Bronchopulmonary Dysplasia: Pathophysiology and the Effects of the Microbiome

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Abstract

Bronchopulmonary dysplasia (BPD) is a chronic neonatal lung disease that occurs in over 50% of premature infants. BPD is characterized by damage to the alveoli and bronchioles and improper vasculature formation. It is primarily caused by overexposure to oxygen through mechanical ventilation, but there are other risk factors that make infants more susceptible to BPD. Microbial composition impacts risk for developing BPD, and research is ongoing about the effects of the microbiome on BPD pathogenesis; this information is also valuable for preventative treatment. This paper reviews the normal function of the lungs, pathogenesis of BPD and how it affects normal lung function, and the current and ongoing research concerning the effects of the microbiome on BPD.

Bronchopulmonary Dysplasia: Pathophysiology and the Effects of the Microbiome

Introduction

Newborns are susceptible to many different diseases, and the risk factor increases for babies born prematurely. One of the most common systems that is affected in premature babies is the respiratory system. While there are several pathologies that can affect the respiratory system, the most common form of chronic lung disease in infants is bronchopulmonary dysplasia. Bronchopulmonary dysplasia (BPD) occurs in babies who are born before 32 weeks, and, although most infants survive, BPD can be fatal for others; it can also have long-term effects through a patient's lifespan. BPD was first characterized by Dr. William H. Northway, Jr. and colleagues in 1967, and research has been ongoing since then (1). In this time, researchers have gained a better understanding of how this pathology occurs and the effects that it can have on the affected individuals, as well as its mechanisms at a microscopic level. One of the emerging areas of research is the microbiome and the role it plays in lung development; the microbiome is important for disease response in the body, which includes lung diseases.

What is Bronchopulmonary Dysplasia?

Bronchopulmonary dysplasia is a chronic disease that primarily affects newborns, especially those born prematurely or in need of oxygen therapy shortly after birth (2). It is characterized by damage to the bronchi, resulting in tissue damage in the alveoli. When it was first characterized by Northway and colleagues in 1967, it was initially used to describe the pathology that resulted from a surfactant deficiency in newborns with ventilator-induced trauma. Today, it refers to the consequences of disrupted and impaired lung development, characterized by damage to the bronchi (1). BPD can be further classified based on supplemental O_2 and positive pressure requirements and categorized as mild, moderate, or severe. Additionally, severe

BPD is further broken down into type 1- supplemental oxygen dependance, and type 2- ventilator dependance.

Normal Physiology of the Lungs

Lung Development

BPD is a condition that primarily affects the lungs and their development. The lungs are part of the respiratory system and are responsible for gas exchange in the body. To do this, the lungs are composed of a large inner surface and conducting airways, which include the trachea, bronchi, and bronchioles; these conducting airways ventilate the alveoli, the gas exchange area. The conducting airways are formed first during development, and then the alveoli are enlarged through a process called alveolarization. Alveolar formation has three main components: classical alveolarization, microvascular maturation, and alveolarization of respiratory bronchioles, as illustrated in Figure 1 (3). In humans, alveolarization takes place in a bi-phasic manner, with classical alveolarization and microvascular maturation occurring in parallel (4).

Classical alveolarization begins around week 36 of gestation and continues until about three years of age (5). During this time, the formation of new septa and division of air spaces occurs. At the beginning of this stage, the lung tissue contains immature septa, which contain a capillary network composed of two layers. The existing airspace is divided as the immature septa are lifted, or upfolded (6). These immature septa are upfolded at sites where precursors have laid elastic fibers and collagen fibrils. The capillary layer that faces the airspace is folded and stays in contact with the site of elastic fibers and collagen fibrils. As the septum rises and reaches its full height, the first alveoli are formed, as shown in Figure 1 (4).

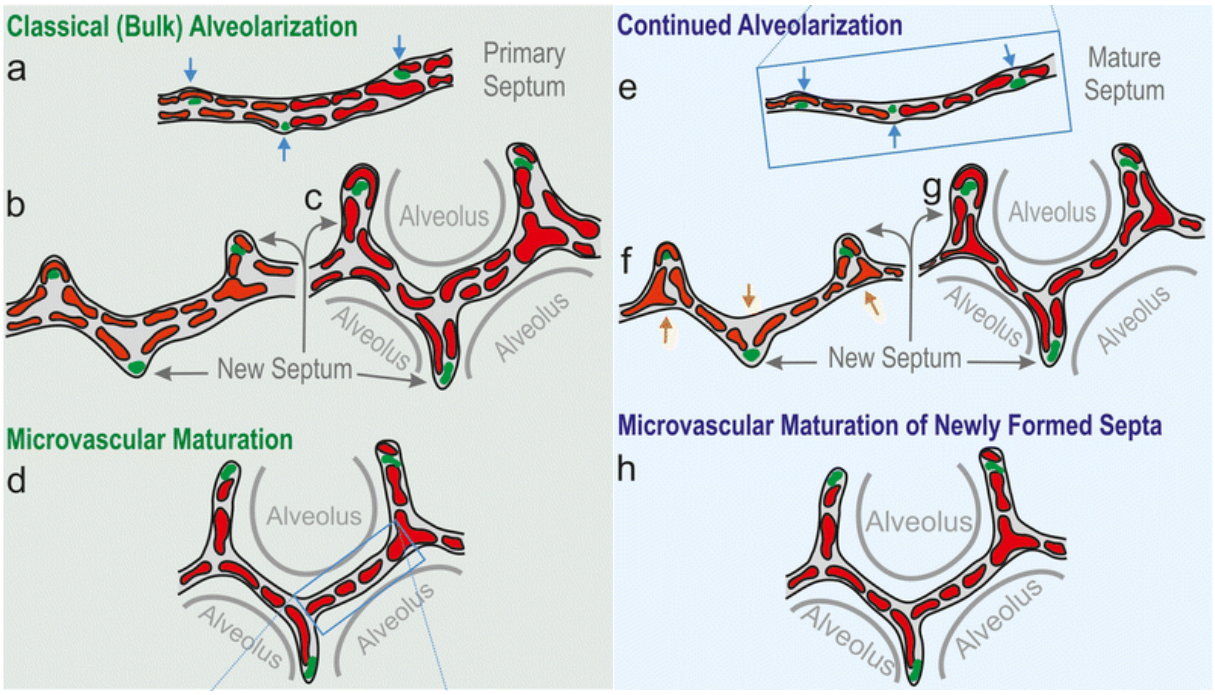

Figure 1

The Processes of Alveolarization and Microvascular Maturation

Note. This figure shows classic alveolarization and microvascular maturation occurring simultaneously, followed by continued alveolarization and further microvascular maturation (5).

Microvascular maturation also begins around week 36, and then continues into early adulthood (3, 6). During this time, the double-layered capillary network undergoes a process of fusion into a single-layered capillary network (7). This process occurs with differential growth and the fusion of capillary segments in multiple places. At the same time, the interstitial tissue volume within the lungs decreases, resulting in the connective tissue and single-layered capillary network interweaving in the septum (5). Microvascular maturation will continue while new septa and alveoli are formed; most alveoli are formed postnatally until about three years of age, and microvascular maturation occurs into early adulthood (3, 4, 8).

In addition to classical alveolarization and microvascular maturation, alveolarization of the respiratory bronchioles also occurs (5). The formation of alveoli of respiratory bronchioles is different than the classical and continued alveolarization otherwise observed. During this phase, future respiratory bronchioles are out-pocketed into the extracellular matrix, forming alveoli; this out-pocketing occurs on the side that is opposite to the pulmonary arteriole that supplies the alveoli. The air-blood barrier is formed when the cuboidal epithelium that lines the cavities formed by out-pocketing flattens (5). Although this is an important part of development, research is limited and has only been studied in rhesus monkeys (4).

As alveolarization occurs, two other significant processes for lung development also occur: vasculogenesis, the formation of new blood vessels from angioblasts, and angiogenesis, the formation of new blood vessels from pre-existing blood vessels (8). Angiogenic growth factors are crucial for vascular development, specifically vascular endothelial growth factor (VEGF) (9). Prenatally, VEGF is necessary for alveolarization and the development of the alveolar capillary bed; when VEGF is inhibited, alveolar growth and development are impaired. Following birth, VEGF signaling is necessary for alveolarization, and without it, lung vascular growth is inhibited (8). In addition to maintaining pulmonary vasculature postnatally, VEGF is necessary for maintenance of alveolar structures into adulthood; however, newborns are more susceptible to VEGF disruptions than adults (4, 9).

Gas Exchange in the Lungs

Factors That Affect Gas Exchange

The lungs are responsible for gas exchange. Based on Fick's law, factors that affect diffusion are surface area, pressure difference, solubility, and membrane thickness. Applied to the lungs and their function in gas exchange, factors that encourage gas exchange are an increase

in membrane surface area, alveolar pressure difference, and solubility of the gas, and a decrease in membrane thickness. This gas exchange takes places at the alveoli. The inter-alveolar septum separates the space within the alveoli and the capillary lumen and serves as the structural basis for gas exchange (10). It consists of an endothelium that faces that capillary lumen, and an epithelium that faces the alveolar lumen. The epithelium consists of type I alveolar cells, which make up the lining of the alveolar lumen and are important for the process of gas exchange, and type II alveolar cells, which are responsible for secretion of surfactant, along with renewal and repair (4, 10). Type I alveolar cells are broad, flat cells in the peripheral lung and are specialized for gas exchange (11). Surfactant produced by type II cells on the alveolar side is important to counter collapsing pressure from epithelial side (12). The inter-alveolar septum provides a larger membrane surface area and a thinner membrane, encouraging gas exchange.

In addition to structural components, alveoli are also stabilized by surfactant released by type II alveolar cells. Surfactant covers the alveolar epithelium and reduces surface tension, preventing alveolar collapse (10). Another important component in lung function is elastin, which is responsible for the elasticity of the lungs, allowing for elastic recoil (13). Together, surfactant and elastin contribute to compliance, the ability of the lungs to expand. Compliance is change in volume over the change in pressure, so it reflects how the lungs expand in relation to pressure changes; the lungs stretch easier when compliance is high and require more force when compliance is low (14). Compliance and elastance are both important properties of the lungs and help them inflate and deflate. When either is affected, breathing complications can result (4).

The vasculature of the lungs is also an essential part of gas exchange; this includes the pulmonary arteries and the pulmonary veins. The pulmonary arteries accompany the airways but have more branches than the airway. The pulmonary arteries branch into different vessels, with

the two main types being conventional and supernumerary vessels (8). Conventional vessels run within an airway and divide with it. The course of supernumerary vessels is shorter; they only supply the alveoli around the pulmonary artery and the related capillary beds. The pulmonary veins converge into larger tributaries; drainage from all intrapulmonary blood vessels goes to the pulmonary veins (4, 8).

The Process of Gas Exchange

The respiratory and circulatory systems work together to facilitate gas exchange. Adult lungs contain about 300 million alveoli, providing a large surface area for gas exchange with the pulmonary capillaries; there are about 1000-4000 pulmonary capillaries in contact with the alveoli, allowing enough blood to be present for gas exchange (15). The alveolar epithelium forms the thin air-blood barrier between the alveoli and pulmonary capillaries. The process of gas exchange is dependent on partial pressure gradients (14). As illustrated in Figure 2, pulmonary arteries carry deoxygenated blood from the heart to the lungs and diverge into capillaries, which are in direct contact with the alveolar epithelial membrane. The Po_2 of the alveolar air is greater than that of the blood, and the Pco_2 of the blood is greater than that of the alveolar air. This results in a partial pressure gradient along which O_2 and CO_2 diffuse until each reaches equilibrium. The blood continues to travel from the pulmonary capillaries, which converge into the pulmonary veins and bring the oxygenated blood to the heart; after being pumped through the heart, the blood is sent through systemic circulation, where gas exchange will occur with all tissues in the body. Here, the partial pressure of gas in the blood will reach equilibrium with that of the tissues, as the Po_2 of the blood is greater than that of the tissue and the Pco_2 of the tissue is greater than that of the blood, resulting in a partial pressure gradient.

This allows for the removal of CO_2 and the oxygenation of the tissues. The deoxygenated blood continues through systemic circulation to the heart, and the entire process begins again (14).

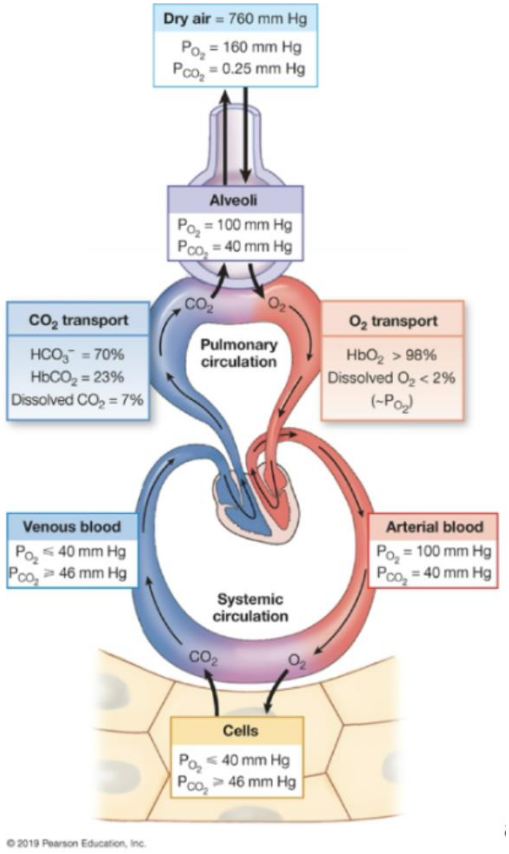

Figure 2

Partial Pressures and Gas Exchange

Note. The diagram below shows the partial pressures of oxygen and carbon dioxide through pulmonary and systemic circulation (14).

The Importance of Gas Exchange

Gas exchange is important not just for the respiratory and circulatory systems, but to maintain homeostasis throughout the entire body. The presence of oxygen and removal of carbon dioxide are necessary for survival; otherwise, the body can fall into a state of hypoxia, with too little oxygen, or hypercapnia, with increased concentrations of carbon dioxide (14). Although the

body is able to make adjustments for short-term states of hypoxia, persistent hypoxic stress can have major health effects and eventually lead to death (16). Cells use oxygen for aerobic respiration, in which organic molecules are converted into energy for the body to use. When the body is in a state of hypoxia, the biggest cellular change is related to energy metabolism; anaerobic glycolysis will be used instead of mitochondrial respiration and oxidative phosphorylation, as both require oxygen (17). While anaerobic glycolysis can be used for short-term purposes, it is not sustainable for extended periods of time, as it has a lower energy yield than aerobic forms of metabolism. When cells are not producing enough energy, they cannot perform all metabolic functions. This can lead to cell death, permanent damage to cells, and eventually death (16).

The removal of CO_2 is also important systemically for pH maintenance and is done through the bicarbonate-carbon dioxide buffer system, which neutralizes 70% of CO_2 in the blood (14). When there is too much CO_2 present, the buffer system can get overwhelmed, leading to a lower pH in the blood and eventually acidosis (18). Additionally, when the concentration of CO_2 is increased, hemoglobin has a lower affinity to oxygen, and oxygen will not be effectively transported in the blood. Hypercapnia has also been observed to inhibit alveolar fluid reabsorption and alveolar epithelial repair and suppress innate immunity and defense in the body (19).

Bronchopulmonary Dysplasia

Defining BPD

Bronchopulmonary dysplasia (BPD) is the most common chronic respiratory disease that occurs in neonatal infants born prematurely (2). It is characterized by arrested development of the alveoli and lung vasculature, typically due to overexposure to oxygen, a state called

hyperoxia. Usually, it occurs because premature infants are placed on ventilators to assist with breathing but are exposed to too much oxygen (20). The general definition of BPD from the NIH is for infants that were born before 32 weeks' gestation and require supplemental oxygen the first 28 days after birth or longer. Mild BPD occurs in 30.3% of BPD cases. It has a mortality rate of 1.5% and is when a patient is completely weaned to room air and does not require supplemental oxygen after 36 weeks postmenstrual age. Moderate BPD occurs in 30.2% of BPD cases and is when a patient needs less than 30% supplemental oxygen after 36 weeks postmenstrual age; it has a mortality rate of 2.0%. Severe BPD, which is further classified as type 1 or type 2, occurs in 16.4% of BPD cases and has a mortality rate of 4.8%. Type 1 is marked by necessity for 30% or more supplemental oxygen or nasal CPAP after 36 weeks postmenstrual age, and type 2 by receiving mechanical ventilation after 36 weeks postmenstrual age (21).

Although infants born prematurely need supplemental oxygen, they often become dependent on it, as their lungs have not completely developed and are reliant on mechanical ventilation. This incomplete development in seen the tissues of the alveoli and the altered process of microvascular maturation, in which the double-layered capillary network forms into a single layer and interweaves with connective tissue in the septum (22). Long term, incomplete development results in inefficient gas exchange and compromised lung function. In addition to hyperoxia, factors that contribute to the pathogenesis of bronchopulmonary dysplasia are disruptions in growth and transcription factors and inflammation of the lungs (20).

Physiological Changes Caused by BPD

Hyperoxia and the resulting effects can cause disturbances in angiogenesis, the formation of pulmonary vasculature, contributing to BPD. Specifically, hyperoxia can cause the walls of pulmonary vasculature to become dysmorphic (23). Remodeling of vasculature often results in

thickened arterial walls, which increases resistance, which then increases blood pressure to the lungs, causing pulmonary arterial hypertension (23, 24). Related to pulmonary arterial hypertension is abnormal cross-linking of collagen and elastin; this can also affect alveolarization. Abnormal cross-linking of collagen and elastin results in stiffening of the pulmonary vasculature, which contributes to pulmonary hypertension. It is debated whether stiffening causes pulmonary hypertension, or if it results due to pulmonary hypertension (20, 24).

In addition to disturbances in angiogenesis, hyperoxia causes arrested alveolarization. Interactions between fibroblasts and mesenchymal-epithelial tissue are important for alveolarization. Hyperoxia damages human fetal lung mesenchymal cells and can also cause the deterioration and destruction of fibroblasts, as well as abnormal fibroblast differentiation (23, 25). Fibroblasts are necessary for the production of the extracellular matrix (ECM), which contains collagen and elastin, and for the maintenance of alveolar structures (23, 24, 26). Abnormal differentiation of fibroblasts results in the remodeling of the ECM, which affects cross-linking of collagen and elastin, and can result in stiffening of the ECM. This affects maturation of epithelial structures, and disrupts septation, the formation of septal walls in the alveoli, which decreases the surface area on the alveoli (23, 25); surface area is critical to optimize gas exchange, and when it is decreased, lung function is compromised (20).

To produce the ECM, fibroblasts are required. Fibroblast growth factor 10 (FGF) is a necessary protein to produce fibroblasts in the lungs and, therefore, the ECM. It is important in branching morphogenesis, in which the lungs and the structures within it are formed (22). Additionally, FGF has both autocrine and paracrine effects, one of which is to promote differentiation of the alveolar epithelium (25). However, when exposed to hyperoxia, FGF expression is reduced (24). Since reduced FGF expression can be caused by hyperoxia and

hyperoxia can cause BPD, the two are associated with one another (20). VEGF is another growth factor associated with lung development and when it is inhibited, formation of pulmonary vasculature and alveolar architecture is impaired (9). Hyperoxia downregulates the expression of VEGF in the lungs and is the cause of BPD (8). Additionally, lower levels of VEGF have been observed in the lungs of infants with BPD (20).

Inflammation is another factor that alters lung development. Although inflammation can occur both pre- and postnatally, BPD is often the result of mechanical ventilation and overexposure to oxygen, and mechanical ventilation has been evidenced to promote the inflammation of lungs in premature neonates (8). Hyperoxia can cause inflammation of the airway, and inflammatory cells are often present in larger amounts in the lungs of premature infants (27). Infants with BPD have more proinflammatory cytokines in their tracheal aspirates and in their blood than infants who do not have BPD (24, 28). Abnormal fibroblasts, commonly found in infants with BPD, can also produce proinflammatory cytokines (26). In addition to the presence of proinflammatory cytokines, inflammation can also lead to elevated production of reactive oxygen species (ROS), which lead to further inflammation and epithelial cell death (23, 28). An abundance of ROS can lead to surfactant oxidation, which interferes with surfactant's function; this includes reducing surface tension of the lungs and reducing inflammation of the lungs, leading to higher surface tension and further inflammation (29). Epithelial cell death interferes with the development of alveolar structures and septa; therefore, inflammation can lead to aberrant alveolarization, which is characteristic of BPD (20, 24).

The most common contributors to BPD are hyperoxia, abnormal regulation of FGF and production of fibroblasts, inhibition of VEGF, and inflammation. These contributors lead to further problems within the lungs that feed into BPD. Remodeling of the ECM caused by FGF

and abnormal production of fibroblasts leads to stiffening, and disrupts septation, reducing surface area available for gas exchange. Inhibition of VEGF stunts the growth of the pulmonary vasculature and capillary network. Inflammation interferes with the function of surfactant and leads to epithelial cell death, causing higher surface tension in the lungs and decreased formation of alveolar structures. These factors work in conjunction with one another to cause disruptions to alveolarization and angiogenesis and lead to reduced pulmonary function and oxygen dependence (20).

Effects of BPD on Gas Exchange

BPD affects gas exchange, as it is the primary function of the lungs. As shown in Figure 3, hemoglobin curves show a rightward shift in infants with BPD, which indicates that hemoglobin has a decreased affinity for oxygen; this can result in decreased levels of oxygen transport in the blood. Infants with moderate and severe BPD show an increased shift compared to infants with mild BPD (30). The ratio of ventilation to perfusion (V_A/Q) represents the ratio of air that enters the alveoli to blood that comes into contact with the alveoli. In infants with BPD, V_A/Q is decreased, indicating the ventilation is decreased and that less air is coming into contact with the alveoli. One study showed that infants with severe BPD that did not require supplemental oxygen had a lesser shift and higher V_A/Q compared to infants with severe BPD who did require supplemental oxygen, showing that oxygen dependance negatively effects gas exchange (30). Additionally, right-to-left shunt is also increased in infants with BPD. Right to left shunt is when deoxygenated blood in the veins is sent directly to the systemic arteries; since deoxygenated blood is not going to the lungs and being oxygenated, less oxygen is delivered to the rest of the body (31).

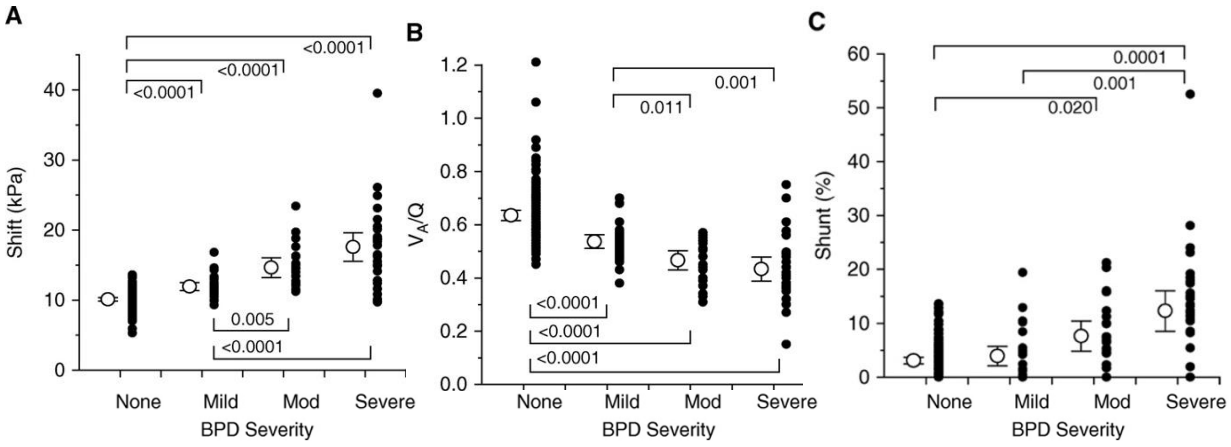

Figure 3

Effects of BPD on Gas Exchange

Note. The graphs above show the effects of BPD on (A) rightward shift of the hemoglobin saturation curve (B) ratio of ventilation to perfusion and (C) right-to-left shunt (30).

Long-Term Effects of BPD

Although BPD is most dangerous in infancy, it can have effects that continue into adulthood. Compared to children without BPD, children with BPD are found to have more respiratory symptoms and need to use respiratory medications (32). Extremely premature children with BPD are more likely to have chest wall deformities and Harrison sulci, an indentation on the chest around the sixth rib often caused by underlying respiratory conditions, as they grow older (33); they are also twice as likely to have asthma, and their asthma symptoms are much more severe with a greater need for medication. Since most pulmonary vasculature forms during the first two years of life and many infants with BPD rely on ventilation during this time, they have abnormal pulmonary vasculature and abnormal diffusion capacity and airway expiratory flows. While children with BPD can still exercise, they have different breathing patterns, such as an increased breathing frequency, higher respiratory rate and minute ventilation,

and lower inspiratory strength (32). As BPD survivors age, their need for hospitalization for respiratory issues decreases.

Because BPD has had changing definitions since its initial definition in 1967, research surrounding adult survivors is limited. However, it has been observed that many adult survivors frequently experience wheezing, coughing, and difficulty breathing (34). Other common findings include asthma-like symptoms, COPD, emphysema, and pulmonary vasculature disease (35). This is most likely due to lung parenchymal abnormalities observed in adult survivors of BPD. Though many abnormalities tend to be minor, adults with moderate or severe BPD have more hypoattenuated areas and opacities, which could impair lung function (34). Hypoattenuated areas show up as less dense areas on CT scans and are typically caused by an irregular increase in air, a decrease of blood flow to the lungs, or the loss of lung tissue (36). The general pattern observed is that adult survivors of BPD continue to have reduced lung function, as BPD affects the development of the lungs (34). Even though BPD does affect some of the lungs' functions into adulthood, adults with BPD can still perform daily functions and manage their symptoms.

Treatment and Management of BPD

Early BPD

Early BPD is when BPD has not fully developed or is just beginning to develop, so most of the strategies for treatment and management focus on prevention and decreasing the severity of BPD. Since BPD only occurs in prematurely born newborns, preventative strategies can be taken immediately after birth as premature newborns are already at risk. The primary strategy is careful monitoring of mechanical ventilation and making adjustments as needed. Oxygen saturation should be monitored and in the 90-95% saturation range, and infants should be weaned off invasive mechanical ventilation after the first week (37). Additionally, intubation

should be used as minimally as possible and only when necessary so that further injury related to mechanical ventilation can be prevented (21). Other strategies include minimally invasive surfactant treatments and LISA treatments for surfactant delivery through nasal CPAP, as premature newborns born before 32 weeks do not have yet have surfactant in their lungs; this will help bring structural integrity to their lungs (37).

In addition to ventilation strategies, there are medicinal treatments that have some efficacy. Caffeine therapy within the first three days of life has been shown to be highly effective in reducing BPD by assisting with expiratory flow; the best dosage and timing is still being studied (37). Postnatal steroids can also be used but are typically reserved for more severe cases; dexamethasone is a steroid that is effective, but does increase the risk for NDI and cerebral palsy, so it is only used in cases with the highest risk (38). Hydrocortisone in low doses within the first week of life produces similar effects to dexamethasone but has not been observed to have negative neurological side effects. There is some research supporting the use of diuretics in infants early in their BPD diagnosis, but there is not yet solid support, so its continued use is only encouraged in infants who have been responsive and have shown improvement with diuretic use (37). There are also nutritional strategies that have been observed to decrease BPD incidence, such as feeding with only maternal breast milk and reducing the fluid intake of the infant at risk. Vitamin A and vitamin D supplementation have also been observed to protect against lung damage due to mechanical ventilation (38).

Established BPD

Once BPD has been established, the focus of treatment is to wean infants off mechanical ventilation, as too much exposure will further damage their lungs and increase the severity of BPD. To wean infants off supplemental oxygen, prednisone has been shown to be effective (37).

For less severe cases, the infant can be weaned at home through supplemental oxygen therapy. In very severe cases, where an infant spends 90-100 days relying on mechanical ventilation and extubation attempts have failed, tracheostomy can be considered (37). If pulmonary hypertension is present, then pulmonary vascular resistance is high; phosphodiesterase inhibitors, endothelin receptor antagonists and prostacyclin analogs can be used to reduce resistance and hypertension (39). For severe BPD with asthma-like symptoms of hypertrophy and inflammation, inhaled bronchodilators have proved effective (21). Children and adult survivors of BPD are more likely to have other pulmonary complications, so treatment they receive is based on those complications.

Current Direction of Research

Since BPD is the most common chronic lung disease that affects premature infants, research is ongoing. Current research on BPD focuses on developing potential treatments or on expanding understanding of the pathology. Recent developments in potential treatments include inhaled vitamin A dosing and continuous distending pressure of CPAP to hyperoxia-exposed lungs. Research shows that inhaled delivery of vitamin A is more effective and less invasive than the intramuscular dosing of vitamin A that is currently used as a preventative strategy for BPD; this could change the way that early BPD is treated (40). Continuous distending pressure of CPAP to hyperoxia-exposed lungs can reduce effects of hyperoxia, such as reduced compliance and elastance, enlarged alveoli, decreased radial alveolar count and more muscular arteries, that are typically present in BPD (41). This has only been demonstrated in animal models so far, but its implications are important for the future of BPD prevention and treatment (20).

Research concerning deepening the understanding of BPD is also ongoing. Mouradian et al. demonstrated that exposure to hyperoxia, such as from mechanical ventilation, makes it more

difficult for infants to recover from states of hypoxia; it can cause decreased tidal lung volume and reduced inspiratory capacity (42). These results show how hyperoxia disrupts control of breathing, leading to a reduction of lung response to short-term stressors and adding to the understanding of its long-term effects. Ariaans et al. conducted research focused on angiogenic peptides present in infants with BPD and found that levels of angiogenic peptides, many of which were growth factors, differed between infants with and without BPD (43). This research is important in understanding which peptides are associated with BPD and can help with preventative strategies in both the identification and treatment of BPD. An emerging area of research is the effect of the microbiome on BPD. Based on recent evidence of crosstalk between the gut and lung microbiomes, Willis et al. focused on how perinatal maternal exposure to antibiotics affected the severity of BPD. This research expanded the understanding of this relationship, showing that the use of perinatal maternal antibiotics increased the mortality of BPD (44). The effect of the microbiome on BPD is a new area of research but is one that could prove to be vital in understanding the development, prevention, and treatment of BPD (20).

What is the Microbiome?

The human microbiome refers to the large microbial population present in or on the human body and is an emerging field of study. The majority of research on the microbiome is on the gastrointestinal microbiome and has established that the microbiome plays a significant role in disease states and that the bacteria present, as well as resulting metabolites, can have important effects on disease pathogenesis. Recently, more research has been conducted on the airway microbiome and the effect it has on respiratory diseases, making the relationship between BPD and the airway microbiome an upcoming area of study. The lungs were once considered to be completely sterile, but research shows that the lungs are exposed to bacteria and have their

own microbiome (45); this information could have major clinical implications and be vital to the treatment of lung diseases and disorders. In addition to the relationship between the airway microbiome and BPD, the gut-lung axis is an important relationship that is also an ongoing area of research and could prove to have significance in the pathogenesis of BPD.

The Airway Microbiome

The airway microbiome consists of microbial communities found in the respiratory tract and the lungs. Since most research on the microbiome is focused on the gut microbiome, research on the airway is new; the lung microbiome has been recently shown to play a role in the development of respiratory diseases such as COPD and asthma (46). The composition of the lung microbiome is constantly changing and evolving, as there is a high rate of microbial immigration and clearance and a low biomass present in the healthy lung (45). Recent research shows that lung microbiota, either dead or alive, are associated with immune response, such as protecting against infection and stimulation of T-regulatory cells. Much of the bacteria in the lung microbiome come from the upper respiratory tract, such as *Firmicutes* and *Bacteroides*; because of this, dysbiosis in the lung microbiome can originate from the upper respiratory tract, or even from bacteria in the bloodstream (47). One of the most common genera found in the lungs is *Lactobacillus*, which is known to have immunomodulatory function and help protect against infections in the respiratory tract; it also helps regulate respiratory immune response (48). The bacteria that are present in the lung microbiome have great importance; changes in the lung microbiome in infancy can lead to disease later in life, even if those changes are with commensal bacteria (45). New research shows that this microbiome is not just affected by respiratory changes but could also be linked to changes in the gut microbiome (47).

The Gut-Lung Axis

The gut-lung axis is an emerging area of study and focuses on the influence that the microbiota of the gut has on the lungs; these interactions are called crosstalk. The gut and lung share an embryonic origin and are structurally similar, providing the basis for research on gut-lung crosstalk (49). In addition to this, the gut and lungs have similar microbial needs. They both need to have many of the same normal commensal bacteria for normal immune function and development (50). They have the same main bacteria present, *Firmicutes* and *Bacteroides*, and are known to exchange bacteria in disease states (51). It has also been observed that antibiotics that lead to dysbiosis in one system can affect the other. For example, the use of antibiotics that cause intestinal dysbiosis can also lead to lung diseases, such as asthma, COPD, and respiratory infections. This is because endotoxins, cytokines, hormones, and microbial metabolites can cross over the gut-lung axis through the bloodstream (52).

The Relationship Between the Microbiome and BPD

One of the largest areas of study about the relationship between the microbiome and BPD is the composition and diversity of the lung microbiomes of babies who developed BPD vs those who did not. Infants with differing severity of BPD show different microbiome composition and diversity, with these differences being more severe on day 1 of life than on day 7. Those with BPD had increased *Stenotrophomonas* in their airway and decreased microbiome diversity, and those with severe BPD also had more *Ureaplasma* and *Proteobacteria*, but less *Staphylococcus, Acinetobacter,* and *Lactobacillus* (53, 54). *Lactobacillus* is important for the respiratory system because it helps with immune response and in protecting against infection, so having less present can leave an infant more susceptible to disease. *Lactobacillus* can produce metabolites that modulate immunity, such as short-chain fatty acids, which increase T regulatory cell (Treg)

generation and IL-10 production, or lithocholic acid, which can increase Treg function and inhibit Th17 responses (48); the specific effects of Treg response in BPD are still being further researched. The phylum *Proteobacteria* contains several potentially harmful bacteria, which leads to an increase in immune response from Th17 cells. Th17 responses are involved in autoimmune diseases, and, when found in the lungs, they are often indicative of poorer lung function (45). Patients with BPD experience more frequent changes within the microbial community of the airway microbiome, but the long-term effects are still unknown (55).

Another upcoming area of study is the relationship between the gut-lung axis and BPD; it has been observed that the dysbiosis of either the gut or lungs can be related to disease in the other, so the possible relation to BPD is an emerging topic of interest. The intestinal and lung microbiome work together to maintain immune homeostasis, and when disrupted, can lead to inflammation and possibly BPD. It has been observed that in infants with BPD, the gut microbiome is disrupted and has less diversity present (56). Antibiotic use and its relationship to BPD pathogenesis is also being studied, as antibiotic use has been associated with BPD (55). It is thought that antibiotic use increases inflammation, which leads to an increased incidence of BPD, as inflammation is a contributing factor of BPD (44). Prolonged antibiotic exposure right after birth is also correlated to a higher incidence and severity of BPD (49). Research to better understand the relationship between the microbiome and BPD is still ongoing.

Review of Current Research on the Microbiome and BPD

Gram-Negative Bacteria

One area of research concerning the microbiome and its relationship with BPD is the presence of Gram-negative bacteria in tracheal aspirate cultures (57). Imanshi et al. sought to examine the relationship between BPD development and the presence of Gram-negative bacteria

at 36 weeks postmenstrual age using tracheal aspirates. Results showed that Gram-negative bacteria, specifically Gram-negative rods, were present in increased amounts in tracheal aspirates of infants with BPD and are associated with BPD development. This research is important because it is known that Gram-negative rods are associated with harmful respiratory symptoms and airway inflammation, which both contribute to BPD (57). Therefore, detection of Gram-negative rods early in infants born before 26 weeks could be helpful in the prevention of BPD.

In a different study, Gao et al. looked at tracheobronchial aspirates to observe microbes in the lower respiratory tract and how they relate to BPD (58). Results showed that more Gram-negative bacteria were detected in infants with BPD than those without. Of those bacteria, several were nosocomial, typical pathogens found in hospital settings, such as *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, and *Acinetobacter baumanii*, all of which can cause pneumonia. This research further demonstrates the prevalence of Gram-negative bacteria in infants with BPD, and also shows that some of these bacteria are associated with hospital-borne diseases, meaning they could be caused by the invasive ventilation that many infants with BPD experience (58). Further research would need to examine infants who did not have endotracheal ventilation and compare the bacteria present in their tracheobronchial aspirates.

Relationship Between Mother and Child

The relationship between the mother and child is another area that could play a significant role in BPD pathogenesis. One area in which microbial signatures could prove to be significant is the amniotic fluid. A study was done by Staude et al. to examine the bacterial signatures of the amniotic fluid from pregnant women with intact pregnancies as opposed to that of premature babies who developed BPD (59). Results from this study showed that the amniotic fluid of infants with BPD is different from infants without BPD, as their 16S rRNA gene

signatures differ and infants with BPD showed an increased bacterial load present in the amniotic fluid (59). This research adds to the knowledge of prenatal origins of BPD, which could eventually be used for BPD prevention. Future research in this direction would be to investigate living bacteria as opposed to just using 16S rRNA sequencing, and to look at the relationship between the mothers' microbiome and their effects on the composition of the amniotic fluid.

When children are breastfed by their mothers, they receive nutrients and antibodies from breastmilk; this composition is affected by the mother's diet. To better understand the dynamics of the intestinal tract and the nutrients infants receive, fecal short-chain fatty acid (SCFA) levels can be used, as they are affected by the microbiome and by diet and are produced by bacteria in the colon. Frazer et al. observed the levels of SCFAs in maternal breastmilk and infant stool samples to see if they were correlated to BPD incidence (60). Results showed that lower levels of acetic acid in infant stool were associated with increased likelihood of BPD. This research is important because it shows how the microbiome of the infant is related to the nutrients they receive from their mother, and future research could concern the use of acetic acid, prebiotics, or probiotics as preventative measures for BPD. Additionally, the relationship between the microbiomes of the mother and baby in BPD pathogenesis is still largely unknown, so research could be conducted to examine this relationship.

The Upper Respiratory Microbiome

The upper respiratory tract serves as a major gateway for bacteria into the body. The presence of bacteria in the nasal microbiome and its impact on BPD is another area of research that is upcoming; this is done on the basis that bacteria of the upper respiratory tract can affect the composition of the lower respiratory tract. Xu et al. conducted a study on the bacteria present in the nasal microbiome of infants with BPD and those without BPD to see if any correlation

with BPD exists (61). In this study, nasal swabs were collected from infants with BPD and those without. 16S rDNA sequencing revealed that there were no significant differences between the nasal microbiome of infants with BPD and those without. This study is the first of its kind, laying the groundwork for future research, as the influence of the nasal microbiome on BPD is understudied. Future research could include researching the metabolic effects of the bacteria present in the nasal microbiome to further understand if there is any significant relationship between them and BPD (61).

Another structure of the upper respiratory tract through which bacteria enter is the mouth. Gentle et al. sought to form a correlation between the oral microbiota and nitrate reductase (NR) activity and their role in BPD pathogenesis, as NR regulates nitric oxide (NO) signaling, which has been shown to impact BPD (62). The results demonstrated that at 29 weeks PMA, infants who developed BPD did not experience any changes in NR activity, but infants who did not develop BPD saw an increase in NR activity, which stayed at consistently higher levels. The relative abundance of the bacteria *Akkermansia municphila 2, Burkholderia*, and *Chryseobacterium* were related to NR activity. This research suggests NR activity in the oral microbiome may be caused by the bacteria present and could contribute to BPD (62). Future research can examine the effects of the gut microbiome on NR and NO activity, as this study was only focused on the oral microbiome.

The Intestinal Microbiome

With the emergence of research on the gut-lung axis and its relationship on lung pathologies, research is ongoing concerning its impact on BPD. To further investigate this relationship, Fan et al. conducted a study using mice to examine the changes of the intestinal microbiome in BPD (63). 16S rRNA sequencing showed that BPD is associated with gut

microbiome dysbiosis; additionally, the study found that changes in lung tissues occurred before intestinal dysbiosis. This study is important to this field because it further investigates the gut-lung axis, a new area of research, and helps provide a foundation on which other research can be conducted; eventually, these findings can be applied to a clinical manner (63). The potential relation of the gut-lung axis to BPD allows for several new areas of research, such as how the presence of certain bacteria influence disease pathogenesis, and the effects of antibiotic use of either the mother or infant and related effects on BPD.

In another study, Ran et al. used mice to examine the effects of antibiotics on intestinal dysbiosis before hyperoxia exposure (64). The results demonstrated that induced intestinal dysbiosis leads to increased susceptibility to BPD, and that developing macrophages polarize to the M1 subtype rather than the M2 subtype in the lungs. This is important because polarized M1 macrophages promote the production of pro-inflammatory substances and can lead to inflammation, a common characteristic of BPD; polarized M2 macrophages are involved in tissue repair and remodeling, so their inhibition can be harmful to the process of alveolarization. Also, this study implies that too much antibiotic exposure can be harmful for infants and increase their chances of developing BPD (64). Future research can look to apply these results in a clinical setting and in humans, as this study was done on mice.

Lung Inflammation

Dysbiosis and lung inflammation are associated with one another and their relationship as it relates to BPD is a new area of research. To further understand the relationship between these two factors, Saie et al. examined the relationship between hyperoxia- and LPS-induced lung inflammation on BPD inflammation; LPS is found in the outer membrane of Gram-negative bacteria, which are associated with inflammation and respiratory symptoms (65). 16S rDNA was

sequenced, revealing four genes that are related to BPD incidence. Additionally, it was determined that interaction between hyperoxia and LPS has a significant impact on the composition of the metabolome (65). This research has potential clinical implications, as the microbiome and metabolome could be potential targets for BPD treatment; it is also useful for future research, as most studies of this nature focus on the adult lungs, and having a baseline of information on infant lungs can provide the basis and background research for further studies.

The presence of Tregs is another area that is being studied in lung diseases, as Treg response can be promoted by bacteria like *Lactobacillus* (48). Pagel et al. sought to examine the effects of Treg levels on BPD during the first four weeks of life and to better understand its suppressive capacity and found that increased levels of Tregs during the first two weeks of life are related to BPD development (66). When examined in other inflammatory diseases, such as asthma, Treg levels have shown varying results and have been present in increased numbers in some cases, but in decreased numbers in others, making it difficult to draw conclusive evidence. However, these results also could suggest that the role Tregs play is dependent on the stage of disease progression (66). Future research could target Treg dynamics for BPD treatment or prevention and could investigate how the timing of disease progression affects Tregs.

Conclusion

Bronchopulmonary dysplasia is the most common chronic lung disease that affects premature newborns. It is caused by overexposure to oxygen through mechanical ventilation, and results in damage to the alveoli and bronchi by interfering with the development of the lungs and vasculature; this leads to reduced lung function and oxygen dependence. The effects of the microbiome, specifically that of the airway and potentially of the gut, on BPD development is an emerging area of study. This is done on the basis that the airway microbiome has an effect on the

pathogenesis of other lung conditions. Recent research has focused on identifying common bacteria found in not just the airway microbiome, but also the nasal, oral, and intestinal microbiomes; identifying the differences between the microbiomes of healthy infants and those with BPD could prove to be useful in a clinical setting by allowing for practitioners to enact preventative strategies on infants who present with bacteria common to BPD. Future research should focus on the gut-lung axis and examine how the use of antibiotics may be related to BPD pathogenesis, and to study the role of a mother's microbiome on an infant's along with any potential correlation with BPD.

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