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Analysis Of Morphometric Indicators Of The Structural Units Of The Lung Tissue Of Premature Newborns With Respiratory Distress Syndrome.

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Abstract
In this scientific study, an analysis of the morphometric parameters of the structural units of the lung tissue in premature infants with hyaline membrane disease was carried out. The lung tissue of premature babies aged 22-37 weeks was taken as a material. Morphometric study of the structural units of the lung tissue was carried out by modifying the method of "counting points" by transferring it to a computer screen.
Key words: respirator distress syndrome, baby, prematurity, lung, structure, alveolus, hyaline membrane, morphology, “scoring - test system”, light interval.

Introduction
One of the main problems in neonatology is respiratory distress syndrome (RDS) and the appearance of bronchopulmonary dysplasia and hyaline membranes (GM) as its morphological sign. The hyaline membranes of the lungs are the most basic and severe form of RDS. The relevance of this problem is due to the growing number of premature births in the world. For example, in Russia it is 5-10%, including the birth of children with extremely low body weight occurs in 0.2-0.4%. Their survival, according to foreign authors, averages 50%, and if intensive therapy is used, it decreases to 25%. The cause of death in such children is mainly respiratory distress syndrome and hyaline membranes.
Purpose of the study: is to determine the features of morphological changes in the lungs of premature infants that develop as a result of RDS.
Materials and methods. As material, the lung tissue of premature infants at 22-37 weeks of age was taken. Morphometric examination of the structural units of lung tissue was carried out by modifying the "point counting" method by transferring it to a computer screen.

Morphometric examination of the structural units of lung tissue was carried out according to the method of G.G. Avtandilov (1984) "counting dots". This method is used by the author, in fact, by placing a grid of 200 cells on images of organs and tissues taken from histological preparations, and counting which tissue structures the points on it correspond to. To ensure the reliability of the obtained data, points are counted in 8-10 figures from each group of material and an average is taken. We modified this method by transferring it to a computer screen, that is, we took 10 pictures in advance from different areas of histological preparations prepared for each group of the examined material, and on the computer monitor, we placed a linear grid of 200 cells corresponding to these pictures, counting the points where the lines intersected, depending on which structural structure of the tissue they corresponded to. Since the points of the mesh placed on the tissue segment are at the same distance, it is known from the essence of this method that they coincide with tissue structures without selection. The distribution of the points of G.G. Avtandilov's grid grid to the structural units of all areas of the fabric pattern surface does not coincide with the law of relativity. The area of all structural units present in the figure is taken as Vv 100%, the area of each of the structural units to be calculated is indicated by the name of this structure, for example: Vaac (air-injected alveolar cavity), Vgac (hyaline membrane alveolar cavity), Vgma (hyaline membrane area), Viat (interalveolear tissue). Thus, as a result of counting the points, the relative area of the studied structural units in the tissue is calculated. The results show the unit of volume of each structural unit in the studied tissue. Thus, if the area occupied by all structural units in the studied tissue is Vv 100%, then the evenly distributed points in it are denoted by z, and if the ratio of

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each point to the structural unit is taken as R, then its formula will be as follows: P = Vv/100. The correspondence of points to other structural units is determined by the formula: Q = 100-Vv/100. If we take the points corresponding to the studied structural units as x, then its degree of error is calculated by the formula: x/z - P, the percentage indicator of absolute error is calculated by the formula:

$$\varepsilon = (x/z - P) . 100 = 100\ x/z - Vv$$

The degree of error of the calculation according to the theory of relativity - x/z - R, in another formula, is calculated as follows: = t. √Rq/z.

In this formula: x - the number of points corresponding to the studied structural units; z - the total number of points in the test system; P - unit of relativity of points falling on the studied structures; q - unit of relativity of points falling on satisfied structural units; t - normalized difference of indicators from each other. Based on the foregoing, the absolute error of the quantitative indicators is calculated using the following formula: $\varepsilon = t \sqrt{Vv\ (100 - Vv) / z}$.

Result and discussion.

Using G.G. Avtandilov's morphometric method "point counting - test system," 3 levels of premature infants with hyaline membrane disease of the lungs were identified: 1) 22-27 weeks, 2) 28-32 weeks, 3) 33-37 weeks. To compare the quantitative indicators of these groups, the tissue structures of the lungs of children who died from extrapulmonary traumatic brain injury were taken as the control group. In these groups, points corresponding to the structural units indicated below were counted in images taken from histological sections stained with hematoxylin and eosin dyes prepared from lung tissue. On average, 10 dots were counted in each group:

- Air-entrained alveolar cavity - Paac;
- Alveolar cavity with hyaline membrane - Pacg;
- area of hyaline membranes - Pagm;
- Interalveolar tissue - Piat;

For each structural unit, the points listed in 10 figures were summed, the average was calculated, and from it, the area occupied by the structural unit (V) was calculated according to the following formula, for example: the area occupied by the alveolar cavity into which the air enters - V Paac; = Paac;/Px100. Thus, the areas occupied by all structural units of lung tissue were calculated: V aac, Vacg, Vagm, Viat. Based on the quantitative data obtained for these indicators, the following coefficients can be calculated:

Coefficient of the ratio of the area of the alveolar lumen to the area of the hyaline membrane - alveolar lumen activity coefficient (ALCA);

1) 22-27 weeks

Number of microphotos	**Number of dots**				**Total number of dots**
	Paac	Pacg	Pagm	Piat	
1	36	19	15	130	200
2	32	20	17	127	
3	34	18	15	130	
4	44	17	14	128	
5	38	20	13	129	
6	41	19	16	130	
7	33	18	17	131	
8	35	22	15	128	
9	34	21	16	127	
10	39	20	14	128	
∑	366	194	152	1288	2000
M±m %	**18,3±1,72**	**9,7±1,32**	**7,6±1,18**	**64,4±2,14**	

V aac = Paac /P x 100 = 366/2000 x 100 = 18,3%,

ε aac = 2,0 x √ 18,3(100 – 18,3) / 2000 = 1,72% (P=0,05)

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Vacg, = Pacg, /P x 100 = 194/2000 x 100 = 9,7%
εacg, = 2,0 x √ 9,7(100 – 9,7) / 2000 = 1,32% (P=0,05)
Vagm, = Pagm, /P x 100 = 152/2000 x 100 = 7,6%
εagm, = 2,0 x √ 7,6(100 – 7,6) / 2000 = 1,18% (P=0,05)
Viat = P iat/P x 100 = 1288/2000 x 100 = 64,4%
εiat. = 2,0 x √64,4(100 – 64,4) / 2000 = 2,14% (P=0,01)
ACAC - 18.3: 7.6 = 2.4 (alveolar cavity activity coefficient)

2) 28-32 weeks

Number of microphotos	**Number of dots**				**Total number of dots**
	Paac	Pacg	Pagm	Piat	
1	57	34	23	84	200
2	62	36	25	88	
3	54	36	22	82	
4	54	39	25	81	
5	58	27	24	79	
6	61	29	24	84	
7	63	36	23	86	
8	55	38	25	82	
9	56	35	22	88	
10	54	38	23	88	
∑	574	348	236	842	2000
M±m %	**28,7±2,02**	**17,4±1,68**	**11,8±1,44**	**42,1±2,2**	

V aac = Paac /P x 100 = 574/2000 x 100 = 28,7%,
εaac = 2,0 x √ 28,7(100 – 28,7) / 2000 = 2,02% (P=0,05)
Vacg = Pacg, P x 100 = 348/2000 x 100 = 17,4%
εacg, = 2,0 x √ 17,4(100 – 17,4) / 2000 = 1,68% (P=0,05)
Vagm = Pagm, /P x 100 = 236/2000 x 100 = 11,8%
εagm, = 2,0 x √ 11,8(100 – 11,8) / 2000 = 1,44% (P=0,05)
Viat = Piat./P x 100 = 842/2000 x 100 = 42,1%
εiat. = 2,0 x √42,1(100 – 42,1) / 2000 = 2,2% (P=0,01)
ACAC - 17.4: 11.8 = 1.47↓ (alveolar cavity activity coefficient)

3) 33-37 weeks

1) 33-37 weeks	**33-37 weeks**				**1) 33-37 weeks**
	Paac	Pacg	Pagm	Piat	
1	28	44	19	108	200
2	29	48	17	118	
3	31	42	21	102	
4	26	43	19	101	
5	28	47	18	109	
6	31	44	20	114	
7	33	42	17	106	
8	25	41	18	112	
9	28	45	22	108	
10	29	43	19	106	
∑	630	332	354	684	2000
M±m %	**31,5±2,07**	**16,6±1,61**	**17,7±1,70**	**34,2±2,12**	

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Vaac = Paac /P x 100 = 630/2000 x 100 = 31,5%,
εaac = 2,0 x √ 31,5(100 – 31,5) / 2000 = 2,07% (P=0,05)
Vacg = Pacg, /P x 100 = 332/2000 x 100 = 16,6%
εacg, = 2,0 x √ 16,6(100 – 16,6) / 2000 = 1,61% (P=0,05)
Vagm, = Pagm, /P x 100 = 354/2000 x 100 = 17,7%
εagm, = 2,0 x √ 17,7(100 – 17,7) / 2000 = 1,70% (P=0,05)
V iat = P iat/P x 100 = 684/2000 x 100 = 34,2%
ε iat = 2,0 x √34,2(100 – 34,2) / 2000 = 2,12% (P=0,01)

ACAC - 16.6: 17.7 = 0.93↓ (alveolar cavity activity coefficient)

Qualitative indicators of hyaline membrane disease of the lungs, which is "respiratory distress syndrome," are assessed depending on the color, size of macroscopic and microscopic changes developed in the lungs, the degree of differentiation from lung tissue, and whether it is filled with air or not. Three stages of hyaline membrane development are distinguished based on patho- and morphogenetic characteristics: Stage I - the "light interval" period lasts several hours (4-6), and the general condition of the newborn manifests depending on prematurity, but respiratory disorders are not observed. During this period, the incompletely formed surfactant is used up and replaced by a hyaline membrane. During this period, a network-like protein substance appears in the alveolar lumen.

Characteristic of stage II is the "manifestation of clinical manifestations," lasting 48 hours and the appearance of the following distinct clinical symptoms: excitation and damage to the nervous system, tachypnea lasting 72 hours, the appearance of sound during exhalation, involvement of accessory muscles in respiration, acrocyanosis, skin cyanosis and the intensification of this sign during the exacerbation of GMC, the appearance of apnea, the appearance of crepitation-friction and small moist chokes on auscultation, spasm of peripheral vessels in the cardiovascular system, an increase in arterial pressure, tachycardia of 180-220 per minute, the appearance of systolic murmurs, a decrease in diuresis, sometimes oliguria and anuria, the appearance of signs of DIC syndrome. A characteristic morphological feature of the second period is the appearance of eosinophilically stained fibrous protein in the alveolar lumen.

Period III - the "revival period" lasts 3-10 days. Signs of respiratory disorders disappear, changes in the central nervous system subside, and peripheral blood circulation is restored. This period can sometimes be in a "terminal" state, in which the child becomes weak, the skin undergoes total cyanosis, acquires a marble appearance, and breathing is severely disturbed, paradoxically turning into apnea and bradypnea. Shortness of breath leads to wheezing and small chills in the lungs. A drop in blood pressure is observed, a systolic murmur appears, and bradycardia and cardiomegaly are observed. Polyorgan insufficiency and DIC syndrome, anuria develop. In the III period, a homogeneous, eosinophilically stained hyaline protein, covering the alveolar wall, appears in the alveolar lumen.

The hyaline membranes that develop in the lungs of newborns can be congenital or acquired in the early postnatal period. Their location has not been fully studied, according to the data of our studies, it was confirmed that hyaline membranes develop more in the upper segments of the right lung and in the middle intermediate segments of the left lung. In our study, we conducted morphometric studies of premature infants, dividing them into 3 groups according to gestational periods.

Table 1

Anthropometric indicators of premature infants, M±m

№	Group of premature infants	n	Average gestational weeks	Body weight, g	Height, cm
1	**22-27- weeks**	12	25,2±0,4	654±24,3	29,5±1,6
2	**28-32- weeks**	19	29,8±0,6*	1067±84,7*	38,4±4,6*
3	**33-37- weeks**	24	35,3±0,7**	1986±124,6**	43,2±8,5**

Appendix: * - P≤0.05 - gestational age, body weight, and height in group 2 compared to group 1 Reliability difference

** - P≤0,05 – Significant difference in gestational age, body weight, and height in group 3 compared to group 1.

Table 2. Indicators of the area occupied by structural units of lung tissue by gestational periods in premature infants with hyaline membranes, M±m % and ACAC coefficient.

Groups	Area occupied by structural units, %				AC AC
	Paac	Pacg	Pagm	Piat	
22-27- weeks	18,3±1,72	9,7±1,32	7,6±1,18	64,4±2,14	2,24
28-32- weeks	28,7±2,02*	17,4±1,68*	11,8±1,44*	42,1±2,2*	1,47
33-37- weeks	31,5±2,07**	16,6±1,61**	17,7±1,70**	34,2±2,12**	0,93

Appendix: * - P≤0.05 - significance indicator relative to the control group
** -P≤0.01 - significance indicator compared to the control group

Along with the qualitative assessment of morphological features characteristic of any pathological process, including hyaline membrane disease of the lungs, the reliability of the criteria for assessing changes inherent in the pathology will be high if they are assessed by quantitative indicators. We calculated and analyzed the structural changes characteristic of hyaline membrane disease in the lungs, dividing premature babies into 3 groups according to gestational ages. 1st group, in which the lungs of premature infants and children who died from respiratory failure at 22-27 weeks of gestation were collected. For morphometric calculation, we calculated the area occupied by the following structural units present and developed in lung tissue using the "point test" method of Avtandilov G.G. (1994). These included: 1) Paac - sign - air-filled cavity of the alveoli, 2) Pacg- sign - air-filled cavity of the alveoli from which the hyaline membrane originates, 3) Pagm - sign - area occupied by the hyaline membrane, 4) Piat - sign - tissue area between the alveoli. In the 1st group, it was established that the area of the alveolar cavity, into which air enters the lung tissue, occupies only 18.3±1.72%. It was established that the area of the air-filled cavity of the alveoli with a hyaline membrane is relatively small, i.e., 9.7±1.32%. It was confirmed that the area occupied by hyaline membranes appeared in relatively few alveoli during this early week of gestation and occupied a small area (7.6±1.18). In these early weeks of gestation, due to the underdevelopment of lung tissue, most alveoli were not yet opened, and due to the dense state of lung tissue, the area occupied by the interalveolar tissue was wider and occupied more space than all other structural units, i.e., 64.4%.

In the 2nd group, the percentage of all the structural units we studied differed from the 1st group. If we examine each of them separately, it is observed that the cavity of the air-entrained alveoli expands 1.6 times compared to the 1st group. Alveoli with the appearance of hyaline membranes also dilated, and the area of the air-filled cavity in them also expanded, reaching 2 times, that is, 17.4%, compared to the previous group. If the area of hyaline membranes in the 1st group was 7.6%, then in the 2nd group it slightly increased, and the area occupied also reached 11.8%. Due to the expansion of the air-injected alveoli and the alveoli with the formation of a hyaline membrane, the area of the interalveolar tissue was significantly reduced compared to the 1st group, occupying 42.1% of the area.

The 3rd group of our study was 33-37 weeks of gestation, and in this group, all the structural units we studied increased compared to the previous groups. Of these, it was found that the air-filled alveolar lumen increased by 1.7 times compared to the first group and by 1.1 times compared to the second group and amounted to 31.5% of the area. The area occupied by hyaline membranes also increased and increased by 2.3 times compared to the 1st group and by 1.5 times compared to the 2nd group, i.e., it occupied 17.7% of the area. At the same time, it was noted that the air-filled cavity of the alveoli, where the hyaline membrane was formed, did not expand compared to the previous group. Due to the relative expansion of the alveoli and increased air filling, a significant reduction in the area occupied by the interalveolar tissue was observed, i.e., it occupied 34.2% of the area, which is 2 times less than in the 1st group and 1.5 times less than in the 2nd group.

Conclusion

It was confirmed that the area of the air-filled alveolar space, indicating the degree of air filling or respiration of lung tissue, morphometrically occupies 18.3% of lung tissue in premature infants at 22-27 weeks, and the area of interalveolar tissue is 64.4%. As the gestational age increases, the area occupied by

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hyaline membranes in the lung tissue expands, and the area of the air-filled alveolar lumen also increases. It was confirmed that the coefficient of activity of the alveolar lumen was 1.63 at 22-27 weeks of gestation, and sharply decreased in subsequent periods of gestation and was 1.47 and 0.93, respectively.

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