

Lung injury patterns in newborns, infants and young children – morphological and immunohistochemical approaches

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Abstract

Lower respiratory infections are an important cause of morbidity and mortality in children, especially in newborns, infants and young children. We conducted a retrospective study and we analyzed the causes of death in newborns, infants and young children, in the necropsy protocols from two Departments of Pathology (Mureș County Hospital and Emergency County Hospital of Târgu Mureș, Romania), between 2016–2018. We performed descriptive statistics: number of necropsies per year, distribution by gender (male/female), by place of origin (rural/urban), by age and leading causes of death in our study. To establish the pattern of lung injuries, we performed a morphological, histopathological and immunohistochemical study [cluster of differentiation (CD) 3, CD14, CD20, CD31, CD34, CD68]. Our study is showing the most frequent and typical aspects of pulmonary pathologies in fetuses, newborns, infants and young children. In this way, we are highlighting the microscopic aspects of the immature lung, amniotic fluid and meconium aspiration, pulmonary distress syndrome in children, pneumonia, bronchopneumonia and vascular pulmonary disease developed in patients with congenital cardiac defects. Most deaths were recorded in the first 30 days or in the first year of life. Primary respiratory diseases were the leading causes of death in these patients. Secondary respiratory diseases were associated with the major causes of death in these patients as an aggravating or precipitating factor.

Keywords: lung injuries, newborns, infants, aspiration of amniotic fluid, pneumonia.

Introduction

Lower respiratory infections are an important cause of morbidity and mortality in children, especially in infants and young children [1–3]. The rate of infant mortality is defined by the ratio between the number of children who have deceased below the first year of age and the number of children born alive in the same year. It is reported to 1000 newborn living children. This rate can be expressed at five years as well and it is represented by the ratio between the number of deaths of children less than 5 years old and the number of newborn living children from the same time lapse [4, 5].

Among with the general natality and mortality, it is the most accurate indicator regarding the evolution of a country's population, but indirectly it also reflects the socioeconomic and cultural status of that population, the level of sanitary education and the value of the health system in that particular country [6, 7].

According to *World Health Organization* (WHO)

statistics, in 2017, more than 6.3 million children have died around the world. There were 5.4 million deaths in children under the age of 5, and 2.5 million deaths in newborns in the first month of living. The main causes of death were perinatal complications [8], pneumonia, birth asphyxia [9, 10], and malaria.

The EUROSTAT statistics shows that Romania occupies the last place in Europe regarding the children mortality, starting from 2006 until 2017 (the most recent report). In 2006, the mean in the European Union (EU) was held at 4.6/1000, while in Romania it reached 13.9/1000. In 2017, EU reached a mean of 3.6/1000 and Romania a mean of 6.7/1000.

The lowest mortality rates in children are encountered in: Cyprus 1.3/1000, Finland 2.0/1000, Slovenia 2.1/1000, Estonia and Norway with 2.3/1000, and Sweden with 2.4/1000. In comparison with 2006, in Romania we can see a reduction of this indicator at half of its value, which shows a significant improvement in child care, starting with the assistance given to the pregnant women, the

nursing of the mother and the child and ending with the development of maternity hospitals and children hospitals. Even with these changes, the number of deaths in children in Romania is alarming.

Aim

The purpose of the study was to analyze the causes of death in newborns, infants and young children, in the necropsy protocols, in our pediatric patients. The particularity of this paper was to establish the pattern of lung injuries that led to death.

Materials and Methods

We conducted a retrospective study and we analyzed the causes of death in newborns, infants and young children in the necropsy protocols from two Departments of Pathology (Mureș County Hospital and Emergency County Hospital of Târgu Mureș, Romania), between 2016–2018. We performed descriptive statistics: number of necropsies per year, distribution by gender (male/female), by place of origin (rural/urban), by age and leading causes of death in our study. To establish the pattern of lung injuries, we performed a morphological, histopathological (HP) and immunohistochemical (IHC) study.

Tissue samples were collected from the necropsy, and were processed by routine HP techniques: were fixed in 10% neutral buffered formalin, paraffin embedded and stained with Hematoxylin–Eosin (HE). An IHC analysis was performed on 4 µm-thick sections prepared from formalin-fixed paraffin-embedded tissues by using an automated immunostainer (BechMark GX, Ventana Medical Systems Inc., Tucson, AZ, USA). IHC assays were performed on a Ventana BenchMark GX automated staining instrument, according to the manufacturer's instructions. Slides were deparaffinized using EZ Prep solution (Ventana Medical Systems, Inc.), at 90°C, and all reagents and incubation times were chosen as directed on antibody package inserts. Slides were developed using the OmniMap 3,3'-Diaminobenzidine (DAB) detection kit (Ventana Medical Systems, Inc.) and counterstained with Hematoxylin. Table 1 shows the antibodies used for immunohistochemistry.

Table 1 – Antibodies used for immunohistochemistry

Antibody (clone)	Source	Reactivity	Dilution
CD3 (2GV6)	Ventana Medical Systems, Inc.	T-lymphocytes	RTU
CD14 (EPR1653)		Macrophages and neutrophils	
CD20 (L26)		B-lymphocytes	
CD31 (JC70)		Endothelial cells	
CD34 (QBEnd/10)		Endothelial cells	
CD68 (KP-1)		Macrophages	

CD: Cluster of differentiation; RTU: Ready-to-use.

For inflammatory cells (T-lymphocytes, B-lymphocytes and macrophages), for cluster of differentiation (CD) 3, CD20 and CD68 reactivity, sections were incubated with anti-CD3 primary monoclonal antibody [clone 2GV6, ready-to-use (RTU), Ventana], anti-CD20 antibody (clone L26, RTU, Ventana), and anti-CD68 antibody (clone KP-1, RTU, Ventana), according with the protocol recommended by the manufacturer. For each run, a positive control slide (tonsil) was performed. Regarding the CD3, CD20 and CD68 expression, we analyzed the cellular expression (membranous).

For CD14 reactivity, sections were incubated with anti-CD14 primary monoclonal antibody (clone EPR1653, RTU, Ventana), according with the protocol recommended by the manufacturer. For each run was performed a positive control slide (tonsil). Regarding the CD14 expression, we analyzed the cellular expression (membranous, cytoplasmic). CD14 was expressed in alveolar macrophages and in monocytes from the blood vessels.

For endothelial cells, for CD31 and CD34 reactivity, sections were incubated with anti-CD31 primary monoclonal antibody (clone JC70, RTU, Ventana) and anti-CD34 antibody (clone QBEnd/10, RTU, Ventana), according with the protocol recommended by the manufacturer. Regarding the CD31 expression, we analyzed the cellular expression (membranous).

The study has been approved by the Ethics Committees of the Clinical Hospitals and of the “George Emil Palade” University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș.

Results

Between 2016 and 2018, the two above-mentioned Departments of Pathology have conducted a number of 226 necropsies in children, from newborns to 14 years old. The distribution per year was 83 in 2016, 89 in 2017 and 54 in 2018 (Figure 1). One hundred twenty-two (53.99%) children were males and 104 (46.01%) were females. Eighty-five (37.62%) children came from urban area and 141 (62.38%) from the rural area (Figure 2).

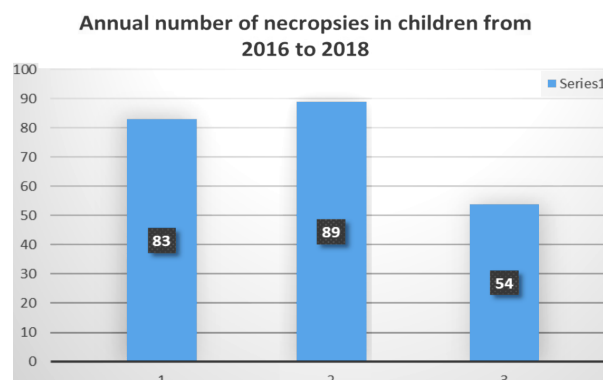


Figure 1 – Number of necropsies in children.

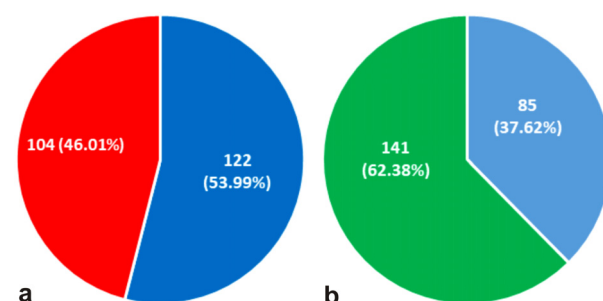


Figure 2 – (a) Gender distribution of deaths; (b) Rural vs. urban distribution of patients.

The distribution based on age interval showed that 88 (38.93%) newborns have died before birth (Figure 3), 74 (32.74%) newborn living children have died in the first 30 days after birth and 53 (23.45%) infants died in their first year of age (Figure 4). Table 2 shows the causes of deaths by age group.

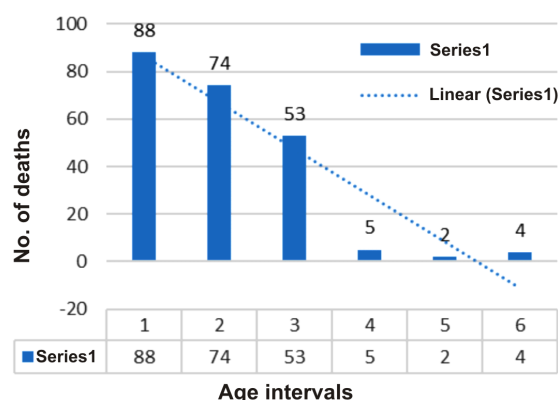


Figure 3 – Age distribution. 1: Dead newborns before birth; 2: Alive newborns (days 1–30); 3: Dead infants (months 1–12); 4: Dead toddlers (1–2 years old); 5: Dead preschoolers (3–6 years old); 6: Dead grade-schoolers (7–14 years old).

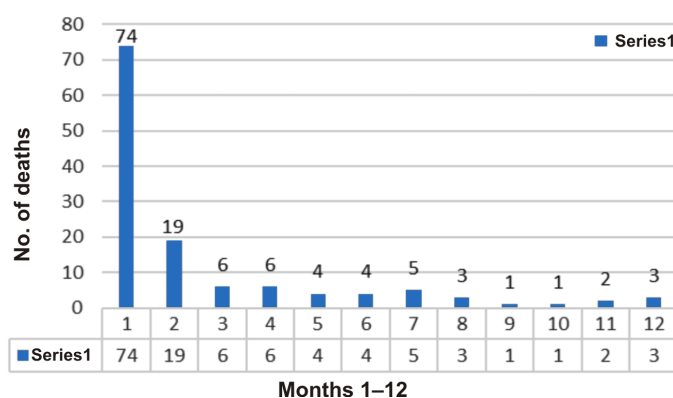


Figure 4 – Number of deaths in the first year.

Table 2 – Causes of deaths by age group

The causes of death in antepartum fetal period	
Fetal causes	Pulmonary anectasis; Non-ventilated lungs; Immature lungs; Amniotic fluid and meconium; Aspiration syndrome.
Maternal causes	Retroplacental hemorrhage; Rh-immunization.
Intrauterine fetal deaths	Medical therapeutic abortion
The causes of death in alive newborns	
Perinatal causes	TORCH syndrome: ▪ Congenital toxoplasmosis; ▪ Perinatal measles (rubella); ▪ Cytomegalovirus infection.
Genetic causes – genetic predisposition (the major risk)	Trisomy 13; Trisomy 18; Edwards syndrome; Turner syndrome.
Central nervous system	Anencephaly; Severe congenital hydrocephalus; Cerebral hemorrhage; Myelomeningocele.
Cardiovascular system	CHD: ▪ Cyanogenic: tetralogy of Fallot, transposition of the great arteries, single congenital ventricle; ▪ Acyanogenic: ASD, VSD; ▪ Associated with severe pulmonary hypertension, with pulmonary vascular disease stages II, III or IV.
Respiratory system	Pneumonia; Bronchopneumonia; Hyaline membrane pneumopathy; Shock lungs.
Digestive system	Duodenal atresia; Jejunal atresia.
Urinary and reproductive system	Congenital hydronephrosis; Malformations of external and internal genitalia.
The causes of death after first year of life	
1–2 years old (toddlers)	Severe CHD associated with cardiovascular and pulmonary complications; Respiratory infections: bronchopneumonia, sepsis.
3–6 years old (preschoolers)	Severe CHD associated with cardiovascular and pulmonary complications; Respiratory infections: bronchopneumonia, sepsis, aspiration pneumonia, laryngomalacia.

The causes of death after first year of life	
7–14 years old (grade-schoolers)	10 years old: brain tumor, left frontal lobe metastasis; 10 years old: generalized peritonitis secondary to multiple intestinal perforations; 12 years old: pluriformative syndrome (trisomy 13 and 18); 13 years old: cachexia, aspiration pneumonia, multiple pulmonary abscesses.

TORCH: Toxoplasmosis, Other agents, Rubella, Cytomegalovirus, and Herpes simplex; CHD: Congenital heart disease; ASD: Atrial septal defect; VSD: Ventricular septal defect.

Along with the descriptive statistics data, our study is also presenting the most frequent and atypical aspects of pulmonary pathology in fetuses, newborns and infants.

In case of pulmonary immaturity, the pulmonary parenchyma can be seen in one of its four developing stages: pseudoglandular, canalicular, saccular or alveolar. The immature lung parenchyma shows aspects from the saccular stage, with autolysis of the bronchial mucosa and intrasaccular cellular content. The reduction of the space between the lumen of the respiratory airways and blood capillaries can be observed. The alveolo-capillary membrane is starting to take a mature aspect that will be observed at birth and during adulthood. The type II pneumocytes can be observed as well, they are cuboidal cells that are able to produce surfactant in this particular stage (Figure 5A).

Pulmonary anectasis shows non-aerated, compressed alveolar spaces, thickening of the septa and congested blood vessels (Figure 5B). In pulmonary dystelectasis, parenchyma had non-aerated alveolar spaces, containing an eosinophilic fluid represented by edema (Figure 5C). In the same patient, in the surrounding areas affected by edema, aspects of compensatory emphysema, with the rupture of the alveolar septa and normal alveoli (Figure 5D).

In our study, the most frequent causes of death during birth or immediately postpartum (during the first days after birth) were the amniotic fluid aspiration, accompanied or not by aspiration pneumonia, if the newborn has survived after birth.

In case of massive aspiration of amniotic fluid were also found squamous epithelial cells and keratin blades, originating from the physiological desquamation of the fetal skin. The remnants of lanugo can also be observed under the form of hairs shafts. Some alveoli are weakly aerated, others are completely compressed, lacking air or containing only fluid. The alveolar septa are thickened and the blood vessels contain erythrocytes. The meconial corpuscles have a round or ovalar shape and are basophilic or brown stained. The pulmonary parenchyma after a massive meconial aspiration presents numerous brown meconial corpuscles, desquamated alveolocytes and congested blood vessels in the thickened alveolar septa (Figure 6, A–D).

The microscopic aspects of pneumonia and bronchopneumonia in newborns and toddlers is showing in our study: aspects of suppurative bronchiolitis, with the complete obstruction of a bronchiole and the destruction of the mucosal epithelium and partially, of the bronchiole wall. They are also presented hyaline, eosinophilic membranes that are partially lining the alveoli (the early stages of the respiratory distress syndrome in newborns).

Inside the alveoli spaces, we can notice an eosinophilic liquid, along with numerous inflammatory cells and desquamated alveolocytes (Figure 7A). It can see areas with rupture of the alveoli septa, partially aerated alveoli and partially obstructed alveoli, containing fluid. In the right side of the image, it can be remarked a medium-sized blood vessel in which we can notice the formation of a fibrinous thrombus and a smaller blood vessel that

presents a hyaline microthrombus, expressions of disseminated intravascular coagulation (DIC) (Figure 7B). The characteristic aspect of massive pneumonia, with suppuration was the presence of a large fibrino-leukocyte exudate (especially granulocytes neutrophils) that occupies the entire surface of the alveolar sacks. The blood vessels are congested. It can no longer observe aerated spaces. This aspect is characteristic for the red hepatization stage in the clinically evolution of pneumonia (Figure 7, C and D). The microscopic aspects of pneumonia and bronchopneumonia were supplemented by the IHC staining of the inflammatory cells: CD3+ for T-lymphocytes, CD20+ for B-lymphocytes, CD68+ for macrophages and CD34+ for endothelial cells (Figure 8, A–D), respectively. The alveolar macrophages, in cases of bronchopneumonia in children younger than one year, were highlighted with CD68+ (Figure 9, A and B) and CD14+ IHC staining (Figure 9, C and D), respectively. With the second marker, CD14, both tissue macrophages (alveolar) and monocytes from the blood vessels can be observed. The vascular pulmonary disease appears in the context of congenital cardiac defects and it will lead to severe pulmonary hypertension and chronic pulmonary heart disease: severe perivascular fibrosis in Masson's trichrome staining (Figure 10, A and B) and in van Gieson's staining (Figure 10, C and D). The same type of lesion was evidenced by the presence of the severe perivascular fibrosis and the vascular endothelium highlighted with the CD31+ IHC staining (Figure 11, A–D).

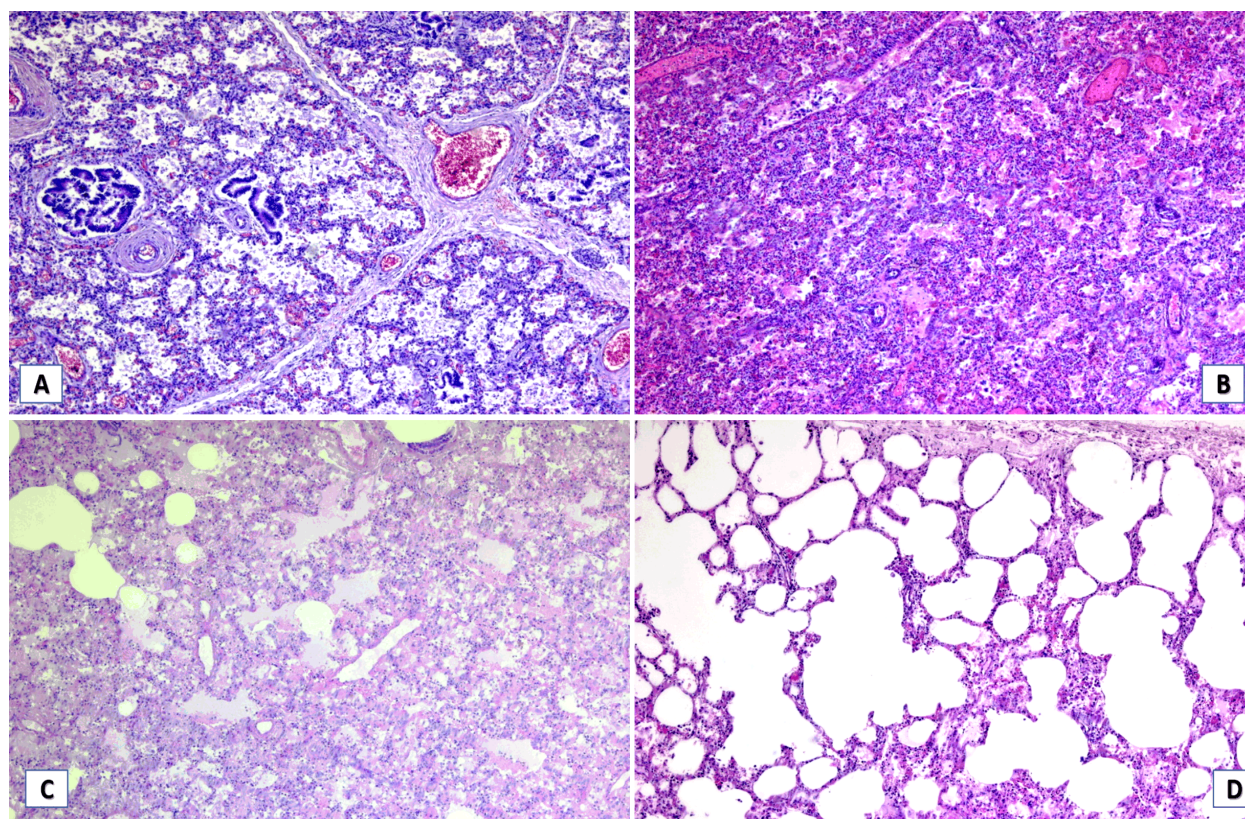


Figure 5 – (A) Immature pulmonary tissue in fetus: sacular stage; (B) Pulmonary anectasis; (C) Pulmonary dystelectasis: non-aerated alveoli, containing fluid; (D) Pulmonary dystelectasis: compensatory emphysema. Hematoxylin–Eosin (HE) staining: (A–D) $\times 50$.

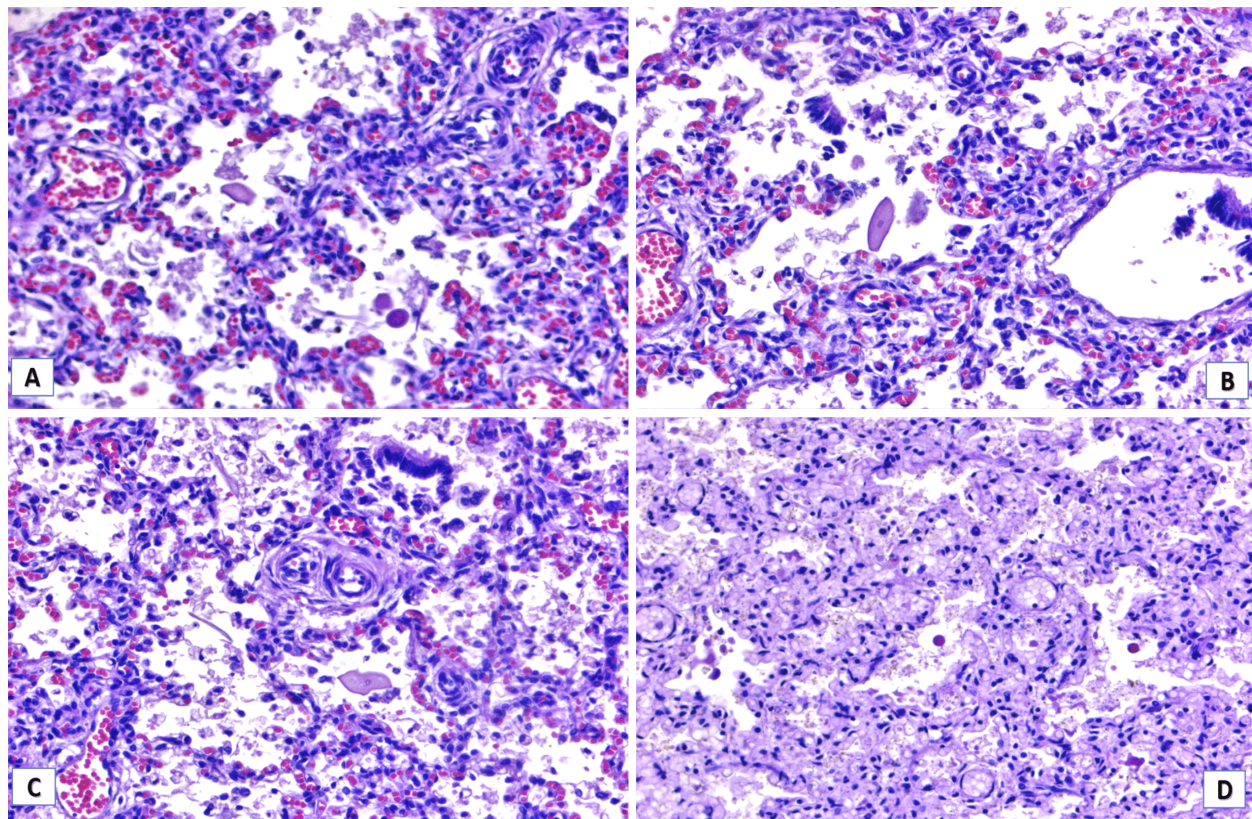


Figure 6 – (A–D) Amniotic fluid aspiration: squamous cutaneous cells and meconium (HE staining, $\times 100$).

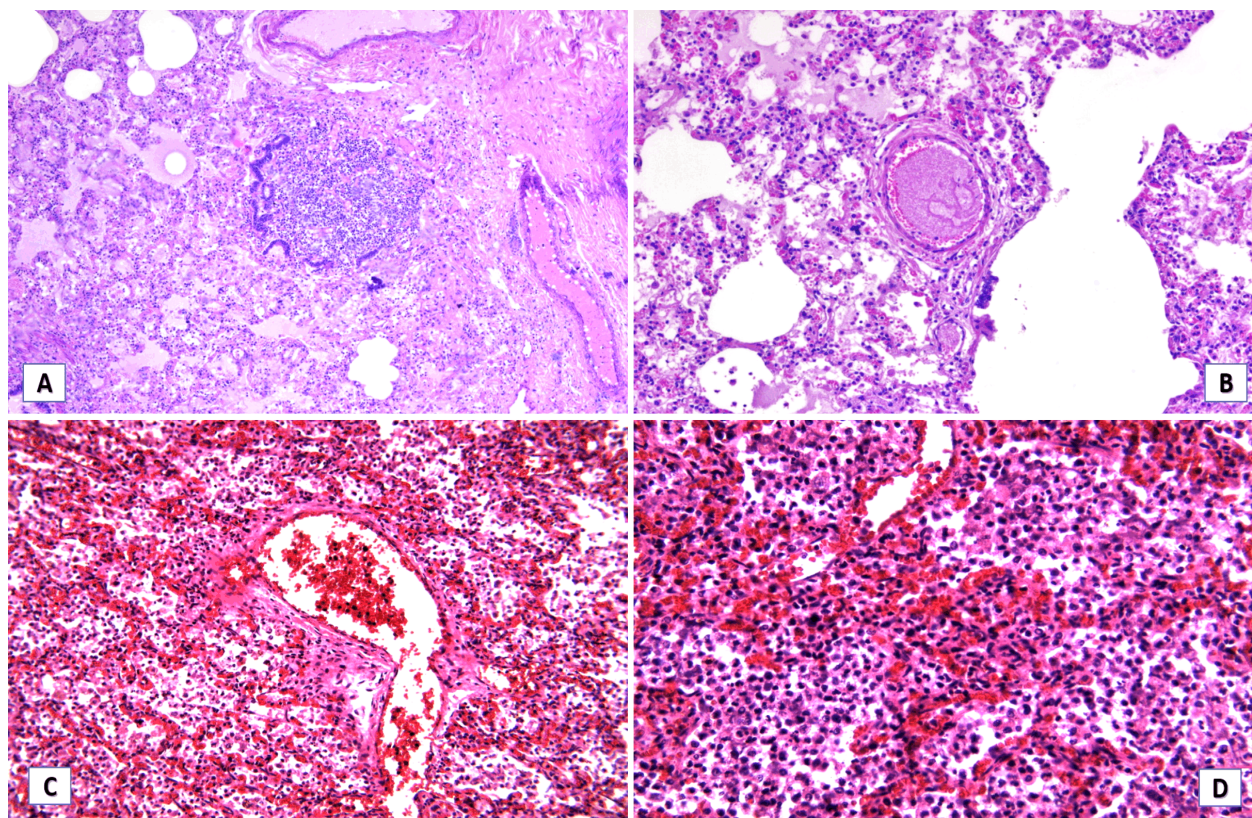


Figure 7 – (A) Bronchiolitis and early hyaline membranes; (B) Fibrinous thrombus; (C and D) Pneumonia: large number of neutrophils in the alveolar spaces. HE staining: (A–C) $\times 50$; (D) $\times 100$.

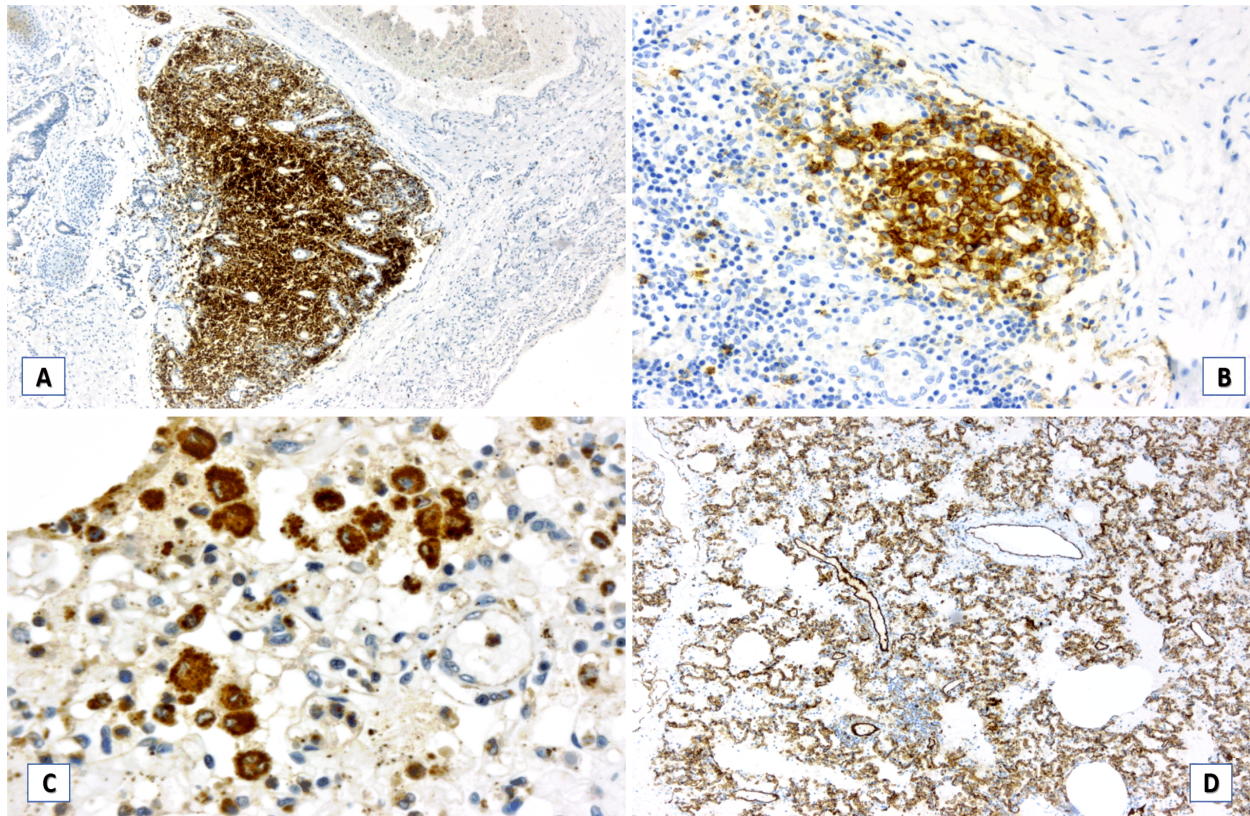


Figure 8 – Bronchopneumonia: (A) T-lymphocytes; (B) B-lymphocytes; (C) Alveolar macrophages; (D) Blood vessels: endothelial cells. Anti-CD3 antibody immunostaining: (A) $\times 50$. Anti-CD20 antibody immunostaining: (B) $\times 100$. Anti-CD68 antibody immunostaining: (C) $\times 200$. Anti-CD31 antibody immunostaining: (D) $\times 50$. CD: Cluster of differentiation.

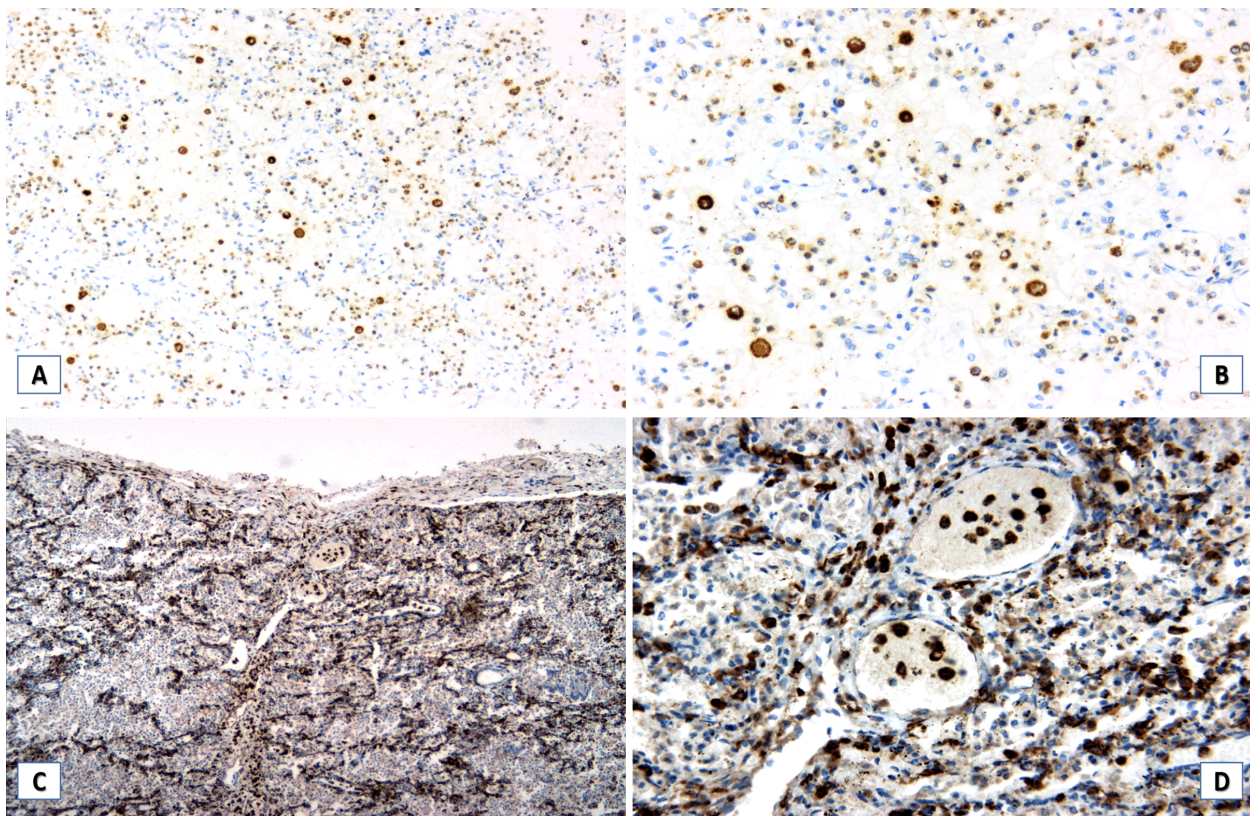


Figure 9 – Bronchopneumonia: (A–D) Alveolar macrophages. Anti-CD68 antibody immunostaining: (A) $\times 50$; (B) $\times 100$. Anti-CD14 antibody immunostaining: (C) $\times 50$; (D) $\times 100$.

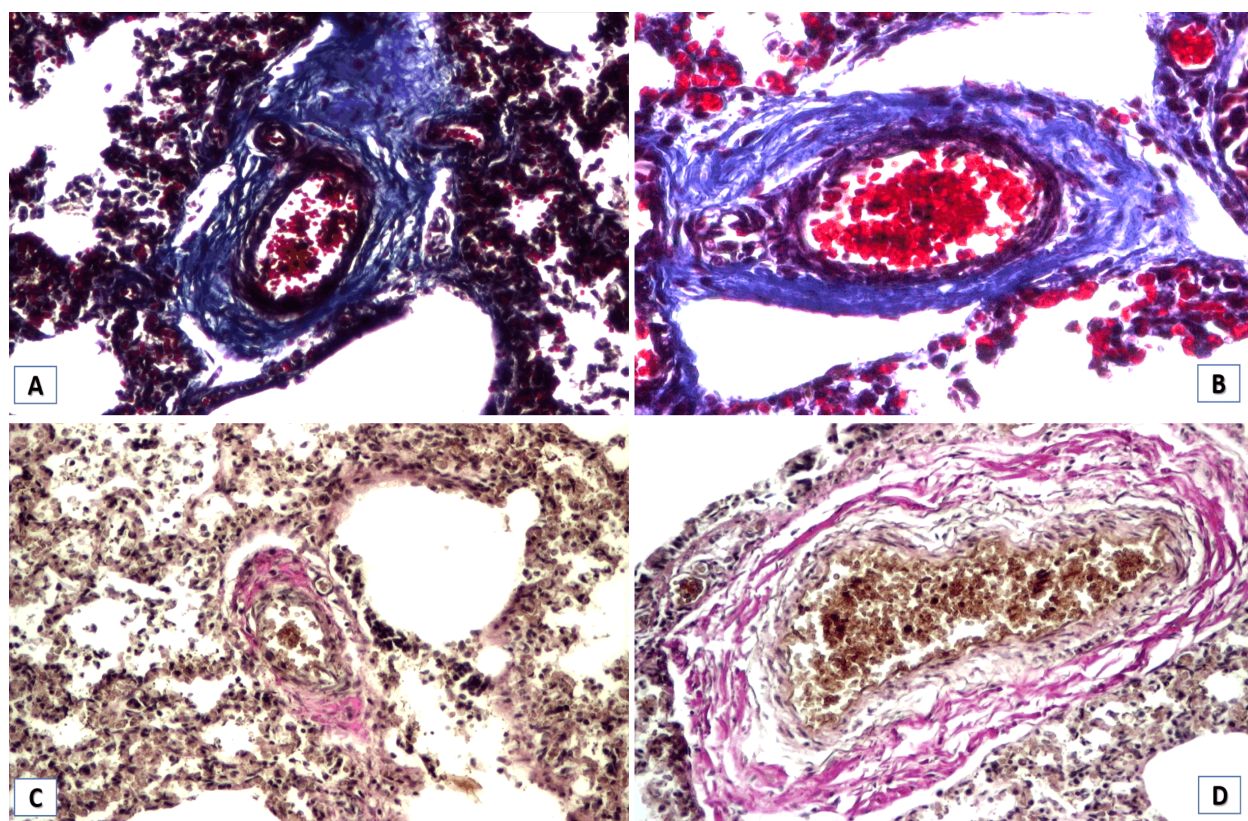


Figure 10 – Pulmonary vascular disease: (A–D) Severe perivascular fibrosis. Masson's trichrome staining: (A) $\times 50$; (B) $\times 100$. Van Gieson's staining: (C) $\times 50$; (D) $\times 100$.

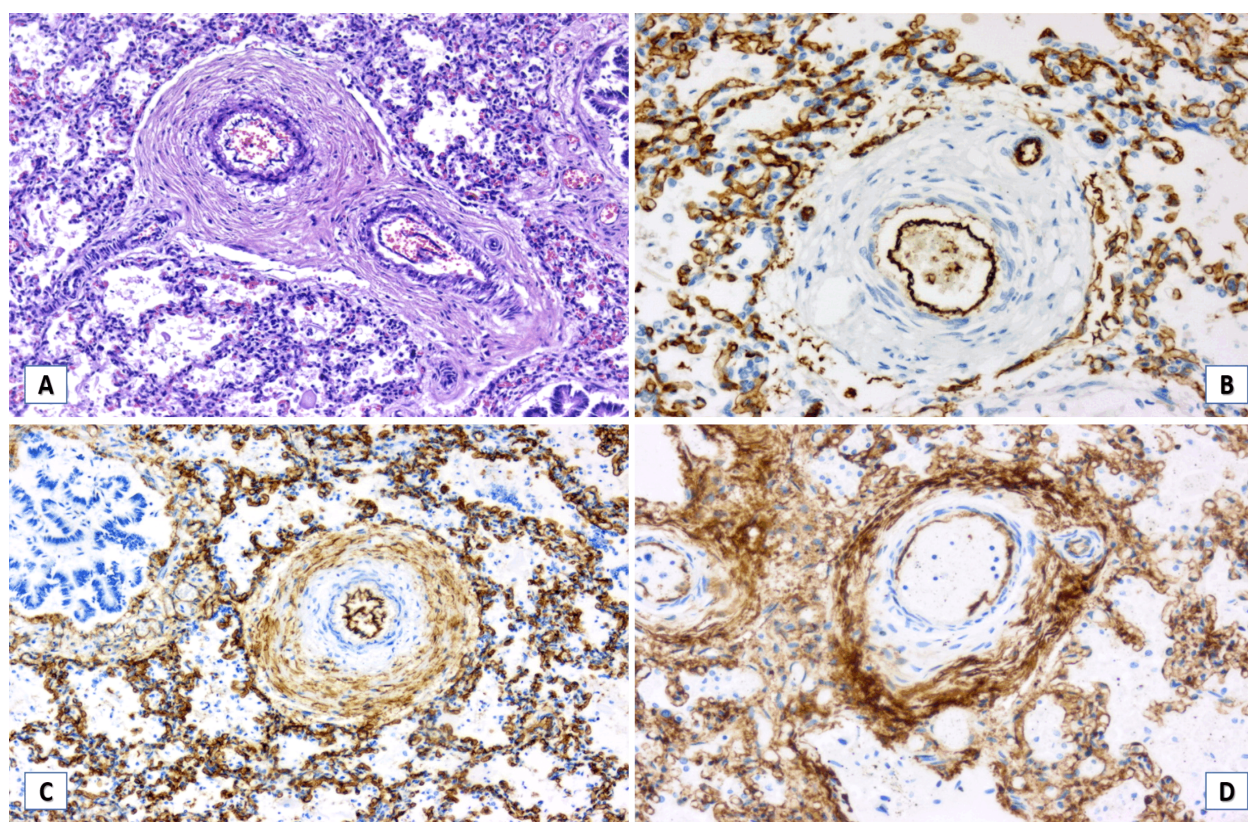


Figure 11 – Vascular pulmonary disease: (A) Severe perivascular fibrosis; (B–D) Blood vessels: endothelial cells. HE staining: (A) $\times 50$. Anti-CD31 antibody immunostaining: (B) $\times 50$. Anti-CD34 antibody immunostaining: (C and D) $\times 50$.

Discussions

Postpartum deaths include, in the first place, causes such as pneumonia or other acute respiratory infections. The risk factors are represented by prematurity, low birth weight (LBW) or very low birth weight (VLBW), the lack of breastfeeding, malnutrition and low socioeconomic status.

For the congenital cardiac malformations, the maximum risk occurs during the embryonic stage [11–13]. For the respiratory tract, the maximum risk occurs during the fetal stage. The last three months in the intrauterine life are essential for the proper development of the respiratory tract and especially for the maturation of the pulmonary parenchyma and surfactant synthesis. This is associated with genetic causes, environmental factors (radiations, temperature), infections [*Toxoplasmosis*, *Other agents*, *Rubella*, *Cytomegalovirus*, and *Herpes simplex* (TORCH) syndrome] [14, 15] or substances consumed by the mother (drugs, medication, alcohol). The pulmonary disease can be unique or associated with other diseases, which can cause the death or they might develop in the context of a systemic illness.

Any cause or damage, maternal or fetal, that leads to the decrease of nutrients in the fetus will be followed by a LBW or premature birth, both being major risk factors for low respiratory tract diseases.

According to *Center for Disease Control and Prevention* (CDCP), in USA, the major causes of perinatal and child-birth deaths are different according to the stage. The fetal causes include: severe congenital cardiac defects, *hydrops fetalis*, placental or umbilical cord damage and intrauterine infection. For the prematures, the main causes include: severe pulmonary undevelopment, acute pulmonary injury, fibrous dysplasia, infection and intraventricular hemorrhage.

For the full term fetuses, the causes of mortality are represented by: birth asphyxia, amniotic fluid and meconial aspiration, pulmonary hypertension, infections, trauma. The highest risk of acute pulmonary injury appears in children born in the 37th week of gestation. This risk is decreasing in children born at the gestational age of 40–42 weeks.

Our study data shows a slightly higher predominance in male patients (122 deaths – 53.99%) in comparison with female patients (104 deaths – 46.01%). Most deceased children came from the rural area (141 deaths – 62.38%) compared with urban area (85 deaths – 37.63%), which is in concordance with the socioeconomic status and the sanitary access.

The most deaths occurred antepartum, in the fetal stage, with a total of newborns deceased at the time of birth in 88 cases, followed by the deaths occurred in the first month (74 living newborns) and the deaths occurred in the first year of life (53 children). In our study, the causes that lead to the antepartum exitus were fetal, determined especially by the pulmonary tract lesions (immature lungs or pulmonary anectasis) or massive aspiration of amniotic fluid and meconium. The data provided by the literature is showing the same types of causes for this group of patients, our study being in concordance with the data [16, 17].

The causes of death in newborns (in the first month of living) and in children younger than one year old were

classified by organ systems. The most common and most serious cause of death reported in the literature was the respiratory distress syndrome [18–21]. Our data are supposable with those from the literature.

To prevent premature births, some authors have proposed either local methods, at the cervical level, with mechanical devices (cerclage), either systemic methods, using hormone therapy [22–24]. A *WHO* study on the causes of maternal death revealed a number of situations that endangered the life of the fetus or the newborn [25].

Pulmonary hypertension in children is a highly studied subject, especially in the context of congenital heart malformations [26–28]. In our study, patients with cyanogenic malformations (tetralogy of Fallot or transposition of great arteries), who did not have timely surgical intervention, developed severe pulmonary hypertension. Studies in recent years explain the pathophysiological mechanisms of this condition and propose new methods for early diagnosis and treatment [29–31].

The causes of death in children older than one year, as our study is showing, are related, first of all, with severe congenital cardiac defects that were not surgically resolved, associated with severe respiratory tract infections. In the group of age between 7 and 14 years old, we had four cases: all of them were either with terminal stage of chronic diseases or genetic diseases, either with a paraneoplastic context.

Conclusions

Most deaths occurring intrauterine had fetal causes. Most deaths were recorded in the first 30 days or in the first year of life. Primary respiratory diseases were the leading causes of death in these patients. Secondary respiratory diseases were associated with the major causes of death in these patients as an aggravating or precipitating factor.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This paper is part of the doctoral thesis supported by Organizing Institution of PhD University Studies (IOSUD) of “George Emil Palade” University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania, between 2016–2019.

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