

Hyaline membrane disease (HMD): the role of the perinatal pathologist

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The role of the clinical pathological dialogue in problem solving

Guest Editors: Gavino Faa, Vassilios Fanos, Peter Van Eyken

Abstract

Hyaline membrane disease (HMD), the pathologic correlate of respiratory distress syndrome (RDS) of the newborn, is an acute lung disease of premature infant caused by inadequate amounts of surfactant. Decreased surfactant results in insufficient surface tension in the alveolus during expiration, leading to atelectasis, decreased gas exchange, severe hypoxia and acidosis. HMD predominantly occurs in infants younger than 32 weeks of gestation and weighing less than 1,200 g. In the interpretation of perinatal lung pathology, it is necessary to consider the development of the immature lung, particularly in the third trimester. Microscopically HMD is characterized by the occurrence of dilated terminal and respiratory bronchioles and of alveolar ducts lined by acellular eosinophilic hyaline membranes. The membranes are composed of necrotic alveolar lining cells, amniotic fluid constituents and fibrin. Retinopathy of prematurity and bronchopulmonary dysplasia are late complications of RDS that usually occur in infants who weigh less than 1,500 g and were maintained on a mechanical respiration more than 6 days. Here a practical approach to a microscopic analysis of the lung in newborns died with the clinical setting of RDS is presented. The most important pathological findings for a complete clinical pathological diagnosis are: the evaluation of the architectural lung development; the endothelial cell lesions; the interstitial edema; the occurrence of disseminated intravascular coagulation; the presence of associated inflammatory lesions. The usefulness of some immunohistochemical stains is also underlined, including anti-

surfactant, anti-smooth muscle actin and anti-CD31 to better evaluate surfactant production, pulmonary artery maturation and endothelial cell damage, respectively. Finally, the prevalent role of endothelial dysfunction and endothelial barrier loss is underlined, representing a major pathological event in the deposition of HMD.

Keywords

Lung, respiratory distress syndrome, hyaline membrane disease, surfactant, premature infant.

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Introduction

Hyaline membrane disease (HMD), the pathologic correlate of respiratory distress syndrome (RDS) of the newborn, is largely an acute lung disease of premature infant caused by inadequate amounts of surfactant. Decreased surfactant results in insufficient surface tension in the alveolus during expiration, leading to atelectasis, decreased gas exchange, and severe hypoxia and acidosis. HMD is one of the most common problems seen in premature babies. The more premature the baby, the higher the risk and the more severe the HMD [1]. RDS is the most common respiratory disorder of premature newborns and its incidence is directly proportional to the degree of prematurity. HMD typically worsens over the first 48 to 72 hours and then improves with treatment. More than 90 percent of babies with HMD survive. The incidence and severity of HMD are inversely proportional to gestational age and birth weight, ranging from 5% at 36 weeks up to 65% at 29 weeks of gestation [2]. HMD predominantly occurs in infants younger than 32 weeks gestation and weighing less than 1,200 g.

Surfactant formation and physiology

HMD is a disorder related primarily to deficiency of pulmonary surfactant. Surfactant is a

complex lipoprotein composed of 6 phospholipids and 4 apoproteins. Surfactant recovered by alveolar wash from most mammals contains 70-80% phospholipids, 8-10% protein, and 10% neutral lipids, primarily cholesterol. Dipalmitoylphosphatidylcholine (DPPC), or lecithin, is functionally the principle phospholipid. About 1% of the 10% protein component comprises surfactant apoproteins; the remaining proteins are derived from alveolar exudate. Among the 4 surfactant apoproteins identified, surfactant protein B (SP-B) and SP-C are 2 small hydrophobic proteins that make up 2-4% of the surfactant mass. The SP-B gene is localized on human chromosome 2, and its primary translation product is 40 kd, which is clipped to become an 8-kd protein in type II cells before entering lamellar bodies to be co-secreted with phospholipids. The SP-C gene is on chromosome 8; its primary translation product, 22 kd, is processed to an extremely hydrophobic 4-kd protein that is associated with lipids in lamellar bodies. SP-A is an innate host defense, hydrophilic lectin coded on human chromosome 10 that regulates lung inflammation. SP-A contributes to the biophysical properties of surfactant primarily by decreasing protein-mediated inhibition of surfactant function. SP-A facilitates phagocytosis of pathogens by macrophages and their clearance from the airways. SP-D is also a hydrophilic protein of 43 kd that is a collectin with structural similarities to SP-A. SP-D is a large multimer that is synthesized by type II alveolar cells and Clara cells, in addition to other epithelial cells in the body. It also binds pathogens and facilitates their clearance. The components of pulmonary surfactant are synthesized in the Golgi apparatus of type II alveolar cells [3]. Its production is regulated by different hormones and growth factor as glucocorticoids, insulin, prolactin, thyroxine and transforming growth factor beta (TGF- β). Its production generally starts around the 24th to the 28th week of pregnancy, and surfactant is found in amniotic fluid between 28 and 32 weeks. By about 35 weeks of gestation, most babies have developed adequate amounts of surfactant. Surfactant deficiency may not be the sole cause of hyaline membrane formation. An early morphological feature of HMD is necrosis of the bronchiolar epithelium. This has been attributed to pulmonary circulatory problems and may explain the development of hyaline membranes in some full-term infants with abruption placentae, placenta previa and fetal malposition, as well as their occurrence in some stillborn infants [4].

Pathogenesis

The basic defect causing the idiopathic RDS in preterm babies is represented by immaturity of the lungs, particularly of type II pneumocytes. Qualitatively and quantitatively, fetal surfactant is less efficient than adult surfactant in lowering the alveolar surface tension and keeping the alveoli open. Because lung compliance is low, the critical negative pressure needed to allow influx of air into the lungs cannot be attained. The collapse of alveoli (atelectasis), not adequately coated with surfactant, reduces the pulmonary surface, allowing exchange of gases only through the walls of alveolar ducts and terminal bronchioles structures that are not suitable for that purpose (**Fig. 1**). Anoxia and hypercapnia cause acidosis, leading to peripheral vasodilatation and pulmonary vasoconstriction. This situation, in turn, leads to the reestablishment of a partial fetal circulatory pattern. Right-to-left shunting of unoxygenated blood through the ductus arteriosus and foramen ovale further contributes to hypoperfusion of the lungs, jeopardizing the respiratory oxygen supply even more. Hypoxia adversely affects pulmonary cells, and necrosis of

endothelial, alveolar, and bronchial cells takes place (**Fig. 2**). Vascular disruption causes leakage of plasma into the alveolar spaces and layering of fibrin and necrotic cells arise from type II pneumocytes (“hyaline membranes”) along the surface of alveolar ducts and respiratory bronchioles partially denuded of their normal cell lining (**Fig. 3**) [5]. In neonatal RDS, there is a notable shortage of neutrophils in the inflammatory reaction associated with these membranes. It represents a significant peculiar finding that distinguishes neonatal HMD from acute respiratory distress syndrome occurring in adults (ARDS) wherein the neutrophils play an important role [6]. The deposition of hyaline membranes in turn further impedes the passage of oxygen from the alveolar spaces across the respiratory surface into the pulmonary vasculature. Moreover, extravasation of blood into the respiratory airways, combined with the collapse of the alveoli, further contributes to the consolidation of the newborn lungs. In terminal stages air is found only in bronchi and dilated bronchioles, whereas the rest of the lung parenchyma is consolidated and airless. Other factors that can increase the risk for developing RDS include:

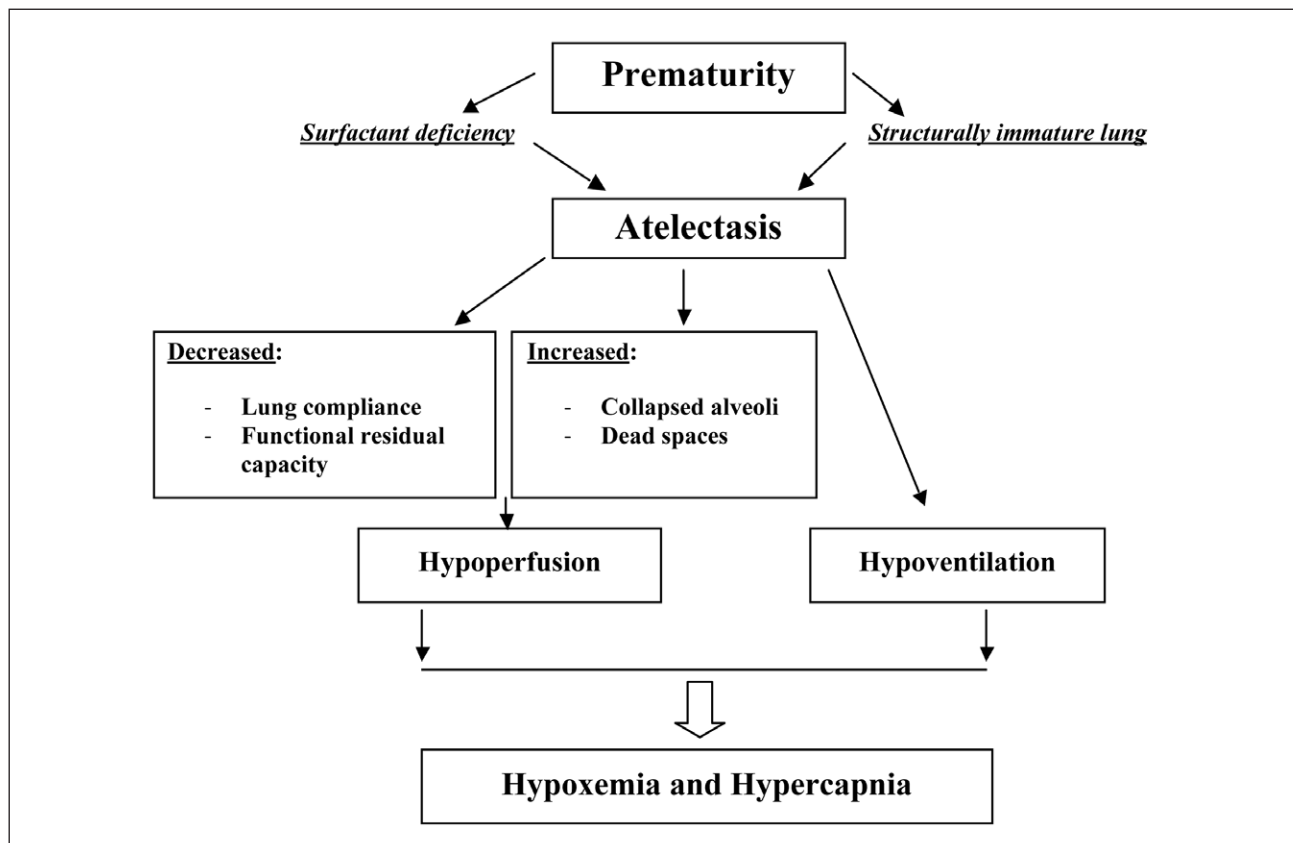


Figure 1. Pathogenesis of respiratory distress syndrome (RDS). First part.

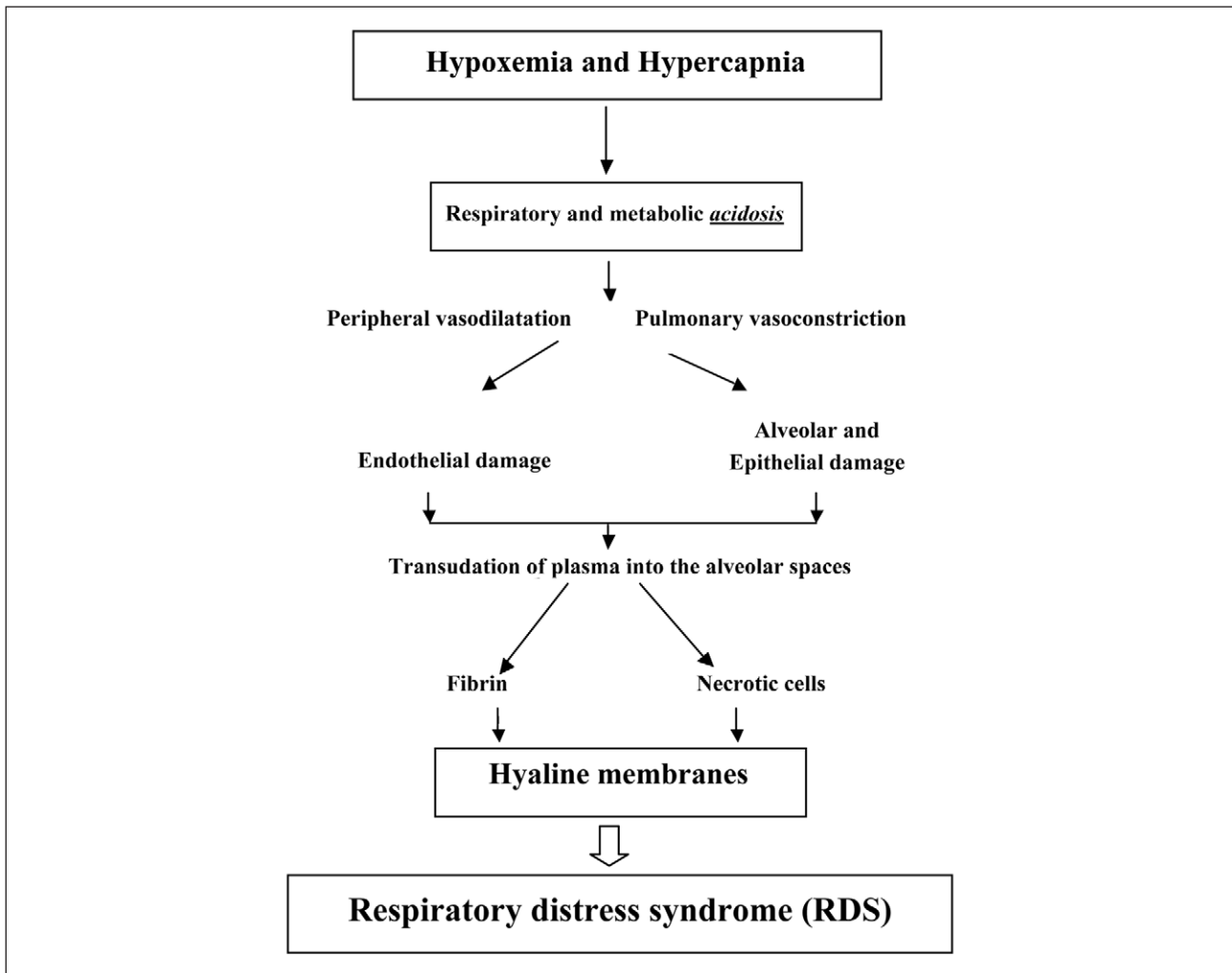


Figure 2. Pathogenesis of respiratory distress syndrome (RDS). Second part.

- Caucasian or male newborns (M:F = 2:1);
- previous birth of newborns with HMD;
- cesarean delivery;
- perinatal asphyxia (lack of air immediately before, during or after birth);
- cold stress (a condition that suppresses surfactant production);
- perinatal infection and growth retardation;
- babies with patent ductus arteriosus;
- multiple births (multiple birth babies are often premature);
- infants of diabetic mothers;
- maternal hypothyroidism;
- mutations in the genes encoding the surfactant proteins [7].

Cesarean delivery has been shown to represent an independent risk factor for RDS, the incidence of HMD being reduced with labor before cesarean, but still elevated. This supports the importance of being certain of fetal lung maturity before cesarean

delivery, particularly when done before labor [8]. Another study, comparing of singleton and multiple-birth outcomes of infants born before 32 weeks of gestation showed a higher incidence of RDS among multiple-birth infants [9]. Infants born to mothers with glucose intolerance (diabetic) are at increased risk of morbidity and mortality related to respiratory distress. Surfactant-associated protein of Mr 28,000 to 35,000 (SAP-35) is an abundant glycoprotein present in the alveolus of the lung, which controls the structural organization of surfactant phospholipids regulating secretion and metabolism. Glucocorticoids both enhance and inhibit SAP-35 expression in fetal lung explants. We know that fetal hyperinsulinemia and hyperglycemia, or both, inhibit the morphologic differentiation of type II epithelial cells in association with decreased phospholipid surfactant synthesis or secretion. Insulin is also a potent inhibitor of SAP-35 expression in fetal lung tissue,

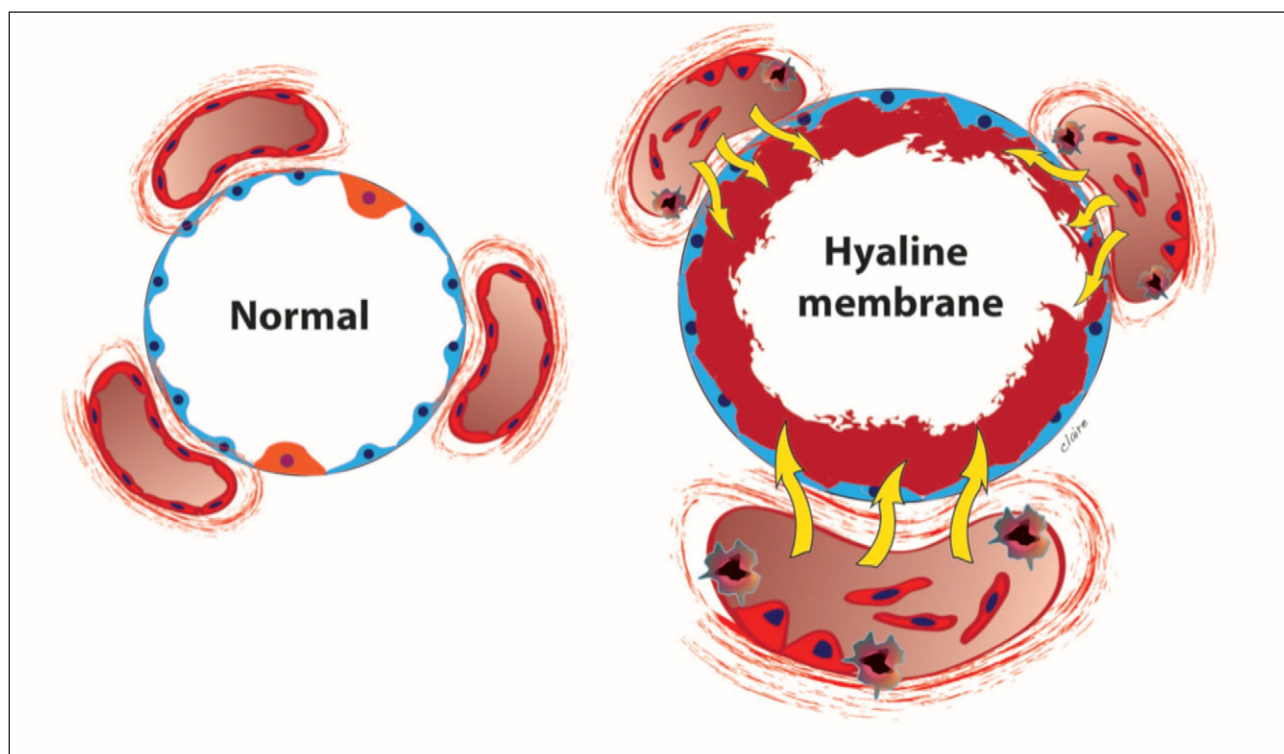


Figure 3. Pathogenesis of hyaline membrane disease (HMD). Vascular disruption causes leakage of plasma into the alveolar spaces and layering of fibrin and necrotic cells arise from type II pneumocytes (“hyaline membranes”) along the surface of alveolar ducts and respiratory bronchioles partially denuded of their normal cell lining.

and decreased SAP-35 was previously observed in amniotic fluid of women with diabetes during pregnancy. Recent progress in the management of diabetes in pregnancy, characterized by more rigorous metabolic control, has decreased the risk of RDS for the infant of the diabetic mother and is associated with normal levels of SAP-35 in amniotic fluid [10]. An association between maternal hypothyroidism and the risk of RDS in newborns has been described. Thyroid hormones are very important for the development of the fetus, and especially for the production of lung surfactant. Recent studies have shown that newborns of hypothyroid mothers need of intensive care after birth, especially due to complications such as premature birth, low birth weight and increased neonatal respiratory distress [11]. Moreover, associations with genetic disorders have been noted. The available studies mainly concentrate on surfactant proteins (SP-A, SP-B); mutations in the genes encoding SP-B, SP-C and the phospholipid transporter are associated with respiratory distress and interstitial lung disease in the pediatric population [12]. Hereditary SP-B deficiency is the most frequent genetic condition associated with HMD [13].

Pathological features in hyaline membrane disease

Grossly, the lungs are reduced in volume and show a solid or “liver-like” consistency. The neonatal lungs appear congested, with marked atelectasis [14].

The most important histological features are presented below (**Fig. 4**):

- dilated terminal and respiratory bronchioles and alveolar ducts lined by acellular eosinophilic hyaline membranes are observed;
- the membranes are composed of necrotic alveolar lining cells, amniotic fluid constituents and fibrin [15];
- the membranes form as early as 2-3 hours after the insurgence of RDS. They are well formed by 8-12 hours; in the absence of high oxygen tensions and mechanical ventilation, resolution by 24-48 hours occurs;
- membranes can be stained bright yellow in infants with kernicterus, in the setting of unconjugated bilirubin, intraventricular hemorrhage, intrahepatic bile stasis, and disseminated intravascular coagulation [4];

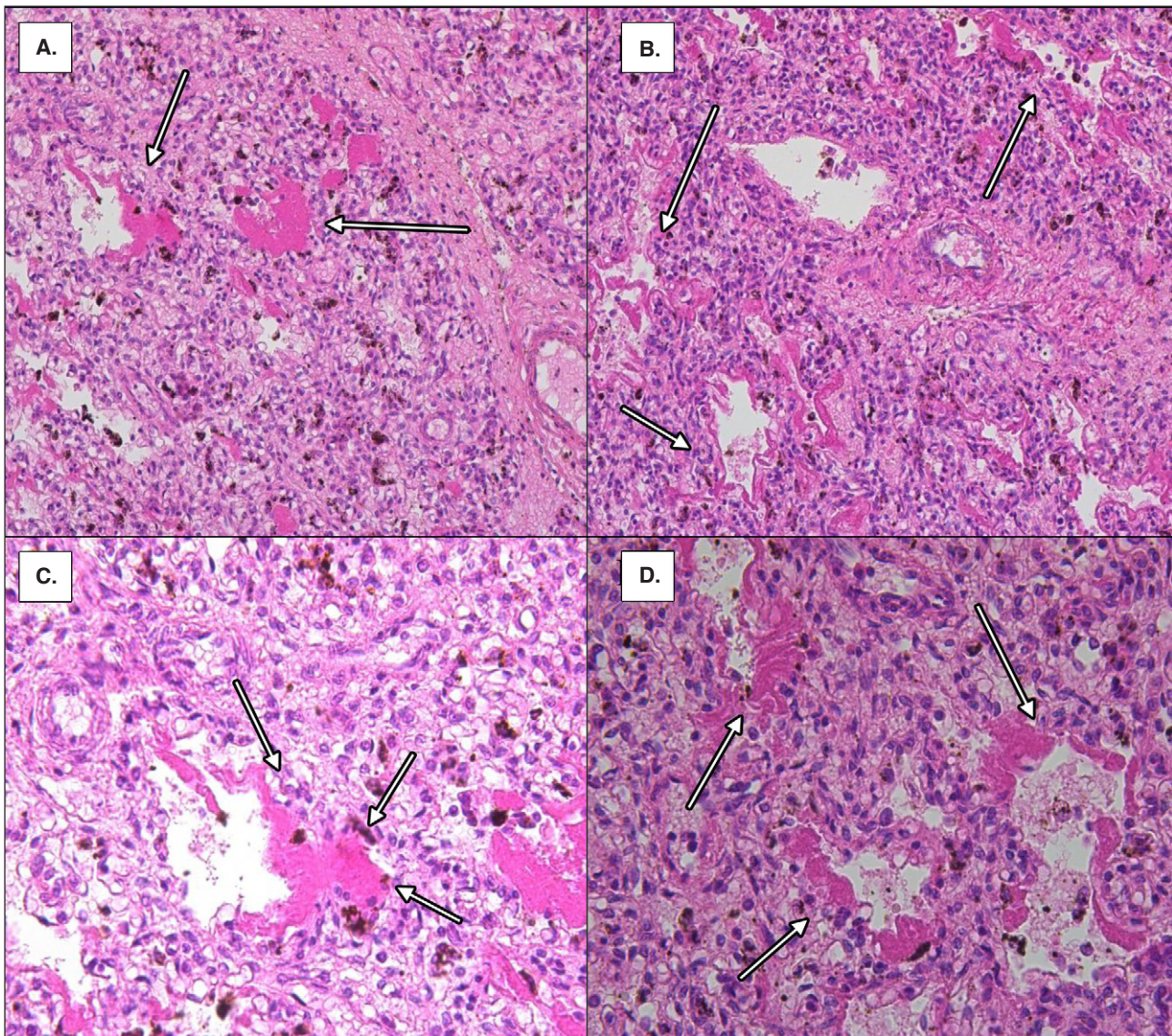


Figure 4. A, B. Preterm human lung: hyaline membrane disease (HMD) are shown (arrows). Hematoxylin-eosin (HE). OMx200. C, D. Preterm human lung: HMD are shown (arrows). HE. OMx400.

- e. alveolar collapse and congestion of alveolar capillaries, associated with epithelial desquamation of the terminal airways are frequently observed;
- f. hyaline membranes are frequently absent in infants affected by RDS who die at less than 4 hours of age;
- g. fibroblastic organization leading to bronchopulmonary dysplasia (BPD) may be present or start within 36 hours after birth, in affected newborns submitted to reoxygenation utilizing high O_2 percentages.

Complications

Major complications of RDS of the neonate, frequently observed at autopsy, are:

- intraventricular cerebral hemorrhage;
- persistence of the patent ductus arteriosus;
- necrotizing enterocolitis (NEC);
- retinopathy of prematurity (ROP) or retrolental fibroplasia (RLF);
- bronchopulmonary dysplasia (BPD).

ROP and BPD are generally considered late complications of RDS that usually occur in infants weighing less than 1,500 g, who were maintained on a mechanical respiration for more than 6 days (**Fig. 5**) [16].

However, very recent data obtained with metabolomics suggest that BPD is a congenital disease and that postnatal interventions only determine severity of the form, not its presence or absence [17-20].

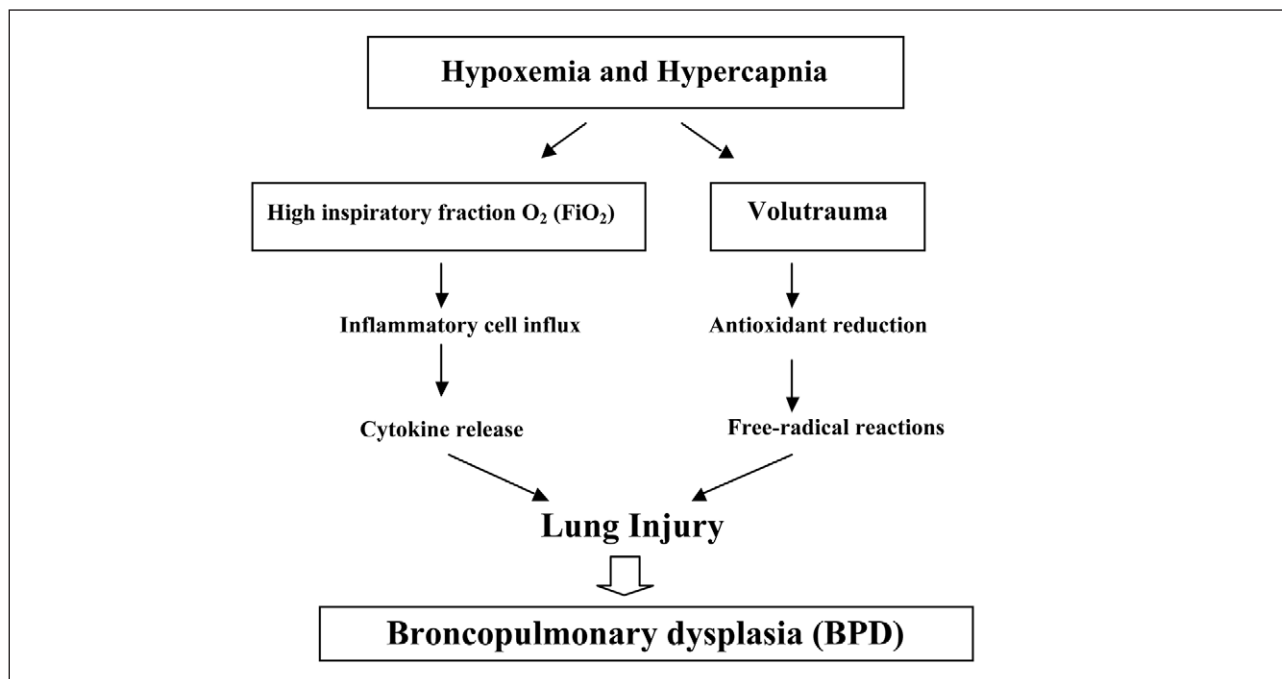


Figure 5. Complications of respiratory distress syndrome (RDS), as classically described.

Differential diagnosis

Although characteristic of HMD, hyaline membranes are a nonspecific finding with a number of causes, which should be considered especially in the term and post-term infant with RDS. Hyaline membranes can be seen associated with meconium aspiration, neonatal pneumonia, pulmonary edema and hemorrhage and with various irritants to the terminal airways and alveoli.

Discussion

The finding of hyaline membranes at the histological examination of lungs is a frequent event in preterm newborns died in the clinical setting of RDS. Even though hyaline membranes should not be considered specific, on the other hand they are characteristic of HMD and, in the majority of cases, allow to confirm the clinical diagnosis.

The role of the pathologist should not be restricted to description of the presence of hyaline membranes in the neonatal lung: other pathological data may be important for reaching a complete clinical-pathological diagnosis. The most important histological features required in these cases are here reported.

Architectural lung development

The first approach to the histological study of a neonatal lung should include the evaluation of lung development. In order to give the clinician useful data regarding the ability of the newborn lung to function in the extrauterine environment, the following features should be reported:

- the degree of alveolarization of saccular structures;
- the presence of reticulin fibers, typically diffuse in the immature lung;
- the presence of elastic fibers, typical of lung maturation;
- the differentiation of alveolar epithelium, with the evolution from cuboidal undifferentiated progenitor cells to type I pneumocytes;
- the degree of maturation of the pulmonary arterial wall and in particular the persistence of fetal arteries, characterized by a narrow lumen and a thick muscular wall;
- the differentiation of the alveolar cells responsible for surfactant production. To this end, immunohistochemistry against surfactant protein-A and B is mandatory.

Vascular changes

After the evaluation of the architectural changes, the pathologist should focus on the most

important pathogenetic lesion in HMD: alveolar capillaries, and in particular to endothelial dysfunction in these structures. The following sequential endothelial morphological changes should be evaluated:

- a. endothelial swelling;
- b. endothelial apoptosis;
- c. endothelial detachment;
- d. complete loss of the endothelial barrier.

The presence of endothelial changes should be looked for in alveolar capillary, in arterial and venous branches. It should also be correlated with the degree of hyaline membranes deposition, endothelial damage representing the most important pathological lesion leading to the formation of hyaline membranes.

Perivascular edema

One of the most important consequences of endothelial dysfunction, and in particular of the endothelial barrier loss, is the leakage of liquid and proteins into the interstitial space, leading to inter-alveolar edema. At histology, it causes the enlargement of inter-alveolar septa.

Intravascular coagulation

The other most important consequence of endothelial dysfunction is represented by diffuse intravascular coagulation (DIC). The presence of multiple thrombi in the peri-alveolar capillaries is associated with hypoxic changes in alveolar cells, aggravating the pulmonary lesions during the development of HMD.

Hyaline membrane morphology

The last phases of the histological study of the neonatal lung in the setting of HMD should focus on morphology of hyaline membranes. The following features should be reported:

- a. the degree of diffusion, from focal to diffuse;
- b. the principal pulmonary structures affected by hyaline membrane deposition, including alveoli, alveolar ducts and respiratory bronchioles;
- c. the shape of membranes, changing from linear to irregular and fragmented;
- d. the presence of macrophages phagocytosing remnants of hyaline membranes, a finding suggestive for the activation of mechanisms responsible for removal of membranes.

Inflammation

The absence of significant inflammatory lesions should be considered typical of HMD. In rare cases, occasional scattered granulocytes can be observed in the alveolar capillary lumen or in the interstitial spaces. The finding of a marked granulocytic infiltrate in the alveolar lumen should be considered as typical of associated pneumonia and evaluated according to clinical data.

Immunohistochemistry

Whereas the most important pathological changes may be clearly evidenced on H&E-stained sections, immunohistochemistry may be useful. Anti-surfactant antibodies may allow an accurate evaluation of lung maturation. Anti-smooth muscle actin (SMA) antibodies may be utilized for evidencing the presence of fetal muscular arteries. Anti-CD31 antibodies, evidencing vascular endothelial cells, are important for the accurate evaluation of the development of the capillary network. Antibodies against cytokeratin 7 may allow the accurate study of alveolar epithelial precursors and of type I pneumocytes.

Conclusion

In conclusion, according with our experience, the histological examination of neonatal lung in the clinical setting of HMD may allow the pathologist to give important data to neonatologists, in order to reach a complete clinical-pathological final diagnosis.

Given that the vast majority of pulmonary histological changes are not specific of a single disease entity, the dialogue between the pathologist and the neonatologist appears mandatory in order to correlate microscopic changes with the clinical picture.

Finally, the distinction at histology between tissue and cell changes due to the incomplete lung development and pathological features related to RDS may help to better understand the pathogenesis of hyaline membranes deposition in a single infant, allowing neonatologist to a better interpretation of the clinical history.

Declaration of interest

The Authors declare that there is no conflict of interest.

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