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Respiratory distress syndrome of the preterm neonate — placenta and necropsy as witnesses

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Abstract

Aim. To assess the agreement between clinical diagnosis of hyaline membrane disease (HMD) and lung necropsy pathological findings of deceased neonates.

Material and methods. Review of clinical files and necropsy studies of 40 newborn infants ≤ 37 weeks gestational age.

Results. The concordance between clinics and necropsy for the diagnosis of HMD was 43% ($n = 17$). At the necropsy study of the lungs, 11 cases (28%) of clinically diagnosed HMD were associated to meconium aspiration, pneumonia, or pulmonary hemorrhage; 12 (30%) cases were pneumonia and/or meconium aspiration and pulmonary hemorrhage without hyaline membranes. Of the 17 pneumonias, 15 (88%) were associated to histological chorioamnionitis, RR 3.76 (95%CI: 1.9–4.2) ($p < 0.001$).

Conclusions. The clinical diagnosis of HMD needs a cautious interpretation, as it may be mistaken, or HMD may occur in association with other pathological situations enhancing a more ominous prognosis.

Keywords: *Respiratory distress syndrome, hyaline membrane disease, autopsy, chorioamnionitis, placenta*

Introduction

Respiratory distress syndrome (RDS) is one of the most common causes of morbidity in preterm neonates, although lack of a precise definition in infants with very low birth weight necessitates cautious interpretation of statistics regarding incidence, mortality, and outcome [1]. There are many causes of acute RDS in the preterm newborn; the most common cause, however, is RDS, also known as hyaline membrane disease (HMD) [2].

Histologically, hyaline membranes occur lining the terminal airways. This gives the condition its alternative name – HMD – which should only be used in the presence of histological confirmation. Other clinical scenarios such as infection (pneumonia)/inflammation or aspiration syndromes may cause an acute respiratory distress, with or without hyaline membranes formation [2].

We have analyzed the lung necropsy findings and placenta histology of deceased newborns whose diagnosis of HMD was made on a combination of clinical and radiographic features, to assess the extent of agreement between clinics and pathological findings.

Material and methods

We reviewed the clinical charts of 40 newborn infants of 37 weeks gestational age or less, whose parents consented to necropsy, and that were deceased between 1997 and 2005, a 9-year period. All the 40 newborns were deceased during the first 7 days of life, and diagnosed with a HMD on a combination of clinical and radiographic features according to the criteria of RDS of the Vermont Oxford Network (VON), once our unit is a collaborative member. The VON criteria of RDS are: (1) $PaO_2 < 50$ mmHg in room air, central cyanosis in room air, a requirement for supplemental oxygen to maintain $PaO_2 > 50$ mmHg, or a requirement for supplemental oxygen to maintain a pulse oximeter saturation over 85% within the first 24 h of life and; (2) a chest radiograph consistent with RDS (reticulogranular appearance to lung fields with or without low lung volumes and air bronchograms) within the first 24 h of life. We use, at our unit, for practical purposes, the classification from I to III (I – light; II – moderate; III – severe), according to the X-ray appearance ranging from a light reticulogranular with air bronchograms to white lungs, adapted from the classification of Couchard et al. [3].

Gestational age (we considered the completed weeks) was assessed by menstrual age (women with regular menstrual cycles), ultrasound examination (when a discrepancy of two or more weeks existed between the age derived by menstrual dating and the age derived sonographically, or in the absence of a menstrual date) [4] or the New Ballard Score (in the absence of obstetrical indexes) [5]. Intrauterine growth restriction (IUGR) was defined as a birth weight below 10th centile of Fenton's fetal growth charts [6]. Histological chorioamnionitis was classified according to the method proposed by Blanc WA [7].

As routine, autopsies are performed between 12 and 24 h of death. In some particular cases, in may be done after 24 h. In this study, complete autopsies were performed in the first 24 h after death, in all cases. The

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routine handling of the lungs during autopsy is as aseptic as possible. Lungs are weighted together, and a floating test in water is performed. If the lungs are inflated, they are prepared for pathological studied inflated. Then, lungs are fixed in formaldehyde. A fragment of each of the five pulmonary lobes is obtained for study. If needed, according to clinical situation or lungs macroscopic appearance, more than one fragment of any particular lobe may be obtained. A piece of each fragment is send to microbiological study. A slide of each lobe, stained with hematoxylin and eosin, is examined microscopically.

HMD is diagnosed in the presence of eosinophilic membranes (hyaline membranes) lining the visible airways, that normally constitute terminal bronchioles and alveolar ducts [2]. Meconium is a granular eosinophilic material with nonnucleated scales. In this study, meconium was diagnosed by the presence of pigment alone, no special stains were used. Pneumonia was diagnosed in the basis of an inflammatory reaction with polymorphonuclear leukocytes in the in airspaces and lung interstitial tissue [2]. Bacteria were infrequently seen in this study. Cultures for bacteria were negative, probably because antibiotics had been administered mothers and newborns.

We collected data related to birthweight, gestational age at birth, gender, IUGR, antenatal steroids (any dose), severity of HMD, use of mechanical ventilation, the presence of perinatal infectious context (maternal fever, urinary tract infection, white blood cell over 12,000/mm^3, rupture of membranes over 18 h, fetid amniotic fluid, leucorrhoea, clinical chorioamnionitis; note: group B streptococci screening has begun after 2005 at 35 weeks gestation, and was not considered in this study), age of death and clinical cause, placental findings, namely the presence of inflammation/ infection (chorioamnionitis, with or without chorionic vasculitis and/or funisitis) necropsy study findings on the lungs and the cause of death given at the autopsy.

The concordance between clinical diagnosis of HMD and that given by necropsy findings was assessed. The association between pneumonia findings at the necropsy study and histological chorioamnionitis (any degree) was assessed using relative risks (RR) and their 95% confidence intervals (95%CI).

The study protocol has been approved by the institute's committee on human research.

Results

Demographics and clinical characteristics of the study population ($n = 40$) are reported in Table I. All neonates, except one, were preterm (<37 weeks gestation). The clinical causes of death and those found at the autopsy examination are reported in Table II, and the degree of total concordance between clinical and necropsy findings was 23% ($n = 9$). The autopsy added some information to clinics in 16 (40%) cases and changed the cause of death in other 15 (37.5%) cases.

All patients were diagnosed a HMD on clinical grounds. The necropsy study revealed some other findings (Table III). Twenty-nine (73%) diagnosis of HMD were made at histological examination. There were 17 (43%) histological diagnosis of HMD in accordance to clinical diagnosis (including those with pulmonary hypoplasia). In 12 (30%) cases, the necropsy histological examination of the lungs added information to the clinical diagnosis of HMD, and in 11 (27.5%) cases the diagnosis of HMD was not confirmed, and other diagnosis were found.

Table I. Demographics and clinical characteristics ($n = 40$).

Gestational age (weeks), median (min–max)	27 (23–37)
Birthweight (g), median (min–max)	978 (460–2745)
Gender M/F, *n* (%)	20 (50)/20 (50)
IUGR, *n* (%)	4 (10)
Any antenatal steroid, *n* (%)	24 (60)
Hyaline membrane disease, *n* (%)	40 (100)
I	8 (20)
II	10 (25)
III	22 (55)
Surfactant, *n* (%)	36 (90)
Mechanical ventilation, *n* (%)	40 (100)
Perinatal infectious context, *n* (%)	11 (28)
Histological chorioamnionitis, *n* (%)	19 (48)
grade I, *n*	2
grade II, *n*	7
grade III, *n*	10
chorionic vasculitis, *n*	12
funisitis, *n*	10
Day of death, median (min–max)	2 (1–7)

IUGR, intrauterine growth restriction.

Eleven cases (28%) of clinically diagnosed HMD were, at the histological study of the lung, meconium aspiration syndromes, congenital pneumonias, or pulmonary hemorrhages. The 12 cases, in which meconium was detected in the lungs occurred in preterm neonates with a median gestational age of 26 weeks (23–34).

We found an evident perinatal infectious context in 11 (28%) cases. Placental histological chorioamnionitis was reported in 19 (48%) cases. Three cases of histological chorioamnionitis had no perinatal evident infectious context. Seventeen pneumonias were diagnosed at the histological exam of lung tissues, in seven cases associated to meconium aspiration. Of the 17 cases of pneumonia, 15 (88%) were associated to histological chorioamnionitis. The relative risk of pneumonia in neonates born from mothers with chorioamnionitis (any degree) was 3.76 (95%CI: 1.9–4.2) ($p < 0.01$).

Discussion

RDS is an acute illness, usually of preterm infants, and the classic clinical presentation is characterized by a respiratory rate over 60/min, dyspnoea (intercostals, subcostal indrawing, sternal retraction, nasal flaring, cyanosis) with a predominantly diaphragmatic breathing pattern and a characteristic expiratory grunt or moan, all presenting within 4–6 h of delivery [8]. Oxygen administration is required to prevent cyanosis, and there is a reticulogranular chest X-ray appearance as a result of widespread atelectasis. The condition is characterized by noncompliant (stiff) lungs, which contain less surfactant than normal and become atelectatic at end-expiration. The characteristic picture is modified in many infants as a result of the early administration of exogenous surfactant and immediate assisted ventilation [9]. The diagnosis can be established pathologically or by biochemical documentation of surfactant deficiency, nonetheless, most series refer only to a combination of clinical and radiographic features [9].

The lungs of infants who succumb from RDS have a characteristic uniformly ruddy and airless appearance, macroscopically resembling hepatic tissue. Microscopically, atelectactic alveoli are poorly developed, and those

Table II. Causes of death ($n = 40$).

Clinical	Autopsy
HMD = 4	HMD = 1
	Pulmonary hemorrhage = 1
	Pneumonia = 2
HMD + anemia (twin-twin transfusion) = 1	HMD + anemia (twin–twin transfusion) = 1
HMD + pulmonary hemorrhage = 1	Pulmonary hemorrhage = 1
HMD + pulmonary hypoplasia = 4	HMD + pulmonary hypoplasia = 3
	HMD + fracture of the spine with severe intra-spinal hemorrhage = 1
HMD + IVH – III = 1	HMD + IVH – III = 1
Pulmonary hemorrhage = 2	HMD = 1
	HMD + esophageal perforation and hemorhage = 1
Pulmonary hypoplasia = 1	HMD + pulmonary hypoplasia + pneumonia + MAS = 1
Bilateral IVH – IV = 5	Bilateral IVH – IV = 1
	Bilateral IVH – IV + HMD = 1
	Bilateral IVH – IV + pneumonia = 2
	MAS = 1
Asphyxia = 1	Asphyxia = 1
NEC = 1	NEC + pneumonia = 1
Sepsis = 9	Pneumonia = 5
	HMD = 2
	Bilateral IVH – IV = 2
CHD = 3	CHD (CoAo) + HMD + pneumonia + MAS = 1
	CHD (mitral anomaly) + HMD = 1
	HMD = 1
Extreme immaturity = 4	Pneumonia = 2
	Hypoxia + IVH – II = 2
Unknown = 3	HMD = 1
	Pneumonia = 2

CHD, congenital heart defect; CoAo, coartation of aorta; HMD, hyaline membrane disease; IVH, intraventricular hemorrhage; MAS, meconium aspiration syndrome; NEC, necrotizing enterocolitis.

Table III. Diagnostic findings in lung histology.

HMD	Lung histology
HMD – I ($n = 8$) (infectious context = 3)	HMD + VILI = 2
	HMD + MAS + pneumonia + pulmonary hypoplasia = 1
	HMD + MAS + pneumonia = 1
	Pneumonia = 2 (fungal = 1; bacterial = 1)
	Pulmonary hemorrhage = 1
	MAS + VILI = 1
HMD – II ($n = 10$) (infectious context = 4)	HMD = 3
	HMD + VILI = 1
	HMD + pulmonary hypoplasia + VILI = 1
	HMD + pneumonia = 2
	HMD + MAS + pneumonia = 1
	Pneumonia = 1
	MAS + pneumonia = 1
HMD – III ($n = 22$) (infectious context = 4)	HMD = 3
	HMD + VILI = 5
	HMD + pulmonary hypoplasia + VILI = 2
	HMD + MAS = 2
	HMD + pneumonia = 2
	HMD + MAS + pneumonia = 3
	Pneumonia = 3 (fungal = 1; bacterial = 2)
	MAS = 2

HMD, hyaline membrane disease; MAS, meconium aspiration syndrome; VILI, ventilation induced lung injury.

that are present are collapsed. When the infant dies early in the course of the disease, necrotic cellular debris is present in the terminal bronchioles and alveolar ducts. Later the necrotic material becomes incorporated within eosinophilic hyaline membranes lining the respiratory bronchioles, alveolar ducts, and random alveoli, mostly the proximal alveoli. The membranes are largely made up of fibrinogen and fibrin admixed with cell debris derived chiefly from necrotic type II pneumocytes. There is a remarkable paucity of neutrophilic inflammatory reaction associated with these membranes. The lesions of HMD are never seen in stillborn infants or in newborns who die within a few hours of birth. The recovery phase is characterized by regeneration of alveolar cells, including type II cells, with a resultant increase in surfactant activity [2].

In this study, we reviewed the necropsy studies of the deceased neonates with the diagnosis of HMD, whose parents consented to autopsy. We focused our attention mainly in lung tissue findings and it was surprising that only 40% of the clinical HMDs were confirmed at the necropsy study. The other 60% of diagnosed HMD were associated to, or were congenital pneumonias, meconium aspiration, or pulmonary hemorrhage.

In this study, as in others, autopsy added information to that given by clinics, or changed the cause of death, in a significant number of cases [10].

The numerous cases of congenital pneumonias are according to inflammation and preterm birth, and it was not surprising that pneumonias and placental histological findings of chorioamnionitis were significantly associated. Numerous studies have shown that the placenta plays an instrumental role in ascertaining the cause of the adverse perinatal outcome. The placenta has also been called the 'diary of gestational life' [11].

Acute chorioamnionitis is defined histologically, as acute inflammatory cells within the fetal membranes, the amnion and the chorion. It is indicative of an ascending bacterial infection. Initially, the inflammatory response to bacteria that enter the uterine cavity is maternal in origin. If the infection continues and goes unchecked, there will be a fetal inflammatory response that includes the production of cytokines and inflammatory mediators, with acute inflammatory cells migrating out of fetal vessels in the umbilical cord (acute funisitis) and chorionic plate. These events generally occur over a period of days. If the fetus becomes infected, decreased pulmonary function and oxygenation may develop due to acute bronchopneumonia, and if the fetus becomes septic, systemic effects such as hypotension and decreased vascular perfusion will result [12].

A surprising finding in this study was the meconium in the alveolar spaces of preterm deceased neonates, some of 23 and 24 weeks of gestation. We know that control of fetal meconium passage depends on hormonal and parasympathetic neural maturation and is more common after 34 weeks gestation [13]. The exact mechanisms for *in utero* passage of meconium remain unclear, but fetal distress and

vagal stimulation are two probable factors. Also, the passage of meconium below 34 weeks gestation may represent bilious reflux secondary to intestinal obstruction, and an aspiration into the airways may occur [13].

Recently, Mortensen and Kearney [14] described meconium aspiration in autopsy studies of mid trimester pregnancy fetuses.

We have admitted other preterm neonates that were deceased over the last years, with the diagnosis of HMD, but whose parents did not consent to autopsy. Probably, some of these cases also reflect infection/inflammation and/or meconium aspiration syndromes or pulmonary hemorrhages, which were not diagnosed on clinical grounds. Also we have admitted many other preterm neonates diagnosed with HMD that had a favorable outcome, and we cannot be sure at what extent infection/inflammation, meconium or hemorrhage were present. It is rational to think that those cases associated with a fatal outcome were those associated to infection/ inflammation or aspiration syndromes, and the cases with good outcome were not.

It is relatively easy to label a preterm newborn as having a HMD and treat it with surfactant and mechanical ventilation, when the clinical signs of respiratory distress are present and the chest X-ray reveals no clear lungs. However, this may not be so straight. Until which extent we are facing an infection, an inflammation, an aspiration syndrome or a pulmonary hemorrhage? I think we really do not know the answer to this question.

Some centers may have the result of placenta histological examination in a few days after birth, and this may help in understanding the course of the disease and response to treatment. It may also help in the decision of keeping on or discontinue antibiotics.

In conclusion, the classical picture of RDS of the preterm neonate necessitates cautious interpretation, as many other causes may be subjacent, and a hyaline membrane may occur 'alone' or it may be associated with a more ominous diagnosis. The histological diagnosis of chorioamnionitis is of great interest in helping clinician's decisions and expectations. A consented necropsy study should be performed to all deceased newborns, as it significantly increases the acuity of diagnosis and it may help in clarifying the cause of death.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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