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ORIGINAL ARTICLE

# Bronchopulmonary dysplasia-associated pulmonary hypertension: clues from placental pathology

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**OBJECTIVES:** Bronchopulmonary dysplasia (BPD) and the associated complication of pulmonary hypertension (PH) leads to increased mortality and a longer length of stay among survivors. Placental histopathology may give early clues of subsequent events. The objective was to evaluate the relationship of maternal vascular underperfusion (MVU) changes on placental histopathology with subsequent development of BPD-associated PH in a cohort of extremely premature infants.
**STUDY DESIGN:** In a cohort of preterm infants '⩽28 weeks' gestational age (GA) and with 'severe' BPD, this retrospective study evaluated specific placental histopathological changes and assessed the relationship with subsequent development of PH. 'Severe' BPD was defined as the need for ⩾30% oxygen and/or positive pressure ventilation at 36 weeks postmenstrual age. Placental and echocardiographic assessments were done by investigators masked to the grouping and clinical outcomes.
**RESULTS:** Fifty six infants with severe BPD formed the cohort; PH was noted in 22 (39.3%) infants. The GA of the infants with and without PH was comparable (25.8 ± 1.6 vs 25.8 ± 1.3 weeks, $P = 0.9$). On placental histopathological examination, 13 (23%) had features of MVU. On univariate logistic regression, the presence of changes consistent with MVU increased the relative risk of subsequent BPD-associated PH by 2.75 (95% confidence interval 1.56 to 4.85, $P = 0.004$). The significance persisted after adjustment for GA. Stratification by the presence or absence of fetal growth restriction, yielded nonsignificant associations ($P = 0.17$).
**CONCLUSION:** Based on the results of the present study, specific placental histopathological changes may give early clues to the subsequent development of BPD-associated PH.

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## INTRODUCTION

Bronchopulmonary dysplasia (BPD) is the most common chronic respiratory morbidity in surviving preterm infants, with a reported incidence of ~45% in infants between 22 and 28 weeks gestational age (GA).[1] In the NICHD Neonatal Research Network, the incidence is ~68% in infants < 29 weeks GA and birthweight (BW) between 400 and 1500 g.[2] Recent data from Australia and New Zealand Neonatal Network has noted this to be 54% in extremely preterm infants (⩽25 weeks GA).[3] Pulmonary hypertension (PH) is a known complication of BPD, the incidence increasing with the severity of BPD and noted in >50% cases.[4,5] This association also increased the mortality in infants, who had PH for >4 months.[6] Our own data have noted that among infants born ⩽28 weeks GA and having 'severe' BPD, PH complicated ~40% of them.[7] Early clinical predictors of BPD *per se* include male gender, infection, greater ventilator requirements and its association with patent ductus arteriosus. B-type natriuretic peptide or NT-pro-B-type natriuretic peptide is being increasingly used for evaluation of PH in BPD.[8] However, B-type natriuretic peptide could be elevated in the absence of echocardiographic (ECHO) signs of PH or vice versa, is more a marker of ventricular strain (not specific to the right ventricle (RV)) and may be elevated in infants with patent ductus arteriosus. Placental pathologic findings related to maternal preeclampsia and fetal growth restriction may be early predictors as chronic placental malperfusion during fetal development may induce pulmonary vascular remodelling, an important marker of PH. Maternal vascular underperfusion (MVU) detrimentally affects the fetus.[9–11] Given not all cases of MVU are accompanied by clinically apparent preeclampsia or extremely preterm birth may occur before maternal signs are manifest, it is worth investigating whether MVU could serve as an independent earlier predictor of BPD and PH. The reduced vessel density and abnormal vaso-reactivity of the pulmonary vasculature in infants with BPD provides clues for the development of PH.[12]

BPD is considered a multifactorial disease with involvement of both antenatal and postnatal risk factors.[13,14] The role of chronic placental insufficiency as a contributor to BPD pathogenesis is a relatively understudied pathway.[11] Severe chronic placental insufficiency in fetal sheep resulted in pulmonary artery endothelial dysfunction, a recognized pathophysiology behind PH.[13] Mestan *et al.*[15] noted that placental changes of MVU may identify BPD infants with increased risk for developing PH. In a 5-year retrospective study on premature infants ⩽28 weeks GA who developed BPD (a cohort similar to our study), they showed that placental MVU may identify BPD infants who were exposed to intrauterine hypoxia ischaemia, which increases their risk for development of PH disease subsequently. This clinically relevant information identifies a subset of infants (among a high risk cohort) who may benefit from individualized management.

This study evaluated the relationship of MVU changes on placental histopathology with subsequent development of severe BPD-associated PH in a cohort of extremely premature infants. We hypothesized that in keeping with the recent literature, a significant association between placental histopathology and

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subsequent development of 'severe' BPD-associated PH may be noticed.

## METHODS

This retrospective study was conducted at Monash Children's Hospital, a quaternary centre for premature infants from 23 weeks GA onwards. Annually, ~175 infants with BW $<1500$ g are admitted to the Unit. Obstetric and neonatal data were collected from the Unit electronic database. Preterm infant's $\leqslant 28$ weeks GA and having 'severe' BPD during the period January 2014 to May 2015 were identified from the Unit's electronic database. In infants born $<32$ weeks GA, Australia and New Zealand Neonatal Network defines BPD as lung disease with ongoing requirement for supplemental oxygen therapy or ventilation support (high-flow oxygen, continuous positive airway pressure or mechanical ventilation) at 36 weeks post-menstrual age. Included infants belonged to the 'severe' BPD' category (need for $\geqslant 30\%$ oxygen and/or positive pressure ventilation at 36 weeks postmenstrual age.[16] Infants with syndromic associations, surgical thoracic conditions such as congenital diaphragmatic hernia and congenital heart diseases patent ductus arteriosus were excluded. Archived ECHO images were evaluated by a single investigator, masked to the placental changes or grouping. The infants were divided into two groups; with PH and without PH based on *a priori* previously published criteria on ECHO done at 36 weeks' corrected GA.[7] Briefly, the parameters studied included tricuspid regurgitation (TR) jet maximum velocity, patent ductus arteriosus right to left shunt, pulmonary artery Doppler time velocity relationship, interventricular septum configuration, TR/velocity time integral (VTI) ratio and left ventricular systolic eccentricity index (LVsEI).[17–22] Presence of any of the above criteria indicated PH. Placental histopathology examination was then performed by a histopathologist, masked to the grouping or clinical outcomes of the infants. Although a descriptive histopathology evaluation of all the preterm placentas is performed at the Institution, for this particular cohort, the histopathologist re-examined them, blinded to the case identity. For the characterization of MVU in this cohort, the criteria defined by Redline *et al.*[23] was utilized. The study was approved by the Institutional Ethics Review Committee.

### Statistics

Continuous variables were compared using Student's *t*-test and categorical variables were compared using $\chi^2$- or Fisher's exact tests. Univariate regression analysis was used to determine relative risk and 95% confidence intervals. Regression model was adjusted for GA. All analyses were performed using Stata software version 13.0 (StataCorp LP, College Station, TX, USA). Significance was set at two-tailed $P<0.05$.

## RESULTS

During the study period, 122 infants' $\leqslant 28$ weeks GA at birth were admitted to the Unit. Fifty-six (44%) infants developed 'severe' BPD and were included in the study cohort. Table 1 depicts salient demographics. The GA of infants with and without PH was comparable. Eleven (19%) of the cohort weighed $<10$th centile and were classified as having fetal growth restriction. Table 2 compares the clinical and demographic features of those with and without PH. The incidence of maternal preeclampsia trended higher in the BPD with PH group ($P=0.07$). The total duration of respiratory support in the BPD with PH group was significantly longer compared with those without ($224\pm130$ vs $132\pm82$ days, $P=0.001$). Scans for the assessment of PH were performed in the infants at $36.7\pm2$ weeks GA. At least one parameter indicative of PH was noted in 22 infants (multiple in 14/22); the incidence of BPD-associated PH in the cohort being 22/56 (39.3%). Among the 16 infants with a measurable TR jet, the ratio of TR/VTI from the right ventricular outflow tract (RVOT) was noted to be $0.28\pm0.03$. Table 3 depicts the distribution of ECHO parameters.

In the 22 infants with ECHO evidence of PH, the GA distribution was fairly even: ((23to 24 weeks; 7/15 = 46.6%), 25 to 26 weeks; 8/24 = 33.3%) and 27 to 28 weeks; 7/17 (41%)). On placental histopathological examination masked to the grouping and clinical outcomes, 13 (23%) had features of MVU (Figure 1). These included mural fibrinoid necrosis/atherosis, muscularized basal plate arteries, mural hypertrophy of membrane arterioles, immature intermediate trophoblast and increased placental site giant cells. In logistic regression analysis, the presence of changes consistent with MVU increased the relative risk of subsequent BPD-associated PH by 2.75 (95% confidence intervals 1.56 to 4.85, $P=0.004$). After adjustment for GA, the association still remained significant (2.45 (95% confidence intervals 1.43, 5.01), $P=0.002$). Stratification by the presence (7/11) or absence (4/11) of fetal growth restriction yielded nonsignificant associations ($P=0.17$).

**Table 1.** Demographic features of the study population

| *Variable* | N = *56* |
|---|---|
| GA (weeks) | $26\pm1.4$ |
| BW (g) | $796\pm190$ |
| Male gender *n* (%) | 33 (58) |
| Antenatal steroids coverage *n* (%) | 47 (84) |
| Caesarean *n* (%) | 27 (48) |
| Treatment for patent ductus arteriosus | 28 (50)[a] |
| Sepsis | 3 (5.3) |
| *Respiratory support at 36 weeks corrected GA* | |
| High-frequency ventilation | 3 (5) |
| Nasal continuous positive airway pressure/nasal intermittent | 30 (53) |
| Mandatory ventilation | |
| High flow $>4$ l min$^{-1}$ | 17 (30) |
| Low flow | 6 (11) |

Abbreviations: BW, birthweight; GA, gestational age. Figures in parenthesis represent percentages. [a]Subsequent surgical ligation in one.

**Table 2.** Comparison of clinical and demographic factors

| *Variable* | *BPD-associated PH* (n *22)* | *No PH* (n *34)* | P-*values* |
|---|---|---|---|
| GA (weeks) | $25.8\pm1.6$ | $25.8\pm1.3$ | 0.9 |
| BW (g) | $776.4\pm226$ | $770.2\pm171$ | 0.96 |
| APGAR score at 5 min[a] | 8 (6,9) | 8 (6,10) | 0.8 |
| Lack of antenatal steroid exposure | 4/22 (18%) | 5/34 (14.7%) | 1 |
| Maternal pre-eclampsia | 10 (45.4%) | 7 (20.5%) | 0.07 |
| Culture-proven sepsis | 1/22 (4.5%) | 2/34 (5.8%) | 1 |
| Treatment for patent ductus arteriosus | 10 (45.4%) | 18 (53%) | 0.78 |

Abbreviations: BPD, bronchopulmonary dysplasia; BW, birthweight; GA, gestational age; PH, pulmonary hypertension. [a]Median (range).

## DISCUSSION

Information from placental histopathology and biomarkers has recently been noted as the key to a better understanding of perinatal diseases pathophysiology. This includes the commonest respiratory morbidity affecting premature infants, namely BPD. PH as a complication of 'severe' BPD has gained recent recognition, with implications for survival, duration of stay and overall health care resources. BPD associated PH is associated with significantly higher death rates compared with those without PH.[4,5,24,25] This study noted that specific placental histopathology changes may hold early clues to the subsequent development of BPD-associated PH in preterm infants. Our findings support the role of placental histopathology markers as early indicators of BPD-

**Table 3.** Echocardiographic features of PH (multiple findings in some) $N = 22$

| *Reference* | *Parameter* | *Criteria* | *Prevalence* |
|---|---|---|---|
| 17 | TR[a] | $V_{max} \geqslant 2.8$ m s$^{-1}$ (~36 mm Hg RVSP) | 13 (59%) |
| 18 | TPV/RVETc | $< 0.31$ | 14 (63.6%) |
| 19 | Interventricular septum configuration | Flat or bowed into left ventricle | 14 (63.6%) |
| 20 | Patent ductus arteriosus | ⩾30% Right to left Shunt in cardiac cycle | 0[b] |
| 21 | LVsEI | $> 1.15$ | 12 (54.5%) |
| 22 | TR/VTI[a] | $> 0.14$ | 13 (59%) |

Abbreviations: LVsEI, left ventricular systolic eccentricity index; PH, pulmonary hypertension; RVET, right ventricular ejection time; RVSP, right ventricular systolic pressure; TPV, time to peak velocity; TR, tricuspid regurgitation; VTI, velocity time integral. [a]Total 16 had measurable TR. [b]Patent duct noted in 5 infants.

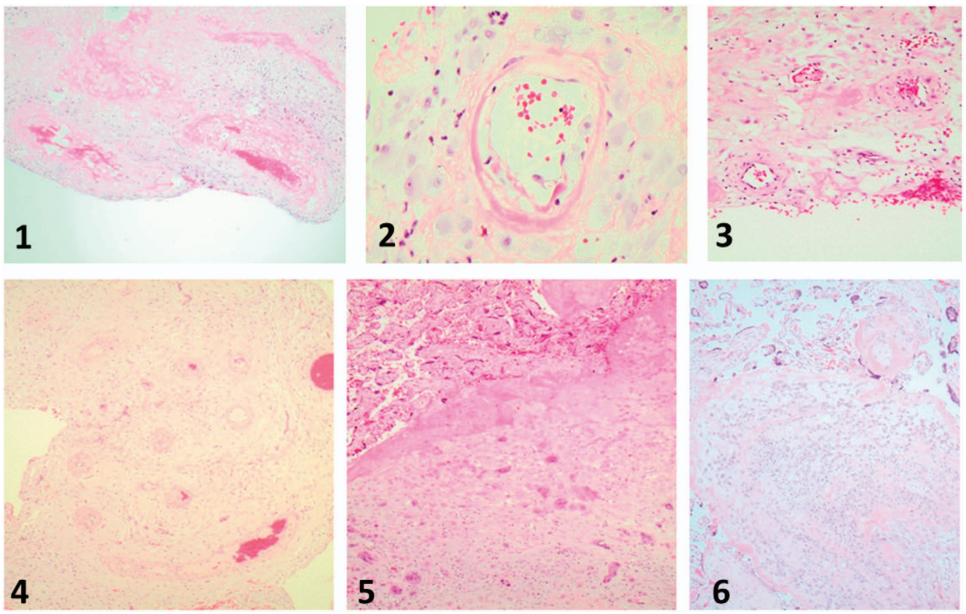


**Figure 1.** Illustration showing salient histopathological characteristics. 1 Mural fibrinoid necrosis. 2 Atherosis. 3 Muscularized basal plate arteries. 4 Mural hypertrophy of membrane arterioles. 5 Increased placental site giant cells. 6 Immature intermediate trophoblasts.

associated PH. Along with providing salient information to the clinician for postnatal management of these premature infants, this adds to the growing evidence of the relevance of chronic placental hypoxia-ischaemia in BPD-associated PH pathogenesis. Whether the premature infant will develop BPD or not, the severity of BPD and the association with PH, is not apparent until several weeks to months after birth. This has guided the search for tangible histopathological and/or receptor level markers, which may provide early indications and potentially be helpful in guiding the medical management principles with the ultimate objective of minimizing (or preventing) BPD.

The pulmonary outcomes associated with MVU in extremely preterm infants are poorly understood and/or under-reported. The characterization of placental lesions seems useful as it has been previously noted that chronic placental vascular mal-perfusion may lead to pulmonary vascular remodelling, the hallmark of subsequent PH disease.[15] Although Redline *et al.*[23] previously reported the association between BPD and placental changes such as fibrinoid necrosis and acute atherosis, the distinction between BPD with and without PH disease was not made. Role of placental histopathology in BPD pathophysiology has been studied in sheep previously. In the presence of chronic fetal hypoxia, it was shown that utero-placental insufficiency leads to the classic histologic lung findings of BPD including pulmonary vascular remodelling.[13] The pathologic vessels were noted to have small lumens (low capacitance) and thick walls (high resistance). The pulmonary vascular components of this model closely resemble the placental histologic description of MVU in human placenta, indicating the role of fetal stressors in adversely affecting pulmonary vascular growth. In a recent longitudinal cohort study on preterm infants, cord blood markers associated with placental MVU as predictors of BPD-associated PH have been studied. Reduced cord blood angiogenic factors (especially placental growth factors and vascular endothelial growth factor-A) were noted as important predictors of BPD-associated PH. Decreased levels of these growth factors correlated with increased MVU severity, indicating the role of delayed feto-placental angiogenesis in the pathophysiology of BPD-associated PH.[26]

Interestingly, it is possible that the vascular architectural changes noted on placental histopathology persist during the post-natal period. Our group has recently examined arterial structure and biomechanical function in a cohort of preterm infants with severe BPD, using a cohort of preterm infants without BPD and a cohort of term infants for comparison. This cohort was born at ⩽28 weeks GA and was evaluated with vascular ultrasound techniques at 36 weeks corrected GA. The arterial wall was found to be significantly thicker and stiffer and with reduced compliance in the infants with BPD, the significance persisting after adjustment for GA and BW separately as well as combined.[27] This is very similar to pulmonary arterial stiffness, which has immense prognostic value in those affected by PH.[28,29] A key implication of these observations is that information from the

vasculature, when used as a measure of cardiovascular risk, may possibly identify novel therapeutic approaches, which may be useful to prevent the onset or progression of PH.[30]

We assessed multiple, pre-defined and previously well studied objective ECHO criteria. Two of them are more recent and merit further discussion. The lack of standardization assigned to ECHO definitions, subjectivity of some parameters such as right heart dilatation, the lack of universal presence of TR on ECHO and a rotated heart affect the sensitivity and identification of PH. Investigators have suggested that a TR jet maximal velocity $\leqslant 2.8\ m\ s^{-1}$ in the absence of an additional variable would indicate an unlikely diagnosis of PH.[17] The ratio of TR to VTI-RVOT has been a well-studied parameter in the paediatric population. Pulmonary vascular resistance (PVR) is calculated invasively by the ratio of trans-pulmonary pressure gradient (Δ p) to *trans*-pulmonary flow (Qp).[31] Thus, TR jet velocity and VTI-RVOT have been proposed as correlates of Δ p and Qp, respectively.[32,33] As the PVR increases, changes in VTI-RVOT and TR jet velocity tend to occur in the opposite direction.[32,34] Multiple studies have noted a strong correlation between ECHO-derived TR/VTI-RVOT and catheter-measured PVR. Among paediatric patients, Ajami *et al.*[35] noted a good correlation with catheter measured PVR ($r^2 = 0.53$, $P = 0.008$) with sensitivity of 71.4% and specificity of 90% for PVR $>6$ Woods Units. Vlahos *et al.*[36] also noted a similar significant correlation ($r^2 = 0.71$), indicating its usefulness in longitudinal tracking these cohorts. In a prospective observational study on children with mean age of 9.7 years, this ECHO-derived ratio correlated well with catheter measured PVR ($r = 0.89$, confidence intervals 0.81 to 0.94, $P < 0.001$). For a PVR of 6 WU, a TR/VTI-RVOT value of 0.14 provided a sensitivity of 96.6% and specificity of 92.8%.[22] We used this cut-off for our study purpose.

The LVsEI is measured in two dimensions from the parasternal short axis view at the mid-papillary muscle level. A ratio of D1/D2 is calculated where D1 is the diameter of the ventricle parallel to the inter-ventricular septum, whereas D2 is the diameter perpendicular to and bisecting septum. It basically measures septal displacement. Normally, the left ventricle is circular; the increased RV pressure shifts the septum to the left, giving the left ventricle a characteristic D-shape. A pressure-loaded RV will deviate the septum in systole, reducing the denominator.[37] Normal LVsEI is 1; it increases in PH, allowing for quantification of a more subjective parameter of septal flattening/bowing. In neonates, systolic septal flattening is recognized at LVsEI $\geqslant 1.15$, whereas greater than half-systemic RV pressure becomes apparent at LVsEI $\geqslant 1.3$. Unlike qualitative assessment of septal flattening, this has a high inter-observer agreement.[21]

Important limitations of this study include its retrospective nature and relatively small numbers. Masked review of placental slides and review by a single perinatal pathologist are among its strengths. This is a single-centre study, and a multicentre study with larger numbers is better placed to further add to this growing body of literature.

We demonstrated a significant association between MVU placental changes and subsequent BPD-associated PH. Given the vascular pathophysiology seems to persist as noted on postnatal vascular ultrasound studies, this seems an important mechanistic link leading to cardiopulmonary disease in early childhood. Early disease predictors may aid preventive strategies in selected populations. These may take the shape of clinical features, radiological findings or laboratory-based test markers that may be useful for early diagnosis, predicting disease severity and aid in the monitoring of disease processes and/or response to therapy. Early initiation of therapies during 'windows of opportunity' or a non-detection of risk may enable the avoidance of therapies and their potential hazards.[38] Hence, the real potential of this work lies in the exciting possibility of identifying subsets of infants at increased risk of BPD-associated PH. Whether this early identification will inform individualized management strategies or use of specific agents such as pulmonary vasodilatory and vascular growth agents needs to be studied prospectively.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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