasia. Semin. Perinatol. 37, 124–131.
Bhandari, A., Bhandari, V., 2013. Biomarkers in bronchopulmonary dysplasia. Paediatr. Respir. Rev. 14, 173–179.
Bhandari, V., et al., 2006. Familial and genetic susceptibility to major neonatal morbidities in preterm twins. Pediatrics 117, 1901–1906.
Bhat, R., et al., 2012. Prospective analysis of pulmonary hypertension in extremely low birth weight infants. Pediatrics 129, e682–9.
Bose, C.L., et al., 2008. Bronchopulmonary dysplasia and inflammatory biomarkers in the premature neonate. Arch. Dis. Child. Fetal Neonatal Ed. 93, F455–61.
Budhiraja, R., et al., 2004. Endothelial dysfunction in pulmonary hypertension. Circulation 109, 159–165.
Carrera, P., et al., 2015. Exome sequencing and pathway analysis for identification of genetic variability relevant for bronchopulmonary dysplasia (bpd) in preterm newborns: a pilot study. Clin. Chim. Acta 451, 39–45.
Cerro, M.J., et al., 2011. A consensus approach to the classification of pediatric pulmonary hypertensive vascular disease Report from the pvri pediatric taskforce, panama 2011. Pulm Circ. 1, 286–298.
Chow, S.S.W., 2014. Report of the Australian and New Zealand Neonatal Network 2012. ANZNN.
Coalson, J.J., 2003. Pathology of new bronchopulmonary dysplasia. Semin. Neonatol.: SN 8, 73–81.
Cracowski, J.L., et al., 2001. Increased lipid peroxidation in patients with pulmonary hypertension. Am. J. Respir. Crit. Care Med. 164, 1038–1042.
Cruickshank, M.N., et al., 2013. Analysis of epigenetic changes in survivors of preterm birth reveals the effect of gestational age and evidence for a long term legacy. Genom. Med. 5, 96.
Cuna, A., et al., 2014. B-type natriuretic peptide and mortality in extremely low birth weight infants with pulmonary hypertension: a retrospective cohort analysis. BMC Pediatr. 14, 68.
De Lemos, J.A., et al., 2003. B-type natriuretic peptide in cardiovascular disease. Lancet 362, 316–322.
De Paepe, M.E., et al., 2010. Angiogenesis-related gene expression profiling in ventilated preterm human lungs. Exp. Lung Res. 36, 399–410.
De Visser, Y.P., et al., 2009. Sildenafil attenuates pulmonary inflammation and fibrin deposition, mortality and right ventricular hypertrophy in neonatal hyperoxic lung injury. Respir. Res. 10, 30.
Delaney, C., et al., 2015. Lack of ec-sod worsens alveolar and vascular development in a neonatal mouse model of bleomycin-induced bronchopulmonary dysplasia and pulmonary hypertension. Pediatr. Res. 78, 634–640.
Donohue, P.K., et al., 2011. Inhaled nitric oxide in preterm infants: a systematic review. Pediatrics 127, e414–22.
Doyle, L.W., et al., 2010a. Dexamethasone treatment after the first week of life for bronchopulmonary dysplasia in preterm infants: a systematic review. Neonatology 98, 289–296.
Doyle, L.W., et al., 2010b. Dexamethasone treatment in the first week of life for preventing bronchopulmonary dysplasia in preterm infants: a systematic review. Neonatology 98, 217–224.
Doyle, L.W., et al., 2017. Increasing airway obstruction from 8 to 18 years in extremely preterm/low-birthweight survivors born in the surfactant era. Thorax 72, 712–719.
Dweik, R.A., 2002. Pulmonary hypertension and the search for the selective pulmonary vasodilator. Lancet 360, 886–887.
Elhawary, N.A., et al., 2013. Tnf-238 polymorphism may predict bronchopulmonary dysplasia among preterm infants in the Egyptian population. Pediatr. Pulmonol. 48, 699–706.
Farquhar, M., Fitzgerald, D.A., 2010. Pulmonary hypertension in chronic neonatal lung disease. Paediatr. Respir. Rev. 11, 149–153.
Fijalkowska, A., et al., 2006. Serum n-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. Chest 129, 1313–1321.
Filep, J.G., et al., 1995. Acute pro-inflammatory actions of endothelin-1 in the guinea-pig lung: involvement of eta and etb receptors. Br. J. Pharmacol. 115, 227–236.
Gamen, E., et al., 2016. The emerging role of epigenetics in pulmonary hypertension. Eur. Resp. J. 48, 903–917.
Gien, J., Kinsella, J.P., 2011. Pathogenesis and treatment of bronchopulmonary dysplasia. Curr. Opin. Pediatr. 23, 305–313.
Gough, A., et al., 2014. Impaired lung function and health status in adult survivors of bronchopulmonary dysplasia. Eur. Resp. J. 43, 808–816.
Hadchouel, A., et al., 2011. Identification of spock2 as a susceptibility gene for bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. 184, 1164–1170.
Hagood, J.S., 2014. Beyond the genome: epigenetic mechanisms in lung remodeling. Physiology (Bethesda) 29, 177–185.
Hansmann, G., et al., 2016. Executive summary. Expert consensus statement on the diagnosis and treatment of paediatric pulmonary hypertension. The european paediatric pulmonary vascular disease network, endorsed by ishlt and dgpk. Heart 102 (Suppl 2) ii86–100.
Hayes Jr., D., et al., 2010. Pathogenesis of bronchopulmonary dysplasia. Resp. Int. Rev. Thoracic Dis. 79, 425–436.
Heilman, R.P., et al., 2015. Right ventricular cyclic nucleotide signaling is decreased in hyperoxia-induced pulmonary hypertension in neonatal mice. Am. J. Physiol. Heart Circ. Physiol. 308, H1575–82.
Herbert, S., Tulloh, R., 2016. Sildenafil, pulmonary hypertension and bronchopulmonary dysplasia. Early Hum. Dev. 102, 21–24.
Hislop, A.A., Haworth, S.G., 1990. Pulmonary vascular damage and the development of cor pulmonale following hyaline membrane disease. Pediatr. Pulmonol. 9, 152–161.
Hislop, A.A., 2002. Airway and blood vessel interaction during lung development. J. Anat. 201, 325–334.
Hislop, A., 2005. Developmental biology of the pulmonary circulation. Paediatr. Respir. Rev. 6, 35–43.
Hoffmann, T.J., et al., 2014. Copy number variation in bronchopulmonary dysplasia. Am. J. Med. Genet. A 164A, 2672–2675.
Howard, L.S., et al., 2012. Echocardiographic assessment of pulmonary hypertension: standard operating procedure. Eur. Respir. Rev. 21, 239–248.
Huston, J.H., Ryan, J.J., 2016. The emerging role of epigenetics in pulmonary arterial hypertension: an important avenue for clinical trials (2015 grover conference series). Pulm Circ. 6, 274–284.
Jobe, A.H., Bancalari, E., 2001. Bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. 163, 1723–1729.
Jobe, A.H., Steinhorn, R., 2017. Can we define bronchopulmonary dysplasia? J. Pediatr. 188, 19–23.
Joss-Moore, L.A., et al., 2011. Epigenetics and the developmental origins of lung disease. Mol. Genet. Metab. 104, 61–66.
König, K., et al., 2016. Bnp, troponin i, and ykl-40 as screening markers in extremely preterm infants at risk for pulmonary hypertension associated with bronchopulmonary dysplasia. Am. J. Physiol. Lung Cell. Mol. Physiol. 00344, 2016 ajplung.
Khemani, E., et al., 2007. Pulmonary artery hypertension in formerly premature infants with bronchopulmonary dysplasia: clinical features and outcomes in the surfactant era. Pediatrics 120, 1260–1269.
Kim, D.H., Kim, H.S., 2014. Serial changes of serum endostatin and angiopoietin-1 levels in preterm infants with severe bronchopulmonary dysplasia and subsequent pulmonary artery hypertension. Neonatology 106, 55–61.
Kim, D.H., et al., 2012. Risk factors for pulmonary artery hypertension in preterm infants with moderate or severe bronchopulmonary dysplasia. Neonatology 101, 40–46.
Kim, G.B., 2010. Pulmonary hypertension in infants with bronchopulmonary dysplasia. Korean J. Pediatr. 53, 688–693.
King, M.E., et al., 1983. Interventricular septal configuration as a predictor of right ventricular systolic hypertension in children: a cross-sectional echocardiographic study. Circulation 68, 68–75.
Kinsella, J.P., et al., 2006. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. N. Engl. J. Med. 355, 354–364.
Kinsella, J.P., et al., 2014. Noninvasive inhaled nitric oxide does not prevent bronchopulmonary dysplasia in premature newborns. J. Pediatr. 165, 1104–1108 (e1).
Knight, A.K., et al., 2016. An epigenetic clock for gestational age at birth based on blood methylation data. Genome Biol. 17, 206.
Kotecha, S.J., et al., 2013. Effect of preterm birth on later fev1: A systematic review and meta-analysis. Thorax 68, 760–766.
Kwon, H.W., et al., 2016. Long-term outcomes of pulmonary hypertension in preterm infants with bronchopulmonary dysplasia. Neonatology 110, 181–189.
Ladha, F., et al., 2005. Sildenafil improves alveolar growth and pulmonary hypertension in hyperoxia-induced lung injury. Am. J. Respir. Crit. Care Med. 172, 750–756.
Lal, C.V., Ambalavanan, N., 2015. Genetic predisposition to bronchopulmonary dysplasia. Semin. Perinatol. 39, 584–591.
Lau, E.M., et al., 2011. Early detection of pulmonary vascular disease in pulmonary arterial hypertension: time to move forward. Eur. Heart J. 32, 2489–2498.
Lavoie, P.M., et al., 2008. Heritability of bronchopulmonary dysplasia, defined according to the consensus statement of the national institutes of health. Pediatrics 122, 479–485.
Li, J., et al., 2015. Exome sequencing of neonatal blood spots and the identification of genes implicated in bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. 192, 589–596.
Madonna, R., et al., 2015. Epigenetic regulation of insulin-like growth factor signaling: a novel insight into the pathophysiology of neonatal pulmonary hypertension. Vascul. Pharmacol. 73, 4–7.
Madurga, A., et al., 2013. Recent advances in late lung development and the pathogenesis of bronchopulmonary dysplasia. Am. J. Physiol. Lung Cell. Mol. Physiol. 305, L893–905.
Martin, J.A., et al., 2013. Births: final data for 2011. National Vital Statistics Reports: from the Centers for Disease Control and Prevention 62. National Center for Health Statistics, National Vital Statistics System, pp. 1–69 72.
McAleese, K.A., et al., 1993. Financial and emotional cost of bronchopulmonary dysplasia. Clin. Pediatr. (Phila.) 32, 393–400.
McEvoy, C.T., et al., 2014. Bronchopulmonary dysplasia: Nhlbi workshop on the primary prevention of chronic lung diseases. Ann. Am. Thorac. Soc. 11 (Suppl 3), S146–53.
Montuschi, P., et al., 1999. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. Am. J. Respir. Crit. Care Med. 160, 216–220.
Mourani, P.M., et al., 2004. Pulmonary vascular effects of inhaled nitric oxide and oxygen tension in bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. 170, 1006–1013.
Mourani, P.M., et al., 2008. Clinical utility of echocardiography for the diagnosis and

management of pulmonary vascular disease in young children with chronic lung disease. Pediatrics 121, 317–325.
Mourani, P.M., et al., 2015. Early pulmonary vascular disease in preterm infants at risk for bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. 191, 87–95.
Mullassery, D., Smith, N.P., 2015. Lung development. Semin. Pediatr. Surg. 24, 152–155.
Nakwan, N., et al., 2009. Successful treatment of persistent pulmonary hypertension of the newborn with bosentan. Acta Paediatr. 98, 1683–1685.
Nold, M.F., et al., 2013. Interleukin-1 receptor antagonist prevents murine bronchopulmonary dysplasia induced by perinatal inflammation and hyperoxia. Proc. Natl. Acad. Sci. U. S. A. 110, 14384–14389.
O'Donovan, D.J., Fernandes, C.J., 2004. Free radicals and diseases in premature infants. Antioxid. Redox Signal. 6, 169–176.
Parasuraman, S., et al., 2016. Assessment of pulmonary artery pressure by echocardiography-a comprehensive review. Int. J. Cardiol. Heart Vasc. 12, 45–51.
Petersen, J.P., et al., 2016. Ugt1a1*28 genotypes and respiratory disease in very preterm infants. A cohort study. Neonatology 109, 124–129.
Piersigilli, F., Bhandari, V., 2016. Biomarkers in neonatology The new omics of bronchopulmonary dysplasia. J. Matern.-Fetal Neonatal Med. 29, 1758–1764.
Poggi, C., et al., 2015. Genetic contributions to the development of complications in preterm newborns. PLoS One 10, e0131741.
Polglase, G.R., Hooper, S.B., 2006. Role of intra-luminal pressure in regulating pbf in the fetus and after birth. Curr. Pediatr. Rev. 2, 287–299.
Polglase, G.R., et al., 2012. The cardiopulmonary haemodynamic transition at birth is not different between male and female preterm lambs. Reprod. Fertil. Dev. 24, 510–516.
Poon, C.Y., et al., 2013. Long term cardiovascular consequences of chronic lung disease of prematurity. Paediatr. Respir. Rev. 14, 242–249.
Pratico, D., et al., 1998. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane f2alpha-iii, an index of oxidant stress. Am. J. Respir. Crit. Care Med. 158, 1709–1714.
Rabinovitch, M., 2010. Ppargamma and the pathobiology of pulmonary arterial hypertension. Adv. Exp. Med. Biol. 661, 447–458.
Reuter, S.D., et al., 2007. Urinary f2-isoprostanes are poor prognostic indicators for the development of bronchopulmonary dysplasia. J. Perinatol. 27, 303–306.
Revanna, G.K., et al., 2016. Bronchopulmonary dysplasia associated pulmonary hypertension: making the best use of bedside echocardiography. Prog. Pediatr. Cardiol.
Rivera, L., et al., 2016. Biomarkers for bronchopulmonary dysplasia in the preterm infant. Front. Pediatr. 4, 33.
Royce, S.G., et al., 2016. Airway remodeling and hyperreactivity in a model of bronchopulmonary dysplasia and their modulation by il-1ra. Am. J. Respir. Cell Mol. Biol.
Rubin, L.J., et al., 2002. Bosentan therapy for pulmonary arterial hypertension. N. Engl. J. Med. 346, 896–903.
Rudloff, I., et al., 2017. Refining anti-inflammatory therapy strategies for bronchopulmonary dysplasia. J. Cell. Mol. Med. 21, 1128–1138.
Sachdev, A., et al., 2011. Right ventricular strain for prediction of survival in patients with pulmonary arterial hypertension. Chest 139, 1299–1309.
Samiee-Zafarghandy, S., et al., 2014. Safety of sildenafil in infants*. Pediatr. Crit. Care Med. 15, 362–368.
Schmidt, B., et al., 2006. Caffeine therapy for apnea of prematurity. N. Engl. J. Med. 354, 2112–2121.
Sehgal, A., et al., 2016. Right ventricular function in infants with bronchopulmonary dysplasia: association with respiratory sequelae. Neonatology 109, 289–296.
Sharma, V., et al., 2015. Persistent pulmonary hypertension of the newborn. Matern. Health Neonatol Perinatol. 1, 14.
Shaw, G.M., O'Brodovich, H.M., 2013. Progress in understanding the genetics of bronchopulmonary dysplasia. Semin. Perinatol. 37, 85–93.
Shinwell, E.S., et al., 2000. Early postnatal dexamethasone treatment and increased incidence of cerebral palsy. Arch. Dis. Child. Fetal Neonatal Ed. 83, F177–81.
Short, E.J., et al., 2007. Developmental sequelae in preterm infants having a diagnosis of bronchopulmonary dysplasia: analysis using a severity-based classification system. Arch. Pediatr. Adolesc. Med. 161, 1082–1087.
Simonneau, G., et al., 2014. [updated clinical classification of pulmonary hypertension]. Turk. Kardiyol. Dern. Ars. 42 (Suppl 1), 45–54.
Slaughter, J.L., et al., 2011. Echocardiographic detection of pulmonary hypertension in extremely low birth weight infants with bronchopulmonary dysplasia requiring prolonged positive pressure ventilation. J. Perinatol. 31, 635–640.
Speer, C.P., 2006. Inflammation and bronchopulmonary dysplasia: a continuing story. Sem. Fetal Neonatal Med. 11, 354–362.
Steinhorn, R.H., 2010. Neonatal pulmonary hypertension. Pediatr. Crit. Care Med. 11, S79–84.
Stoll, B.J., et al., 2010. Neonatal outcomes of extremely preterm infants from the nichd neonatal research network. Pediatrics 126, 443–456.
Tan, K., et al., 2015. Sildenafil therapy in bronchopulmonary dysplasia-associated pulmonary hypertension: a retrospective study of efficacy and safety. Eur. J. Pediatr. 174, 1109–1115.
Teng, R.J., Wu, T.J., 2013. Persistent pulmonary hypertension of the newborn. J. Formos. Med. Assoc. 112, 177–184.
Thebaud, B., 2007. Angiogenesis in lung development, injury and repair: implications for chronic lung disease of prematurity. Neonatology 91, 291–297.
Thomae, K.R., et al., 1995. The effect of nitric oxide on fetal pulmonary artery smooth muscle growth. J. Surg. Res. 59, 337–343.
Trittmann, J.K., et al., 2014. Arginase i gene single-nucleotide polymorphism is associated with decreased risk of pulmonary hypertension in bronchopulmonary dysplasia. Acta Paediatr. 103, e439–43.
Trittmann, J.K., et al., 2016. A single nucleotide polymorphism in the dimethylarginine dimethylaminohydrolase gene is associated with lower risk of pulmonary hypertension in bronchopulmonary dysplasia. Acta Paediatr. 105, e170–5.
Um-Bergstrom, P., et al., 2017. Lung function development after preterm birth in relation to severity of bronchopulmonary dysplasia. BMC Pulm. Med. 17, 97.
Van Marter, L.J., 2009. Epidemiology of bronchopulmonary dysplasia. Semin. Fetal Neonatal Med. 14, 358–366.
Vera, K.B., et al., 2012. Significant differences in markers of oxidant injury between idiopathic and bronchopulmonary-dysplasia-associated pulmonary hypertension in children. Pulm Med. 2012, 301475.
Vogel, E.R., et al., 2015. Perinatal oxygen in the developing lung. Can. J. Physiol. Pharmacol. 93, 119–127.
Vyas, J., Kotecha, S., 1997. Effects of antenatal and postnatal corticosteroids on the preterm lung. Arch. Dis. Child. Fetal Neonatal Ed. 77, F147–50.
Walsh, M.C., et al., 2003. Safety, reliability, and validity of a physiologic definition of bronchopulmonary dysplasia. J. Perinatol. 23, 451–456.
Walsh, M.C., et al., 2006. Summary proceedings from the bronchopulmonary dysplasia group. Pediatrics 117, S52–6.
Walsh, M.C., et al., 2010. Two-year neurodevelopmental outcomes of ventilated preterm infants treated with inhaled nitric oxide. J. Pediatr. 156, 556–561 e1.
Wang, H., et al., 2013. A genome-wide association study (gwas) for bronchopulmonary dysplasia. Pediatrics 132, 290–297.
Wang, X., et al., 2014. Gstm1 and gstt1 gene polymorphisms as major risk factors for bronchopulmonary dysplasia in a chinese han population. Gene 533, 48–51.
Wardle, A.J., Tulloh, R.M., 2013. Paediatric pulmonary hypertension and sildenafil: current practice and controversies. Arch. Dis. Child. Educ. Pract. Ed. 98, 141–147.
Wedgwood, S., Steinhorn, R.H., 2014. Role of reactive oxygen species in neonatal pulmonary vascular disease. Antioxid. Redox Signal. 21, 1926–1942.
Williamson, D.J., et al., 2000. Hemodynamic effects of bosentan, an endothelin receptor antagonist, in patients with pulmonary hypertension. Circulation 102, 411–418.
Yanagisawa, M., et al., 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332, 411–415.
Yang, I.V., Schwartz, D.A., 2011. Epigenetic control of gene expression in the lung. Am. J. Respir. Crit. Care Med. 183, 1295–1301.
Yang, Q., et al., 2015. Igf-1 signaling in neonatal hypoxia-induced pulmonary hypertension: role of epigenetic regulation. Vascul. Pharmacol. 73, 20–31.
Yu, K.H., et al., 2016. The genetic predisposition to bronchopulmonary dysplasia. Curr. Opin. Pediatr. 28, 318–323.
Zhang, Z.Q., et al., 2014. Early biomarkers as predictors for bronchopulmonary dysplasia in preterm infants: a systematic review. Eur. J. Pediatr. 173, 15–23.