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Quantitative analysis of inflammatory and adhesion molecules in lungs of neonates with chronic lung disease (bronchopulmonary dysplasia) receiving mechanical ventilation

Análise quantitativa de moléculas inflamatórias e de adesão em pulmões de neonatos com doença pulmonar crônica (displasia broncopulmonar) submetidos à ventilação mecânica

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ABSTRACT

Introduction: Chronic lung disease (CLD), clinically known as bronchopulmonary dysplasia (BPD), is a major cause of morbidity in premature newborn and were submitted to oxygen therapy. **Objective:** Immunohistochemical identification of inflammatory molecules in the lung tissue of premature neonates that died with CLD. **Methods:** Immunohistochemical analysis of 51 samples of premature newborn lungs – grouped in: without CLD, “classic” CLD and “new” CLD. **Results:** Neutrophil influx and the number of CD4+ and CD45RO+ cells were higher in the “classic” CLD group ($p < 0.001$). **Conclusion:** Our findings suggest that the inflammatory response is mediated by neutrophils and CD45RO+ and CD4+ T lymphocytes in the “classic” CLD.

Key words: bronchopulmonary dysplasia; immunochemistry; premature birth; oxygen therapy; neonatology.

INTRODUCTION

Chronic lung disease (CLD) or, clinically, bronchopulmonary dysplasia (BPD) is a chronic neonatal pulmonary disorder and a major cause of morbidity affecting premature newborn⁽¹⁻³⁾. The classic presentation of this disorder was described in 1960 as a consequence of respiratory distress syndrome, the use of higher concentrations of inspired oxygen and aggressive mechanical ventilation produced characteristic radiological changes. Histological findings of “classic” CLD are emphysema, atelectasis, fibrosis, squamous metaplasia and smooth muscle hypertrophy in the airways and pulmonary vasculature⁽⁴⁾.

The use of prenatal corticosteroids therapy, exogenous surfactant and technological advances in mechanical ventilation have resulted in a significant increase in the survival rates of

newborn with very low birth weight; however, the incidence of CLD has not changed as expected⁽⁴⁻⁷⁾. Instead, another type of CLD, known as “new” CLD emerged, particularly in newborn with less than 30 weeks of gestational age who continued to require supplemental oxygen after 28 days of age⁽³⁻⁶⁾.

In 2001, a consensus suggested that the clinical criteria for the diagnosis of BPD/CLD (“new” or “classic”) in a preterm newborn must depend on the use of respiratory support at 28 days of postnatal life or 36 weeks postmenstrual age^(3,6-9). Although the pathological criteria for “classic” CLD are characterized by an intense inflammatory response and septal fibrosis, in the “new” CLD there is decreased alveolar septation and abnormal pulmonary vascular development compatible with impaired pulmonary growth. Septal fibrosis, inflammatory response and interstitial edema are rarely observed. The alterations in alveolar formation may be very subtle and sometimes not detectable in routine morphological tests.

The characteristics of the “new” CLD may be more related to morphometric data than to the morphological aspects⁽¹⁰⁻¹⁴⁾.

Over the last 10 years, the pathological basis of CLD have predominantly been associated with the following two processes: inflammation and arrest of lung development^(2, 4, 5). The inflammatory response in CLD is exacerbated by mechanical ventilation and exposure to supplemental oxygen. Several studies seem to indicate that neutrophils prevail in the earliest stages of CLD, followed by an abundance of macrophages and other inflammatory cells, increased pulmonary vascular permeability and damage to endothelial and epithelial cells, typically leading to apoptosis⁽¹⁵⁻²¹⁾.

The objectives of this study were to investigate the subtypes of inflammatory cells and adhesion molecules, using immunohistochemistry in lung samples of preterm newborn receiving mechanical ventilation (less than 34 weeks gestational age) and to correlate the immunoexpression of these biomarkers with the presence or absence of CLD.

METHODS

Sample selection

The sample consisted of 51 specimens of preterm newborn lung autopsies performed between 1994 and 2005 (after the pre-surfactant era); from the archives of the Department of Pathology Medicine of Universidade Federal do Paraná (UFPR). The research ethics board of the Hospital das Clínicas (HC)-UFPR reviewed and approved the study (registration number 1099.138/2005-08; approved on August 30, 2005). The following inclusion criteria were used: complete autopsy; absence of major malformations; absence of meconium aspiration; less than 34 weeks of gestational age and use of oxygen therapy for at least 2 hours. The samples that were unsuitable for testing or for which the medical records were not available were excluded. The medical records were analyzed to collect data related to antenatal risk factor for CLD including gender, gestational age, birth weight, maternal age, number of pregnancies, prenatal care, hypertensive event in pregnancy, gestational diabetes, chorioamnionitis, premature amniotic sac disruption, type of birth and twin pregnancy. Additionally, other potential postnatal risk factors for CLD include patent ductus arteriosus, cardiopulmonary resuscitation, Apgar score at the first and fifth minute, antibiotic therapy, surfactant replacement therapy, necrotizing enterocolitis, bronchopneumonia, pulmonary hemorrhage, pulmonary hypertension, choking, intracranial hemorrhage, pneumothorax, sepsis and corrected

gestational postnatal age were recorded. Survival time and cause of death were also recorded⁽¹⁰⁾.

Morphometric analysis

The slides of the 51 patients were stained with hematoxylin and eosin (HE) and divided into the following three groups: 1) histopathological findings of “classic” CLD ($n = 11$); 2) histopathological and morphometric findings of “new” CLD ($n = 5$); and 3) “without” histopathological and morphometric criteria for CLD ($n = 35$). Since the alterations in alveolar formation may be very subtle, the samples of groups 2 and 3 were analyzed only to confirm the existence of the morphometric criteria of “new” CLD in group 2^(9, 10, 22, 23).

The digital images of 10 nonadjacent fields ($200\times = 106$ micrometers in diameter) in slides of the pulmonary parenchyma from the 40 cases (group 2 and 3) were captured using Image Pro Plus softwareTM (Rockville, MD, USA). It measures the alveolar perimeter in micrometers and the number of alveoli per field^(10, 22, 23). The alveoli in each of the 10 digitalized fields were measured. Means and standard deviations for each case were calculated. One blinded observer performed these analyses^(10, 23).

Immunohistochemical staining

Paraffin blocks of the studied cases ($n = 51$) were used to construct the tissue microarrays (TMAs)⁽²³⁾. Lung samples from all the patients were inserted into multi-sample paraffin blocks (TMAs), with four samples of each case and 3 mm diameter for each sample (analyzed area = 28.26 mm^2). Histological sections of TMA blocks were used for immunohistochemical analysis (immunoperoxidase staining). The protocol for the immunoperoxidase assay was standardized with positive and negative controls. The cases were tested with the following antibodies: anti-CD14 (clone 7, mouse monoclonal); anti-CD4 (clone 4B12, kappa 1, mouse monoclonal); anti-CD8 (clone 1A5, mouse monoclonal); anti-CD74 antibody (clone LN-2, mouse monoclonal antibody); anti-CD25 antibody (clone 4C9, mouse monoclonal antibody, alpha subunit of the interleukin-2 [IL-2] receptor); anti-CD54 or anti-intercellular adhesion molecule-1 (ICAM-1) antibody (clone 23G12, mouse monoclonal antibody); and anti-CD106 or anti-vascular cell adhesion molecule (VCAM) antibody (clone 1.4C3, mouse monoclonal antibody), all from NovocastraTM (Leica Biosystems, Buffalo Grove, USA); and anti-CD45RO (clone UCHL, kappa, mouse monoclonal antibody) and anti-CD20 (clone IF5; mouse monoclonal antibody) from Dako (Denmark A/S; Glostrup, Denmark)^(21, 23, 24).

Staining was interpreted as follows: for anti-CD4, CD8, CD14, CD20, CD25, CD74 and CD45RO antibodies, the number of positive cells in four random high-power fields ($400\times = 53$ micrometers in diameter) were counted for each of the four samples of each case^(23, 24). The cells that had a characteristic membrane-staining pattern when observed under Olympus BX50 microscope (Olympus Optical Co, Ltd., Japan) and which were located in the alveolar septum or intra-alveolar space were considered positive. The cells in the intravascular space or in regions that usually contain peribronchial lymphoid aggregates were disregarded⁽²⁴⁾.

For the anti-ICAM-1 (CD54) and anti-VCAM (CD106) antibodies, the slides were assigned a positive score (0 = negative; 1 = weak positive; 2 = strong positive), and the positive score for each case was obtained by the sum of the scores for each of the four samples for this case. The stains were considered positive if they were observed in alveolar septa vessels and in macrophages/monocytes in the septa and alveolar lumina. The positivity of these reactions was assessed in all fields of the four samples of each case using the Olympus BX50 microscope (Olympus Optical Co, Ltd., Japan) at $400\times$ magnification⁽²³⁾.

Statistical methods

Data were analyzed using SPSS 20.0 (IBM, São Paulo, SP, Brazil). Kruskal-Wallis and nonparametric Mann-Whitney test were used for testing quantitative variables. Categorical variables were analyzed using Pearson's chi-square test. Analysis of covariance (Ancova) was used for gestational age adjustment. Statistical significance was accepted for p values < 0.05 .

RESULTS

Histopathological and morphometric data

In group 1 ("classic" CLD), the predominant histopathological characteristics were hyaline membranes and interstitial fibrosis⁽¹⁰⁾.

Cases with "new" CLD (group 2) had minimal histopathological alterations, including subtle septal edema. The alveolar changes caused by insufflation and a very subtle inflammatory response were present in these cases⁽¹⁰⁾. Since the histological findings for the group with "new" CLD were often indistinguishable from those for the group "without" CLD (group 3), a morphometric analysis was required, which showed

a decreased in the number and in the perimeter of the alveoli in the "new" CLD group, $p = 0.033$ and 0.018 , respectively (**Table 1** and **Figure**).

TABLE 1 – The number of alveoli and the alveolar perimeter (in micrometers) in the "without" CLD and "new" CLD groups

Parameters	New CLD* (n = 5)	Without CLD* (n = 35)	<i>p</i> value
Number of alveoli	40.9 \pm 15.1	57.7 \pm 16.2	0.033 [§]
Alveolar perimeter [‡]	481.5 \pm 182.4	611.3 \pm 206.2	0.018 [‡]

*: average \pm standard deviation; [§]: Mann-Whitney non-parametric test ($p < 0.05$); [‡]: square micrometers; [†]: Ancova ($p < 0.05$) (gestational age adjustment).

CLD: chronic lung disease; Ancova: analysis of covariance.

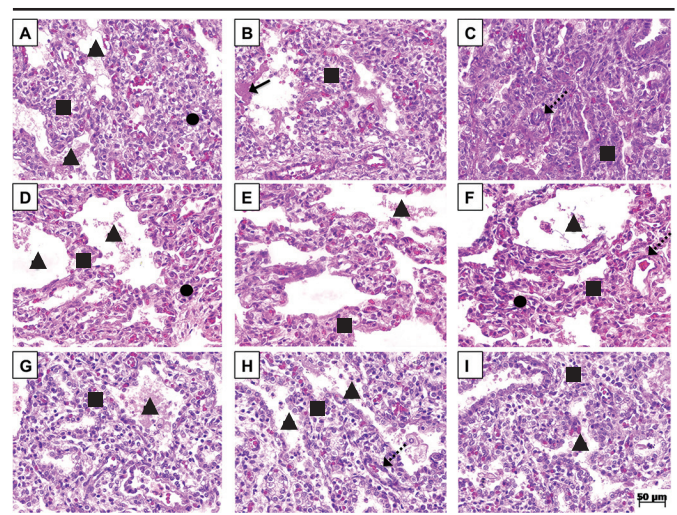


FIGURE – Photomicrograph of groups

A, B and C) photomicrograph of group 1 (classic BPD), here we observe areas of hyperinflation (\blacktriangle) interspersed with areas of atelectasis (\bullet) associated with marked fibrotic septal thickening and chronic inflammatory process (\blacksquare). In other areas hyaline membranes (arrow) are observed as well as septal arterioles muscle layer thickening (dashed arrow); D, E and F) photomicrograph of group 2 (new BPD), here we observe areas of hyperinflation (\blacktriangle) interspersed with areas of atelectasis (\bullet) associated with minimal septal thickening with mild edema and mild chronic inflammatory process (\blacksquare). There are no hyaline membranes and the septal arterioles muscle layer thickening (dashed arrow) is very mild; G, H and I) photomicrograph of group 3 (without BPD), in which we observed few areas of hyperinflation (\blacktriangle) associated with discrete septal thickening with mild edema (\blacksquare). There are no hyaline membranes and septal arterioles muscle layer thickening (dashed arrow) are minimal. The alterations found in group 3 are more associated with pulmonary immaturity than neonatal hypoxic injury.

BPD: bronchopulmonary dysplasia.

Clinical data

The "classic" CLD group received oxygen therapy for the longest period of time ($p < 0.0001$). The risk factors/therapeutic profile of the study sample, including oxygen therapy parameters, gender, gestational age, maternal age and birth weight are shown in **Table 2**, **3** and **4**.

TABLE 2 – Clinical data for the three groups

Parameters	Total samples (n = 51)	Classic CLD (n = 11)	New CLD (n = 5)	Without CLD (n = 35)	p value
Gender[†]					
Female	33	6	4	23	
Male	18	5	1	12	--
Gestational age (weeks)	27 (28.9 ± 2.6)*	27 (27-34.5) [‡]	33 (27-34.5) [‡]	27 (25-33) [‡]	0.04 [§]
Birth weight (g) (n = 49/51)	1760 (972.9 ± 342.7)*	1021 (685-1650) [‡]	1340 (900-1760) [‡]	870 (515-1695) [‡]	0.03 [§]
1-minute Apgar score (n = 45/51)	3 (3.5 ± 2.7)*	2.6 (1-5) [‡]	6 (1-8) [‡]	3.4 (1-8) [‡]	--
5-minute Apgar score (n = 47/51)	7 (5.9 ± 2.9)*	6.2 (2-9) [‡]	8.4 (5-10) [‡]	5.5 (0-10) [‡]	--
Maternal age (years) (n = 48/51)	25 (25 ± 6.4)*	26.2 (16-36) [‡]	29.8 (18-36) [‡]	24.1 (15-37) [‡]	--
Number of pregnancies (n = 50/51)[†]					
1 or 2	30	6	2	22	
≥ 2	20	5	3	12	--
Follow-up prenatal visits (n = 50/51)[†]					
Yes	27	7	4	16	
No	23	4	1	18	--
Hypertensive event in pregnancy (n = 50/51)[†]					
Yes	12	3	3	5	
No	38	8	2	29	0.01 ^{§§}
Gestational diabetes (n = 51/51)[†]					
Yes	1	0	1	0	
No	50	11	4	35	--
Chorioamnionitis (n = 49/51)[†]					
Yes	5	1	0	4	
No	44	10	5	29	--
Premature amniotic sac disruption (n = 45/51)[†]					
Yes	15	1	1	13	
No	30	9	4	17	--
Type of birth (n = 51/51)[†]					
Normal delivery	29	6	2	21	
Cesarean delivery	22	5	3	14	--
Twin pregnancy (n = 51/51)[†]					
Yes	12	5	1	6	
No	39	6	4	29	--

CLD: chronic lung disease; *: median (average ± standard deviation); †: number of cases; ‡: median (min-max); §: Kruskal-Wallis non-parametric test ($p < 0.05$); §§: Pearson correlation; --: not statistically significant data.

Immunohistochemical data

The neutrophil influx was much higher in “classic” CLD group than in “new” CLD or the “without” CLD groups; this difference was statistically significant ($p < 0.001$) (Table 5).

There were more CD25+ lymphocytes in the group with “new” CLD than in the other groups ($p = 0.0400$). There were more CD74+ cells and CD4+ T lymphocytes in “classic” CLD group ($p = 0.0500$ and 0.0160 , respectively). The CD45RO+ T cell count was significantly higher in “classic” CLD group than in “without” CLD and “new” CLD groups ($p = 0.0004$). The CD14+ cell count was higher in the alveolar septum in “new” CLD group than in “classic” CLD or “without” CLD groups, with a trend toward significance ($p = 0.0523$), as shown in Table 5. There was no statistically significant difference between the groups in terms of CD20+ cell and CD8+T cell counts.

Whereas ICAM-1 expression was higher in the “new” CLD group than in the other groups ($p = 0.0200$), there were no

statistically significant differences in VCAM expression between the groups.

DISCUSSION

The pathogenesis of BPD/CLD is complex and remains unclear despite numerous studies on the subject. The lungs of very premature neonates have immature distal airways with a thick blood-air barrier and a small area for gas exchange. Furthermore, they are deficient in surfactant because of the predominance of undifferentiated epithelial cells and the scarcity of type II alveolar cells⁽²⁵⁾.

Several other factors act additively and synergistically, triggering the inflammatory response and lung injury. These factors include oxygen supplementation, mechanical ventilation, prenatal and postnatal infections, and persistent patency of the

TABLE 3 – The postnatal factors for the three groups

Parameters	Total samples (<i>n</i> = 51)	Classic CLD (<i>n</i> = 11)	New CLD (<i>n</i> = 5)	Without CLD (<i>n</i> = 35)	<i>p</i> value
Patent ductus arteriosus [†]					
Yes	15	10	2	3	--
No	36	1	3	32	
Cardiopulmonary resuscitation [†]					
Yes	40	8	3	29	--
No	11	3	2	6	
Antibiotic therapy [†]					
Yes	33	10	4	19	--
No	18	1	1	16	
Surfactant replacement therapy [†]					
Yes	15	7	0	8	--
No	36	4	5	27	
Necrotizing enterocolitis [†]					
Yes	11	7	2	2	0.0001 ^{§§}
No	40	4	3	33	
Bronchopneumonia [†]					
Yes	12	6	0	6	0.0165 ^{§§}
No	39	5	5	29	
Pulmonary hemorrhage [†]					
Yes	9	3	1	5	--
No	42	8	4	30	
Pulmonary hypertension [†]					
Yes	7	2	1	4	--
No	44	9	4	31	
Choking [†]					
Yes	28	6	1	21	--
No	23	5	4	14	
Intracranial hemorrhage [†]					
Yes	11	4	2	5	--
No	40	7	3	30	
Pneumothorax [†]					
Yes	3	1	0	2	--
No	48	10	5	33	
Sepsis [†]					
Yes	24	11	4	9	0.0001 ^{§§}
No	27	0	1	26	
Corrected gestational age (weeks)*	29 (29.7 ± 3.2)*	32 (29-39) [‡]	34 (28-36)*	27 (25-33) [‡]	0.0002 [§]
Survival time (days)	3 (7.6 ± 10.7)*	23.4 (11-50) [‡]	12.4 (8-21) [‡]	1 (0-6) [‡]	0.0001 [§]
Cause of death [†]					
Sepsis	24	11	4	9	
Intracranial hemorrhage	4	0	1	3	
Perinatal asphyxia	11	0	0	11	--
Others	22	0	0	22	

CLD: chronic lung disease; *: median (average ± standard deviation); ‡: median (min-max); †: number of cases; §: Kruskal-Wallis non-parametric test (*p* < 0.05); §§: Pearson correlation; --: not statistically significant data.

TABLE 4 – The parameters of oxygen therapy for the three groups

Parameters	Classic CLD [‡] (<i>n</i> = 11)	New CLD [‡] (<i>n</i> = 5)	Without CLD [‡] (<i>n</i> = 35)	<i>p</i> value
Mechanical ventilation (days)	12.9 (1-29)/1	3.4 (1-9)/1	1.4 (0.02-6)/2	--
CPAP (days)	5.6 (0.04-19)/1	4.40 (1-8)/0	1.91 (0.04-5)/25	--
Oxygen tent (days)	3.8 (1-30)/5	1.8 (1-5)/1	-/35	--
Catheter oxygen therapy [†]				--
Yes	1	1	0	
No	10	4	35	
Oxygen therapy (days)	21.36 (11-50)	10.40 (8-15)	1 (0.02-6)	< 0.001 [§]
FiO ₂ max (%)	95 (40-100)	85 (40-100)	98.57 (60-100)	--
PIPmax (mmHg)	21.16 (18-30)	20 (20-20)	22.93 (17-35)	--
PEEPmax (mmHg)	4.78 (4-5)	5 (5-5)	5 (5-6)	--

CLD: chronic lung disease; ‡: mean (max-min)/median; †: number of cases; CPAP: continuous positive airway pressure; FiO₂ max: maximum fraction of inspired oxygen; PIPmax: maximum peak inspiratory pressure; PEEPmax: maximum positive end-expiratory pressure; §: statistical analysis not significant (*p* > 0.017); Fisher test (Bonferroni correction).

TABLE 5 – The median neutrophils count, the positive cells per high power field for each marker and the mean VCAM and ICAM-1 positive scores for each group

Marker	Classic CLD (<i>n</i> = 11)	New CLD (<i>n</i> = 5)	Without CLD (<i>n</i> = 33)	<i>p</i> value [§]
Neutrophils count	3.11	1.49	0.59	< 0.001
CD20+	1.5	2.12	1.25	0.938
CD25+	0.3214	0.95	0.3839	0.04
CD45RO+	5.25	1.36	2	0.0004
CD14 (alveolar septum)	3	341.5	15	0.0523
CD14 (alveolar space)	2	61	11	0.138
CD4+	10.887	2.766	2.86	0.016
CD8+	0.472	2.5	3.991	0.17
CD74+	12.25	9.625	7.187	0.05
ICAM	0.106	0.6	0.4292	0.02
VCAM	0	0.2	1	0.076

VCAM: vascular cell adhesion molecule; ICAM-1: intercellular adhesion molecule-1; CLD: chronic lung disease; §: Kruskal-Wallis non-parametric test ($p < 0.05$); *p* value is between classic and without CLD and new and without CLD.

arterial duct, malnutrition, vitamin A deficiency and genetic factors. Inflammation, however, is the common final pathway for the factors that cause lung injury in CLD⁽²⁶⁾.

In this study, the “new” CLD group was distinguished from the “without” CLD group by morphometric analysis. This analysis revealed statistically significant differences between the two groups, with a decrease in the number and the perimeter of the alveoli in the “new” CLD group^(10, 12-14, 22).

The clinical, histopathological and morphometric patterns of CLD have changed with the emergence of the concept of underdeveloped distal airways. The histopathological findings for “new” CLD were recently described and were observed in our study^(10, 12). As observed in this study, although the clinical criteria for the diagnosis of CLD should depend on respiratory support at 28 days of postnatal life, the pathological changes seem to precede this clinical concept based on the pathological changes found in the infants with 7-28 days of oxygen therapy.

The imbalance between pro-inflammatory and anti-inflammatory activity and its close relationship with cellular apoptosis and proliferation may affect alveolar formation and pulmonary vascular growth, eventually leading to pulmonary hypoplasia^(19, 26, 27).

Furthermore, neutrophils may produce recruiting factors for other leukocytes, such as interleukin-8 (IL-8), as well as for tumor necrosis factor (TNF), which is responsible for prolonging neutrophil survival by inhibiting the activation of the apoptotic cascade. Some authors have shown that there is increased evidence of CD4+ and CD8+ T lymphocyte involvement in the mobilization and maintenance of neutrophils at the injury site, which may be a key factor, particularly in CLD, bronchial hyper-reactivity and bronchiectasis⁽²⁷⁻³⁰⁾.

Our findings show that the neutrophils influx was higher in the “classic” CLD group than in the “without” CLD and “new” CLD groups ($p < 0.001$). Furthermore, the number of CD4+ T lymphocytes was higher in the “classic” CLD group than in the “new” CLD group ($p = 0.016$), corroborating the hypothesis that the inflammatory response is more intense in the “classic” CLD form^(20, 28, 29, 31).

The physiopathological relationship between the accumulation of neutrophils and the presence of CD4+ T lymphocytes has not been fully clarified. There is some evidence that interleukin-17 (IL-17) plays a significant role in the action orchestrated by these two cells⁽³¹⁻³³⁾.

IL-17 released by the CD45RO+ T lymphocytes may activate T cells, which act as mediators in the increased neutrophil recruitment and activation in the damaged tissues, particularly the lung tissue^(34, 35). This finding was confirmed in studies in which azithromycin was used as an inhibitor of IL-17 secretion, and a reduction in tissue damage was observed⁽³⁵⁻³⁸⁾.

In our study, the CD45RO+ T lymphocyte count was higher in the “classic” CLD than in the “new” CLD and “without” CLD groups ($p = 0.0004$). It was followed by an increased neutrophil count in the same group ($p < 0.001$), corroborating the hypothesis of an initial inflammatory response mediated by neutrophils and maintained by T lymphocytes in this form of CLD^(20, 28, 29, 31).

CD14 is a glycoprotein expressed on the myelomonocytic lineage cells, including monocytes, macrophages and Langerhans cells, and CD14 acts as an opsonin receptor to promote the release of pro-inflammatory cytokines, particularly those associated with Toll-like receptors (TLR)^(39, 40). In our study, the number of CD14+ cells was higher in the “new” CLD group than in the “classic” CLD or the “without” CLD groups, particularly in the alveolar

septum, for which the results showed a trend toward a statistically significant difference ($p = 0.0523$). Some studies have shown that CD14 expression in peripheral blood is lower in premature infants than in term neonates⁽⁴¹⁻⁴³⁾.

The anti-CD74 antibody detects a major histocompatibility complex (MHC) class II receptor on the macrophage membrane that is involved in macrophage migration inhibitory factor (MIF) induction. CD74+ cells are usually macrophages, histocytes or monocytes, as well as type II pneumocytes, and they are correlated with the induction of type II pneumocyte proliferation in alveolar epithelium repair⁽⁴⁴⁾.

Furthermore, Kevin and Bandhari found elevated levels of CD74+ cells and MIF in the lungs of premature rats with respiratory distress syndrome and in tracheal aspirate from premature neonates. Both factors are related to a slight probability of developing CLD. This finding may be explained by the role played by CD74+ cells in pneumocyte proliferation, exacerbation of angiogenesis, increase in leukocyte migration, and therefore the maintenance of inflammatory response⁽⁴⁵⁾.

We found more CD74+ cells in the “classic” CLD group than in the other groups, which may be explained by the fact that during the process of alveolar damage that is observed in “classic” CLD, there is an extensive damage to type I pneumocytes, which are replaced by hyaline membranes. The acute phase is followed by the subacute phase, in which the area is cleaned by abundant macrophages and the alveolar lining is regenerated by type II pneumocyte proliferation. The macrophages and type II pneumocytes have the MIF receptor (CD74), and therefore they reacted with the anti-CD74 antibody used in this study⁽⁴⁵⁾.

CD25+ T-cells were more abundant in the “new” CLD group. This finding can be explained by the fact that regulatory T cells modulate the inflammatory response, resulting in less inflammation and fibrosis in this form of the disease⁽⁴⁶⁾.

Neutrophil migration across the endothelium and epithelial cell barrier depends on the ICAM-1 adhesion receptor, a molecule stimulated in inflammatory disorders that can be induced by cytokines and detected in soluble form in plasma, which has been proposed as a marker of inflammatory activity. The endothelium plays a fundamental role in acute lung injury, particularly in the activation of pro-inflammatory cytokines. Some authors have proposed that the presence of interleukin-1 (IL-1) and TNF supports the presence of activated macrophages, which stimulate the production of other cytokines and increase the expression of adhesion molecules such as ICAM-1^(39, 46, 47).

Little *et al.* showed that plasmatic levels of ICAM-1 in premature neonates at 14 days may be correlate with chronic neonatal lung disease and its severity⁽⁴⁶⁾. Ballabh *et al.* reported similar findings of elevated ICAM-1 levels in neonates who developed CLD on approximately the 28th day of life⁽⁴⁷⁾. In our study, the lung samples of premature neonates from the “new” CLD group had higher levels of ICAM-1 than the lung samples from the other groups. It reasonable to hypothesize that ICAM-1 is an early biomarker for CLD because it appears before the 28th day of oxygen supplementation, i.e., before a clinical diagnosis of CLD. These results contrast with the findings of other authors regarding the plasma levels of ICAM-1⁽³⁹⁾.

In our study, high levels of ICAM-1 in “new” CLD may have been the determining factor for the increased number of CD14+ cells in the alveolar septum. Whether this conclusion is correct, it is reasonable to assume that the increased number of CD14+ cells can influence the increase in apoptotic cascade activity, which seems to be a major cause of the reduced alveolar formation that occurs in “new” CLD. Further immunohistochemical and molecular studies are required to clarify this hypothesis^(46, 47).

Few studies have correlated B-lymphocytes and VCAM with CLD, and no statistically significant differences in the number of CD20+ cells and VCAM immunoexpression were found between the groups in our study⁽⁴⁴⁾.

Our findings seems to corroborate the hypothesis that the initial inflammatory response is mediated by neutrophils and sustained by CD45RO+ and CD4+ T lymphocytes in the “classic” CLD form. Furthermore, the results suggest that CD14 may be a biomarker to connect inflammatory and apoptosis process, particularly in the lung injury associated with decreased alveolar volume observed in “new” CLD. Further studies are needed to better elucidate the pathogenesis of CLD, especially regarding the apoptotic process of the “new” CLD.

ETHICAL CONDUCT OF RESEARCH

The authors state that they have obtained approval of appropriate institutional review board and have followed the principles established by the Declaration of Helsinki for all human or animal experimental investigations. The research ethics board of the HC-UFPR reviewed and approved the study (registration number 1099.138/2005-08; approved on August, 30 2005).

RESUMO

Introdução: A doença pulmonar crônica (DPC), conhecida clinicamente como displasia broncopulmonar, é uma das maiores causas de morbidade em neonatos que nasceram prematuros e foram submetidos à oxigenioterapia. **Objetivo:** Identificar moléculas inflamatórias em tecido pulmonar de recém-nascidos prematuros que morreram com DPC por meio do método de imuno-histoquímica. **Métodos:** Análise imuno-histoquímica de 51 amostras de pulmões de recém-nascidos prematuros – formando os grupos: sem DPC, DPC “nova” e DPC “clássica”. **Resultados:** O influxo de neutrófilos e o número de células CD4+ e CD45RO+ foram maiores no grupo DPC “clássica” ($p < 0,001$). **Conclusão:** Os resultados sugerem que o processo inflamatório é mediado por neutrófilos e linfócitos CD45RO+ e CD4+ na DPC “clássica”.

Unitermos: displasia broncopulmonar; imuno-histoquímica; nascimento prematuro; oxigenoterapia; neonatologia.

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