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Serum activity of MMP-2 and MMP-9 and stromielisin-1 concentration as predictors in the pathogenesis of bronchopulmonary dysplasia in preterm neonates

Surowicza aktywność metaloproteinaz-2 i -9 oraz stężenie stromielizyny-1 jako czynniki predykcyjne w patogenezie dysplazji oskrzelowo-płucnej u noworodków urodzonych przedwcześnie

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Aim: Bronchopulmonary dysplasia (BPD) is one of the most severe respiratory diseases, mainly related to premature neonates. Previous studies indicated the role of matrix metalloproteinases (MMPs) in the development of BPD. The aim of the study was to determine the relationship between MMP-2, MMP-3, MMP-9 with their tissue inhibitors (TIMP-1 TIMP-2) and BPD occurrence in premature neonates.

Material/Methods: Eighty-one patients, divided into four study groups, numbered from 1 to 4, depending on gestational age (25–28; 29–32; 33–36; 37–40 weeks), were enrolled. Venous blood was collected between 5 and 7 days after birth. The activity of MMP-2 and MMP-9 were determined with usage of gelatin zymography, whereas MMP-3, TIMP-1 and TIMP-2 was determined using the immunoassay ELISA.

Results: BPD was diagnosed in 50% of patients from group 1 and 11% from group 2. The increase of MMP-2 activity in Group 2, and a decrease in MMP-2/TIMP-2 ratio was noticed in Group 1 compared to Group 2 and 4. A significantly lower incidence of BPD in patients with higher (above the median) values for MMP-2/TIMP-2 (OR = 0.02, CI = 0.00 – 0.55; p <0.05) was noticed in Group 1. The decreased occurrence of BPD in patients with higher MMP-3 concentration, higher MMP-9 activity and the higher value of MMP-9/TIMP-1 did not reach statistical significance.

Conclusions: It has been shown that elevated activity of collagenolytic enzyme in serum, especially MMP-2, may have the effect of decreasing the risk of bronchopulmonary dysplasia in premature neonates.

Keywords: **matrix metalloproteinases • stromielisin-1 • tissue inhibitors of metalloproteinases • bronchopulmonary dysplasia • preterm infants**

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Abbreviations: **BPD** – bronchopulmonary dysplasia; **CI** – confidence interval; **CLD** – chronic lung disease; **CRP** – C-reactive protein; **ECM** – extracellular matrix; **EGF** – endothelial growth factor; **EGF-r** – receptor for EGF; **ELISA** – enzyme-linked immunosorbent assay; **eNOS** – endothelial form of the nitric oxide synthase; **FGF** – fibroblast growth factor; **IUGR** – intrauterine growth retardation; **MMPs** – matrix metalloproteinases; **MT-MMPs** – membrane type matrix metalloproteinases; **nCPAP** – continuous positive airway pressure, nasal method; **NGAL** – neutrophil gelatinase-associated lipocalin; **OD** – optical density; **OR** – odds ratio; **PDGF** – platelet derived growth factor; **RSV** – respiratory syncytial virus; **SD** – standard deviation; **SOD** – superoxide dismutase; **TGF** – transforming growth factor; **TIMPs** – tissue inhibitors of metalloproteinases; **TORCH** – acronym which stands for Toxoplasmosis, Other (Parvovirus B19, Varicella-Zoster virus infection, Syphilis, Hepatitis B), Rubella virus, Cytomegalovirus infection and Herpes Simplex virus infection; **VEGF** – vascular endothelial growth factor; **WBC** – white blood cells.

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a chronic inflammatory disease of the respiratory system of premature infants, characterized by developmental disturbances and damage to the lung and vesicular follicles [45]. BPD is a risk factor for serious infections, including Respiratory Syncytial Virus (RSV), which, despite extensive use in the recent prophylaxis, is still an extremely important problem in the care of prematurely born babies [5]. From a socioeconomic point of view, BPD is also an important issue not only for the patients themselves, but also for their families and society as a whole, and here for research on the pathogenesis of this disease and its effective treatment, which is an important challenge for modern neonatology [30]. According to the classic definition, formulated in 1967 by Northway, bronchopulmonary dysplasia is a chronic respiratory disease, a complication of neonatal respiratory disorder, subjected to substitute mechanical ventilation and prolonged oxygen therapy with high oxygen concentrations in the breathing mixture [33]. On the basis of radiological criteria, there are basically 4 stages of bronchopulmonary dysplasia. Stage 1 essentially corresponds to the respiratory disorder II or III with respiratory edema and vitreous membranes. Stage 2 is an image of disseminated focal atelectasis, against which, in the further course of the disease, emphysema develops. Stage 3 includes the image of bubble emphysema developing on the substrate of previously diagnosed emphysema, while the beginning bronchial repair processes give an image of increased interstitial drawing. Stage 4 includes the image of massive pulmonary fibrosis with damage to the alveolar walls, as well as an increase in the vascular and bronchial drawings, which results from the hypertrophy of the muscular wall of the bronchi and blood vessels [1, 33].

The new definition of BPD was developed by Jobe and was accepted at the National Institute of Health conference in the United States in 2000. According to the new definition, BPD is also understood as chronic lung disease of premature newborns (CLD), but it should be noted that this is a more general term. This definition is based on the requirement for supplemental oxygen and positive pressure at 36 weeks of corrected gestational age [14, 22, 23]. Based on this definition, BPD is classified as: mild BPD- supplemental oxygen requirement at >28 days but not at 36 weeks postmenstrual age, moderate BPD- supplemental oxygen requirement at >28 days and <30% at 36 weeks postmenstrual age, severe BPD – supplemental oxygen requirement at >28 days and >30% at 36 weeks postmenstrual age or positive pressure at 36 weeks postmenstrual age or both procedures together [10, 13, 24].

Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes that have the ability to digest extracellular matrix (ECM) proteins and play an important role in ECM remodeling under both physiological and pathological conditions. MMPs are involved in the initiation and regulation of inflammatory and carcinogenic processes, in embryogenesis and maturation of organs, including the lungs [12, 16]. In human pathophysiology, gelatinases, MMP-2 and MMP-9 and

their tissue inhibitors (TIMP-1 and TIMP-2) are of considerable importance. Other metalloproteinases, such as stromelysin-1 (MMP-3), have a significant effect on gelatinase activation and indirectly on their biological activity. One of the main substrate for gelatinases is type IV collagen, which is a component of the basal membrane of vascular endothelium. Local degradation of the basal membrane is essential for cell penetration into and from the vascular bed. The effect of this process is, for example, the propagation of the inflammatory process and the formation of metastatic tumors [7, 12, 17]. Gelatinases, due to their role in modeling interstitial tissue of various organs, including the lungs, may play a role in the development of bronchopulmonary dysplasia in prematurely born newborns.

The aim of the study was to determine the serum MMP-2 and MMP-9 activity and MMP-3, TIMP-1 and TIMP-2 concentration in preterm neonates at different age intervals as well as the relationship between biochemical parameters and bronchopulmonary dysplasia of premature infants.

MATERIAL AND METHODS

The study was conducted at the Department of Medical Chemistry, Medical University of Lublin and the Department of Neonatology and Neonatal Intensive Care Unit of the Independent Public Clinical Hospital No 4 in Lublin in the period from 1 December 2013 to 31 December 2014. The consent of the Bioethical Committee at the Medical University of Lublin (consent no. KE-0254/300/2013) was granted. Each time the parents agreed to include the newborn in the study.

Patients: 81 newborns were enrolled in the study, of which four groups were identified on the basis of gestation duration: Group 1 (n = 18) – newborns from 24 weeks + 0 days to 27 weeks + 6 days of gestational age, Group 2 (n = 18) – newborns from 28 weeks + 0 days to 31 weeks + 6 days of gestational age, Group 3 (n = 27) –newborns from 32 weeks + 0 days to 35 weeks + 6 days of gestational age, Group 4 (n = 17) – newborns from 36 weeks + 0 days to 41 weeks + 6 days of gestational age.

The duration of the pregnancy was determined on the basis of ultrasonography and the date of the last menstrual period. Criteria for exclusion from the study were early (up to 7 days of life) systemic steroid therapy, congenital malformations, transfusions of blood products, intraventricular and periventricular hemorrhage III-IV grade in Papille classification [37] and TORCH infections. Antibiotic therapy, non-invasive respiratory support, mechanical ventilation, oxygen therapy, supply of exogenous surfactant were not exclusion criteria. Detailed patient characteristics are presented in Table 1.

The clinical condition of the patients was assessed on the basis of Silverman's respiratory failure scale [21] and on the basis of analysis of ventilation parameters and acid-base balance. All patients were treated according to the current standards of neonatal care in Poland [6].

BPD was diagnosed on the basis of diagnostic criteria consistent with the new definition of BPD [24] in the 11 patients included in the study.

Blood collection: A sample of serum was obtained from venous blood taken between 5 and 7 days after birth. Due to the specificity of the patients, no blood was collected separately for use only in the study. Obtaining a trace amount of material (over a dozen microliters) was left after the planned tests. It was sufficient to carry out the planned indications. Serum was transferred to Eppendorf tubes and stored at -70°C for further analysis.

Biochemical procedures: MMP-2 and MMP-9 activity was determined using gelatin zymography as a substrate for the reaction, which was described elsewhere [28]. Briefly, this method is based on the catalytic degradation of the substrate incorporated in the polyacrylamide gel (in this case gelatin) by both gelatinases. In the next step, the spots of gelatine appear as bright, unmarked bands on blue background (Coomassie Blue staining). The width of the resulting bands corresponds to the activity of the enzyme [44]. Identification of gelatinases was based on their specificity for the substrate and by comparison of their localization with known molecular weight proteins (Fermentas, SM0441). Gels were scanned with a resolution of 600 dpi and a quantitative analysis was made using the ImageJ Software (National Institutes of Health, USA). The activity of the enzyme was expressed in optical density (OD) units.

Commercially available ELISA kits were applied to determine the concentration of MMP-3, TIMP-1 and TIMP-2 (R&D Systems, Minneapolis, MN). The procedures were carried out according to the manufacturer's recommendation. All analyses were made in duplicate. The results were read using a standard microplate reader at a wavelength of 450 nm (540 nm)(correction 540 nm). Due to the fact that the ratio of enzyme concentration to inhibitor concentration with the highest affinity for a given metalloproteinase is often used as a measure of the activity of metalloproteinases in the calculations, the MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios were used in the calculations [25, 28].

Statistics: For each group of results obtained, the normality of the distribution was determined by means of the Kołomogorow-Smirnov test with the Lillefors correction. In the normal distribution of the results of both groups compared, the parametric tests (ANOVA) with post-hoc Tukey's test were used to estimate the difference between these and the values were expressed by mean and standard deviation. For nonparametric decomposition, the Kruskal-Wallis test and post-hoc Dunn's test were used and the values were expressed using median and lower and upper quartile. For the selected parameters, the Chi

Table 1. Patient characteristics. The type and parameters of ventilation, oxygen concentration in the respiratory mixture, acid-base balance parameters, severity of respiratory failure, and type of antibiotic therapy included in the table were assessed on the day of collection

Test parameter	Group 1	Group 2	Group 3	Group 4
Duration of pregnancy (weeks + days)	24 weeks + 0 days – 27 weeks + 6 days	28 weeks + 0 days – 31 weeks + 6 days	32 weeks + 0 days – 35 weeks + 6 days	36 weeks + 0 days – 41 weeks + 6 days
Number of patients	18	18	28	17
Birth weight in grams (average, min-max)	794 (380-1270)	1217 (770-1600)	2109 (1440- 3740)	3264 (2240- 3850)
Apgar in 1 minute (quartile 0, 2, 4)	1, 4, 8	1, 7, 8	5, 8, 10	8, 10, 10
Apgar in 5 minute (quartile 0, 2, 4)	3, 7, 8	4, 8, 9	7, 9, 10	9, 10, 10
Degree of severity of respiratory failure on Silvermann scale (quartile 0, 2, 4)	1, 2, 3	0, 1, 2	0, 1, 2	0, 0, 1
pCO_2 in capillary blood in mmHg (medium, min-max)	55.0 (40-70)	54.0 (40-68)	52.5 (40-70)	51.5 (40-63)
FiO_2 in% (average, min - max)	47 (21-73)	43 (21- 65)	40 (21-58)	21 (21-21)
Respiratory support n-CPAP (number of patients)	1	6	11	2
Conventional mechanical ventilation (number of patients)	13	3	3	0
High frequency ventilation (number of patients)	3	0	1	0
Antibiotic therapy (numer of patients)	14	8	18	3
- amikacin	8	4	12	3
- ampicillin with sulbactam	12	8	17	3
- meropenem	2	0	1	0
- vancomycin	2	0	1	0
Surfactant therapy (number of patients)	18	15	5	0
- including INSURE	7	8	2	0
CRP ng/ml (± SD)	2.9 (5.8)	0.1 (0.3)	0.7 (2.3)	3.7 (8.1)
WBC cells^3/ml (± SD)	13.9 (8.2)	11.4 (4.2)	13.2 (4.1)	12.5 (4.1)

FiO2 - concentration of oxygen in the breathing mixture, pCO2 - carbon dioxide partial pressure, N-CPAP - continuous positive airway pressure, nasal method, INSURE - Intubation-Surfactant-Extubation.

square test was used with the odds ratio (OR) calculated using a Confidence Interval (CI) of 95%. For statistical calculations, InStat GraphPad (La Jolla, USA) was used. Statistically significant differences were found for values less significant than 0.05.

RESULTS

A serum zymographic analysis revealed the presence of several gelatinolytic activities, such as proforms of MMP-2 and MMP-9 (at molecular mass 72 kDa and 92

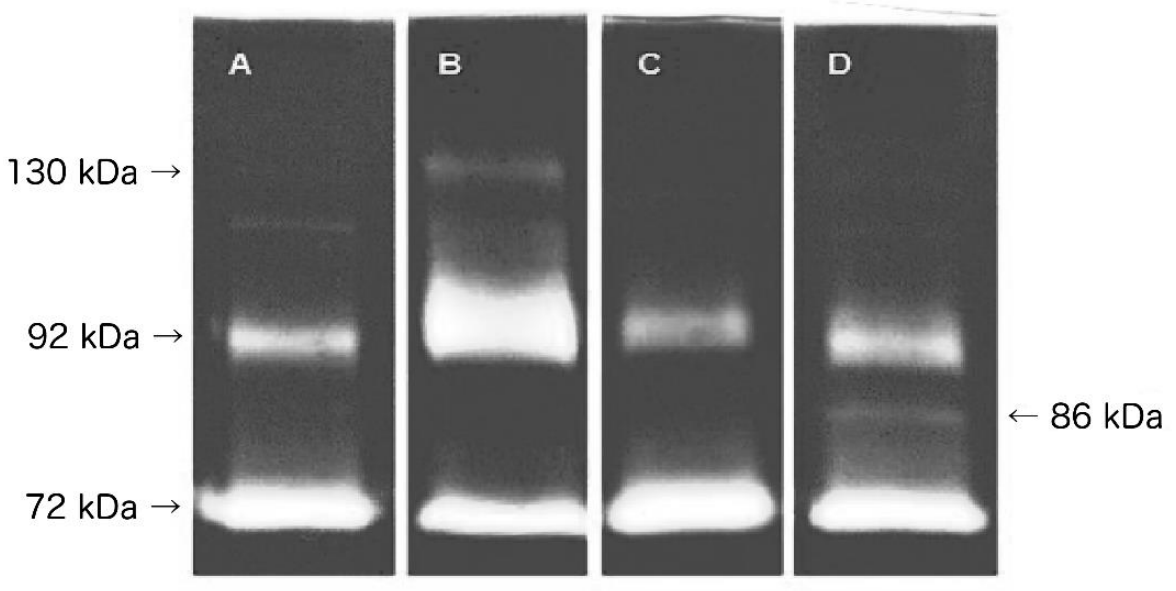

Fig. 1. A representative zymogram. The 72 kDa band corresponds to the pro-MMP-2 form, the 92 kDa pro-MMP-9 band. A – an example of a gelatinous serum activity with a visible pattern of three bands. B – increased activity of pro-MMP-9, C –increased activity of pro-MMP-2, D – the presence of active MMP-9 form with a mass of 86 kDa

kDa respectively) and heterodimer composed of MMP-9 with neutrophil gelatinase-associated lipocalin (NGAL, about 130 kDa). The active form of MMP-9 of 86 kDa was observed in one neonate, female, born at 27 weeks gestation. The remaining samples did not reveal the presence of active form of MMP-2 (66 kDa) and MMP-9 (86 kDa). A representative zymogram is given in Figure 1.

Statistically significantly higher MMP-2 activity was observed in Group 2 compared to Group 3, as well as the higher MMP-9/TIMP-1 ratio was noticed in Groups 2 and 4 compared to group 1. The remaining observed differences in the concentrations or activities in the each study groups did not reach statistical significance. The results of biochemical evaluations are shown in Table 2.

Bronchopulmonary dysplasia was reported in 50% of Group 1 patients and 11% of Group 2 patients. No dysplasia was demonstrated in patients in Groups 3 and 4. The occurrence of bronchopulmonary dysplasia in individual groups is shown in Figure 2.

To determine the effect of the biochemical parameters on the occurrence of BPD, each group with BPD occurrence (Group 1 and 2) was divided into two subgroups, depending on the value of the parameter – above or below the median. A statistically significantly lower incidence of BPD was found in neonates with higher MMP-2/TIMP-2 ratio values (above the median) in group 1 (OR 0.02, CI 0.00 – 0.55, $p < 0.05$). Also, lower incidence of BPD was observed in patients with a higher MMP-9, MMP-9/TIMP-1 ratio as well as higher concentrations of MMP-3 and a lower concentration of TIMP-1. However, the above relationships did not reach statistical significance. The dependence of the analyzed biochemical parameters and BPD occurrence was shown in Table 3. The occurrence of BPD in patients in Group 1 depending on the serum MMP-2/TIMP-2 value is shown in Figure 3.

DISCUSSION

Among the etiological factors of BPD, inflammatory response seems to play an important role. Inflammatory reaction is a cause of interfering of physiological mechanisms of the formation of pulmonary vesicle with correct structure and function. Inflammation especially negatively affects by respiratory development of newborns born prematurely. It is caused by numerous endogenous and exogenous factors. These include congenital and acquired bacterial and viral infections, barotrauma and volutrauma, caused by mechanical ventilation using high volume and inspiratory pressure. Barotrauma and volutrauma lead to excessive and rapid stretching of the unformed wall of the alveoli resulting in pressure on the delicate structures of the blood vessels and secondary ischemia and hypoxia initiating the development of inflammation. High inspiratory pressures and large respiratory volumes directly damage the walls of the alveoli, induce perfusion disturbances within them, activate vesicular macrophages, and increase leukocyte diapedesis to extravascular space. Proteolytic enzymes secretion, which is dominated by elastase and MMPs, is destructive for the extracellular matrix proteins. Chronic and severe inflammatory processes result in pulmonary fibrosis, bronchial hyperresponsiveness and increased respiratory resistance, leading to severe exacerbation of respiratory failure [4, 11, 15, 35].

Table 2. Analyzed biochemical parameters in study groups

	Group 1	Group 2	Group 3	Group 4	ANOVA
MMP-2 (OD) average ± SD	11293 ± 3769	13806 ± 5876*	9999 ± 2868	9883 ± 2261	p<0.05
MMP-9 (OD) average ± SD	5839 ± 5211	9966 ± 7592	6916 ± 7438	12285 ± 3686	p<0.05
TIMP-1 (ng/ml) average ± SD	382.1 ± 178.5	389.5 ± 131.3	458.1 ± 255.0	362.1 ± 79.0	p>0.05
TIMP-2 (ng/ml) average ± SD	198.6 ± 50,7	223,0 ± 71,1	186,0 ± 53,3	197,8 ± 17,4	p>0.05
MMP-3 (ng/ml), median (1^{st}-3^{rd} quartile)	6.93 (3.37-9.27)	5.58 (3.65-6.71)	5.32 (2.76-7.52)	5.38 (3.09-6.60)	p>0.05
MMP-9/TIMP-1 average ± SD	15.0 ± 11.1	31.1 ± 18.9*	18.1 ± 11.8*	37.1 ± 15.1	p<0.05
MMP-2/TIMP-2 average ± SD	52.6 ± 22.0	72.6 ± 26.3	61.6 ± 37.2	60.2 ± 7.2	p>0.05

Significant statistically higher MMP-2 activity in group 2 compared to group 3 and higher MMP-9/TIMP-1 ratio in groups 2 and 4 compared to group 1 were noticed (* Tukey-Kramer post-hoc test, p <0.05)

Despite many studies, it was not possible to establish unambiguously the influence of genetic factors on the development of BPD [41, 46]. In studies that were conducted on newborns with twin pregnancies, a higher incidence of BPD was observed in both monozygotic twins. This relationship was not found between the dizygotic twins. This suggests a significant contribution of genetic factors in the development of the disease. In another study, polymorphisms of genes VEGF and epithelial nitric oxide synthase (eNOS) encoding have been identified, which may be important in the development of BPD [29, 38]. However, in the majority of conducted studies, in which the influence of polymorphism of genes peroxide dismutase (SOD), surfactant proteins and cytokines such as TNF and TGF-β encoding were assessed, the relationship between the tested parameters and the risk of BPD were unequivocally demonstrated [32, 39, 42].

An important role in the process of respiratory morphogenesis is also attributed to retinoic acid derivatives and metalloproteinases of the extracellular matrix, mainly MMP-7, MMP-8, MMP-9 and MMP-2, but also MMP-14 (MT1-MMP), responsible for the activation of MMP-2 *in vivo*. MMP-14 (MT1-MMP) is involved in the physiological processes of cell migration, remodelling of the stromal tissue, vascularisation and repair processes, the expression of which is largely regulated by EGF [3, 20]. In mammalian fetuses with an experimentally blocked EGF receptor (EGF-r), disorders of the vascularization processes were observed, resulting in impairment of bronchiolar differentiation and alveolar ducts, suggesting an important role of EGF-r and its ligands in the regulation of MMP-14 expression (MT1-MMP) and its influence on angiogenesis processes [26]. Fetuses of mice with low MMP-14 (MT1-MMP) activity showed a decrease in the pulmonary alveolar space by approximately 40% compared to fetuses in which MMP-14 (MT1-MMP) activity was normal. Differences were also observed in the diameter of the alveoli and total lung weight. In mice with low MMP-14 (MT1-MMP) activity, pulmonary hypoplasia and pleural defects were observed, whereas pulmonary alveolar diameter was 46% lower compared to fetuses with high MMP-14 activity (MT1-MMP). In fetuses of mice with normal MMP-14 (MT1-MMP) activity, the diameter of the pulmonary alveolar pores varied between 5–20 μm, while the low activity of MMP-14 (MT1-MMP) was closely related to the reduced amount and significantly smaller diameter of respiratory pores in the alveoli studied in the electron microscope [3]. This clearly indicates the important catalytic role of MMP-14 (MT1-MMP) in the reconstruction of stromal tissue and the formation of respiratory pores. In addition, MMP-14 (MT1-MMP) has the ability to activate pro-MMP-2 to the active form; hence, the mechanisms leading to the induction of MMP-14 (MT1-MMP) expression, including EGF interaction on the cell, indirectly affect the activity of MMP-2 in the lungs. The above phenomenon may be important in the pathophysiology of BPD in newborns [27].

Our study showed higher MMP-2 activity in newborn infants born from 28 weeks + 0 days to 31 weeks + 6 days of gestational age compared to activity observed in the group of elderly newborns born from 32 weeks + 0 days

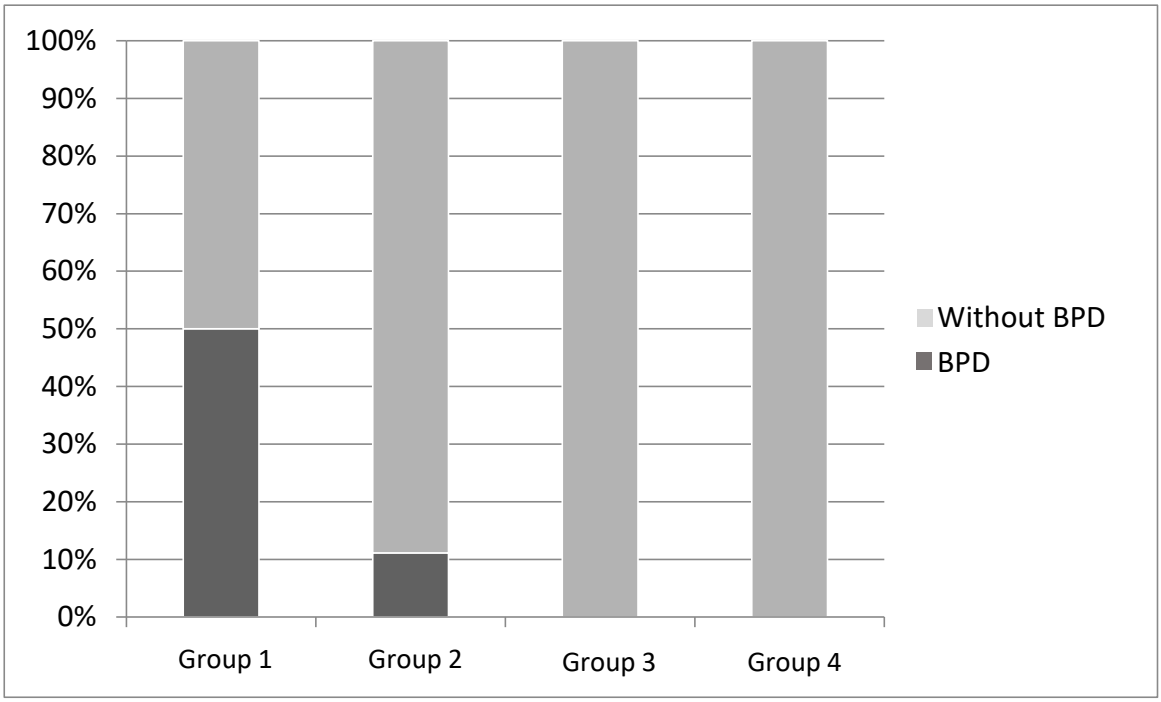

Fig. 2. The occurrence of bronchopulmonary dysplasia in individual groups

to 35 weeks + 6 days of gestational age. The difference in MMP-2 activity is likely to be associated with intensive redevelopment of the respiratory system at this stage of fetal development, leading to the formation of primitive follicular tubules and terminal bags, as well as with the differentiation of type II pneumocytes, responsible for the most intense surfactant synthesis during that period. Given that expression of MMP-2 is constitutive and not induced by pro-inflammatory cytokines, the structural and functional changes of the respiratory system that occur at this stage of fetal development are likely associated with physiological and genetically conditioned increase in activity of MMP-2 [18, 27].

In the conducted research no statistically significant differences in MMP-9 activity were observed in each study group, but an upward trend in MMP-9 activity was observed in the group of the most mature neonates included in the study. This fact suggests that MMP-9 can play a role in the reconstruction of the respiratory system. In MMP-9 immunoassay evaluations studies, MMP-9 has been shown to express significantly in epithelial cells, type II pneumocytes and in pulmonary macrophages at almost every stage of respiratory system development [18]. In rat fetal lung MMP-9 activity was observed from week 26 of intrauterine life and no significant changes were observed during further developmental stages, indicating a relatively constant MMP-9 activity during various stages of respiratory system development. The demonstrated higher MMP-9 activity in older infants may be associated with physiologically increasing numbers of epithelial cells, type II pneumocytes, and ripening of the vascular network [18]. In the conducted studies, the median values for C-reactive protein (CRP) and white blood cells (WBC) concentrations are similar in all groups of newborn infants; hence, the

Table 3. The influence of MMPs, TIMPs and MMP/TIMPs ratio on BPD occurrence in study group 1 and 2

Parameter value > median	Group 1	Group 2
MMP-2	OR 1.0; CI 0.14–7.10	OR 1.0; CI 0.05–18.31
MMP-9	OR 0.16; CI 0.01–1.62	OR 1.0; CI 0.05–18.31
TIMP-1	OR 3.16; CI 0.36-30.7	OR 6.33; CI 0.26-152.98
TIMP-2	OR 0.56; CI 0,06-4.67	OR 6.33; CI 0.26-152.98
MMP-9/TIMP-1	OR 0.16; CI 0.01-1.62	OR 6.05; CI 0.25-142.15
MMP-2/TIMP-2*	OR 0.02; CI 0.00-0.55	OR 1.0; CI 0.05-18.31

OR - odds ratio, CI - 95% Confidence interval. Chi-square test. * $p<0.05$.

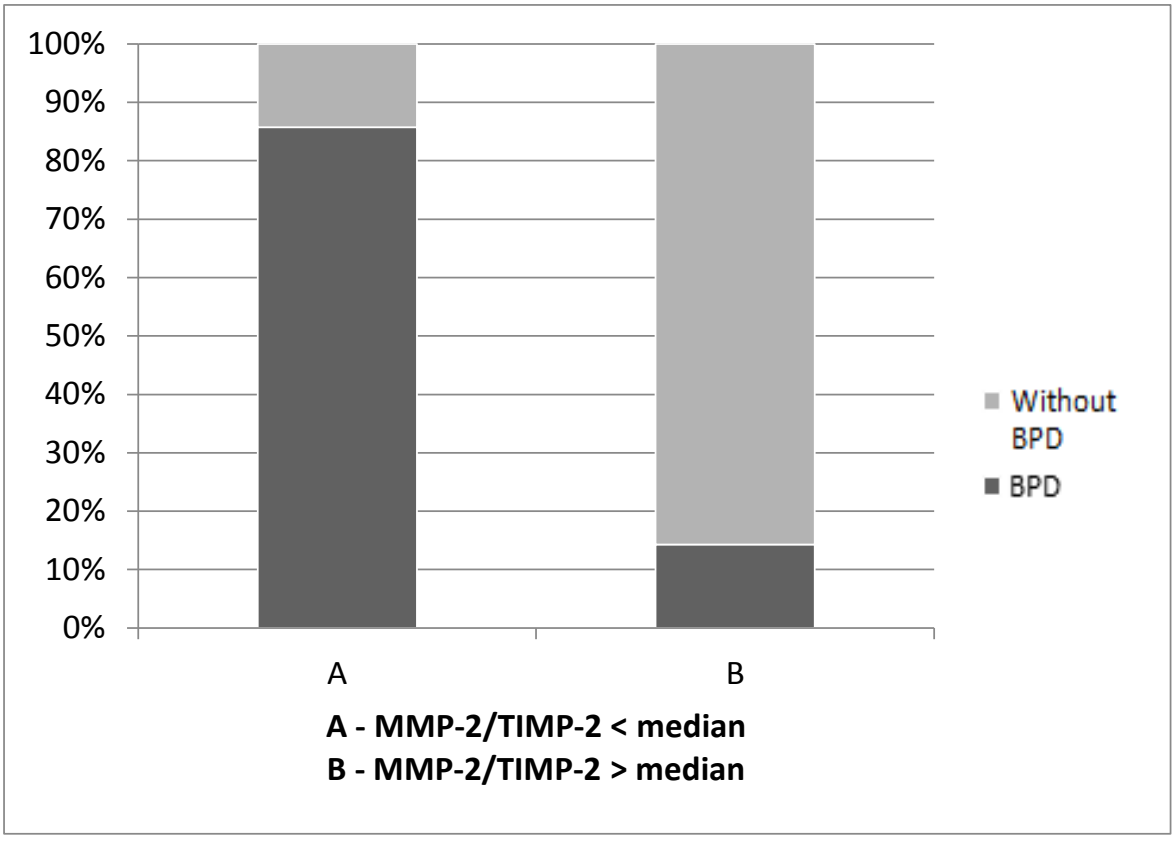

Fig. 3. The Occurrence of BPD in patients in group 1 depending on the serum MMP-2/TIMP-2 value

MMP-9 is highly likely to have an extra-leukocyte origin and is associated with the physiological reconstruction and maturation of the newborn's lungs.

MMP-3 concentrations did not show statistically significant differences between groups of premature infants as well as term infants. MMP-3 expression can be both constitutive and inducible depending on cell type. The enzyme is constitutively synthesized in neurons and astrocytes, while in the respiratory system it is characterized by an inducible expression mechanism [27]. Based on ELISA tests in each neonatal test groups, there were no statistically significant differences between serum TIMP-1 and TIMP-2 concentrations, indicating that their potential role in the development and reconstruction of the respiratory system is not reflected in changes in their serum concentrations.

However, the MMP-9/TIMP-1 ratio value, indirectly indicating MMP-9 activity in vivo, was statistically lower in the youngest preterm (Group 1), among which the highest proportion of patients with BPD have been shown. The result suggests MMP-9 participation in BPD counteracting processes.

Studies have shown a lower incidence of BPD in case of higher (above median) MMP-9 activity, MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios values and MMP-3 concentration. Although only the odds ratio of BPD for MMP-2/TIMP-2 ratio values was statistically significant, it can be stated that high serum metalloproteinase activities in the first days after birth is associated with a reduced risk of BPD.

As has been shown in numerous studies, reduced gelatinases activity is closely related to disorders of the degradation and conversion of the extracellular matrix, which may eventually lead to excessive proliferation of connective tissue [2, 3, 19, 20, 36, 40]. The effect of these processes may be increased fibrosis not only within the lung, but also in various organs. The effect of collagenolytic enzymes on fibrosis processes has been demonstrated in studies on gelatinase activity in the development of liver cirrhosis. In a study of 26 patients with cirrhosis, in whom serum MMP-2 and MMP-9 activity was determined, a statistically significant reduction in MMP-2 was found in the serum of patients with Child-Pugh B and C cirrhosis in comparison with the control group [31].

Similar conclusions were drawn from research conducted by Davey et al. in 2011. The role of metalloproteinases in lung injury in the pediatric population was assessed in a zymography study. It has been shown that in patients in whom gelatinase activity was inhibited by tetracycline, respiratory distress epithelial abnormalities and uncontrolled and excessive fibrosis have been reported, leading to irreversible changes in interstitial tissue analogous

to changes in BPD [9]. Similar conclusions were made by examining the BPD model in mice. Low expression and low activity of MMP-9 have been shown to result in increased lung damage and increased respiratory failure, most probably due to progressive interstitial fibrosis [3, 20, 34]. Studies on the activity of metalloproteinases in aging human lungs have shown that low MMP-2 activity and increased TIMP-1 concentrations are associated with the development of asthma, chronic obstructive pulmonary disease and pulmonary fibrosis [8, 43].

Referring to the data presented in Table 1, it should be noted that the groups differed from each other. However, no statistical analysis was carried out which would show intergroup statistical significance of the analyzed parameters. Frequent use of respiratory support (nCPAP and conventional mechanical ventilation) and antibiotic therapy in newborns in Groups 2 and 3, not directly related to the clinical condition (definitely better Apgar score of newborns from Group 3 with the same rating on the Silvermann scale), in the conducted studies resulted from the coexisting hypotrophy, conditioned by intrauterine growth retardation (IUGR).

In relation to the results of the study presented above, it should be noted that the performed determinations gave the results of static values of the parameters examined, determined by the 5-7 day of life. This is due to the numerous difficulties in collecting material for testing from neonates born with the lowest birth weight and essential bioethical aspects. In order to obtain a dynamic picture of the analyzed parameters, during further tests, it is necessary to perform determinations in blood samples collected from patients in the following days or even weeks of treatment, for example at the end of the 2nd, 4th, 8th and 12th week of life. Conducting further research, prenatal steroid therapy should be included, based on current obstetric recommendations. Acceleration of fetal lung maturation as a result of properly performed prenatal steroid therapy perhaps could be important in reducing the incidence of BPD in the examined newborns. This is the subject of current research in the world of research.

Conclusions from the performed experiments may be a starting point for the tests evaluating the therapeutic use of exogenous enzymes from the group of metalloproteinases in the prophylaxis and therapy of BPD. Maybe someday the treatment of BPD with exogenous collagenolytic enzymes will be common, as is the use of a surfactant in the respiratory distress syndrome.

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