MEDICAL UNIVERSITY OF VARNA

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DEPARTMENT OF OPHTHALMOLOGY AND VISUAL SCIENCES

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Screening program of retinopathy of prematurity – regional application, results, perspectives

THESIS SUMMARY

For the award of the educational and scientific degree "DOCTOR" in the scientific program "Ophthalmology"

Research supervisor

Assoc. Prof. Yana Manolova, MD, PhD

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The dissertation has 202 pages and is illustrated with 34 figures and 17 tables. The bibliography includes 481 references, from which 13 on Cyrillic script, and 468 on Latin script.

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The research on the dissertation was conducted at the University Specialized Hospital of Eye Diseases for Active Treatment - Varna and Specialized Hospital of Obstetrics and Gynecology for Active Treatment prof. Stamatov - Varna

RESEARCH JURY:

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CONTENT:

ABBREVIATIONS USED:

BEAT-ROP – Bevacizumab Eliminates the Angiogenic Threat for Retinopathy of prematurity

CRYO-ROP – The Multicenter Trial of Cryotherapy for Retinopathy of Prematurity

ETROP – Early Treatment for Retinopathy of Prematurity

IVF – In Vitro Fertilization

PVN – Per Vias Naturalis

ROP – Retinopathy of prematurity

SC – Sectio Cesarea

TNF-α – Tumor Necrosis Factor-α

VEGF – Vascular Endothelial Growth Factor

1. INTRODUCTION

Human interaction with the outside world is performed through our five senses – sight, hearing, smell, taste and touch. Of these five, sight undoubtedly plays a key role – starting with the physical survival of the individual at the dawn of human civilisation and extending to the opportunities that modernity provides for movement, the transmission of information and communication.

Preserving eye health is a top priority for health professionals who have dedicated their careers to this small organ. Protecting children's eye health is a task that holds even more challenges. One of these is a disease called retinopathy of prematurity (ROP).

It affects the youngest and most vulnerable patients - children born prematurely. It is a multifactorial vasoproliferative disease that results from abnormal processes occurring in the immature retina (1).

Retinopathy of prematurity is a leading cause of preventable childhood blindness. It has been estimated that by 2010, globally, almost 185,000 preterm children had developed ROP, 20,000 had lost vision, and another 12,300 had developed mild to moderate visual impairment. 65% of children with visual impairment are in developing economies, which includes Bulgaria. According to the same study, there are 50 000 children under the age of 15 worldwide who have lost their sight due to this disease. (2)

The struggle of ophthalmologists with ROP has been going on for more than 80 years. The progress of medicine in this period is, of course, undeniable. Whereas in the 1940s and 1950s, the disease was simply described in its terminal phase and vision was irretrievably lost, in the following decades treatment techniques were tried, aiming at the earliest possible intervention, the natural course of the condition was studied and the pathophysiological mechanisms that suppress abnormal processes without affecting the normal maturation of ocular structures were sought. Developments in digital technology offer new rapid, convenient methods of diagnosis, including at a distance. At present, there are still many question marks. One difficulty comes from the small number of specialists trained to deal with this specific pathology, as well as purely logistical difficulties – remoteness of the intensive care units for premature babies from the ophthalmology units, the need for coordination between specialists from separate medical structures. The increasing number of children who are affected, as well as the legal aspects - claims by parents in case of an unfavourable outcome, form a palette of problems facing the medical community. Of particular concern is the fact that fewer practicing ophthalmologists

are willing to work in this field, and a number of them are planning to quit, according to the American Ophthalmological Association (AOA) (3).

Meanwhile, children around the world continue to lose their sight, with all the social and economic consequences that entails.

The strategy that could effectively combat this problem should absolutely be aimed at providing the highest quality of neonatal care, as well as a precise screening program aimed at early detection and timely treatment of children at risk for the most severe course of the disease.

In order for these measures to be successful in Bulgaria, it is urgent to ensure a certain level of communication between the different treatment centres in the country to standardise the approach to the disease and ensure access to consultative or curative care when needed. An indisputable step towards this goal is the creation and promotion of an electronic registry containing information on the ocular status of preterm children with signs of ROP, to facilitate the transfer of information between the different specialists involved in the care of preterm children. An optimal option would be to integrate the information into the National Health Information System, which would provide a comprehensive picture of the neurological, ocular, hearing and motor status of these children, refining their needs, ensuring their timely treatment and rehabilitation.

2. AIM AND TASKS

2.1 Aim of the current thesis

To establish the epidemiological and clinical characteristics of ROP among premature infants in the Varna region according to the current screening program for ROP adopted in the Republic of Bulgaria, and to propose a platform to improve the awareness of parents and medical professionals about this disease.

2.2 Tasks

1. To establish the frequency and characteristics of ROP among premature infants screened in the Neonatology Department of the Specialized Hospital of Obstetrics and Gynecology "Prof. Dimitar Stamatov" - Varna and The University Specialized Hospital for Active Treatment in Ophtalmology - Varna;

2. To analyze the risk factors on the fetal side, which could be related to the development and progression of the disease;

3. To analyse the maternal risk factors that could be relevant to the development and progression of the disease;

4. To monitor the early post-therapeutic effect of intravitreal administration of anti-VEGF medication;

5. To determine the place of ROP as a cause of severe visual impairment and blindness among the students at the Special School for Students with Visual Impairment "Prof. Dr. Ivan Shishmanov", Varna;

6. To offer an internet-based information portal for ROP.

3. MATERIALS AND METHODS

3.1 Clinical material according to the ROP screening programme

This study was approved by the Research Ethics Committee of the Medical University - Varna (No. 94/25.06.2020). All procedures were in accordance with the requirements of good clinical practice and the ethical standards of the World Medical Association (Declaration of Helsinki on the Rights of Human Subjects).

The clinical material includes the preterm children who were screened for ROP through the the Neonatology Department of the Specialized Hospital of Obstetrics and Gynecology "Prof. Dimitar Stamatov" - Varna and followed up in The University Specialized Hospital for Active Treatment in Ophthalmology - Varna; for the period January 2017 - December 2020, a total of 124 preterm children.

The examinations were carried out on the territory of:

- Department of Neonatology of the Specialized Hospital of Obstetrics and Gynecology "Prof. Dimitar Stamatov" - Varna

- University Specialized Hospital for Active Treatment in Ophtalmology - Varna,

In case of necessity of treatment, it was carried out in the clinic of neonatology at Neonatology of the Specialized Hospital of Obstetrics and Gynecology "Prof. Dimitar Stamatov" - Varna.

For the purpose of the study, the children were divided into two groups:

Group I - patients who did not develop ROP;

Group II - patients who developed ROP.

The second group in turn was divided into two more subgroups:

Group II A - patients who had developed ROP who had spontaneous regression (untreated);

Group II B - patients with progressive ROP requiring treatment (treated).

The inclusion criteria for the study were consistent with the standard adopted at the National Workshop on Screening and Treatment of ROP in Bulgaria in 2009 (4).

1.Live, preterm infants born before 32 years of age and/or weighing less than 1500 grams;

2. All premature infants weighing 2000 g and less at:

- Apparatus ventilation;

- Severe intrapartum asphyxia;

- Intracranial hemorrhage;

- Exchange blood transfusions;

- Sepsis.

3. At the discretion of the neonatologist.

Exclusion Criteria:

1.All premature infants not falling into the relevant groups

3.2 Clinical material associated with incidence of ROP in Specialized School for visually impaired children “Prof. d-r Ivan Shishmanov“ Varna

The study was conducted in the period August 2022 - November 2022 among the students of the school for children with visual impairment "Prof. Dr. Ivan Shishmanov" in the city of Varna, after receiving permission from the school principal. Questionnaires were provided in advance to enable the school management to familiarize themselves with the nature of the survey and to ensure that there was full transparency regarding the information required concerning the personal details and health status of each pupil. Data was collected on the age, gender and cause of visual impairment as per the available medical information of the visually impaired students attending the school in the academic year 2022/2023.

A special questionnaire was prepared for children with severe visual impairment due to ROP disease. It was marked with the age, sex, current visual acuity of each pupil, as well as data concerning the presence of ongoing treatment for ROP, its type, information on comorbidities affecting neuro-mental development, and hearing status. The questionnaire for each of the covered children was completed with the close collaboration of the school principal as well as the class teachers of the respective students.

3.3 Examination methods

Diagnostic methods used in the study included:

- Identification of preterm infants to be screened;

- Examining medical records – current medical history, documents from birth, and from neonatal units where the child was treated;

- Ensuring maximum medical mydriasis;

- Administering topical anesthesia and eye speculum;

- Examination by binocular indirect ophthalmoscopy at the SBAGAL "Prof. Dimitar Stamatov" and/or photodocumentation of the clinical findings with RETCAM at the SBOBAL-Varna;

- Choice of management- follow-up or treatment.

Identification of preterm children subject to screening:

These are the children who meet the inclusion criteria in the study. They are referred to the screening program by the staff at the neonatology clinic, no earlier than 4 weeks after the child's birth. On the day of the study, maximal medical mydriasis is aimed to be achieved in order to view the posterior pole, middle and extreme periphery via BIO and/or RetCam. The protocol used was a triple drip of a midriatic "cocktail" of Tropicamide 0.5% and Phenylephrine 2.5% over 10 minutes. The drip was performed by a neonatology nurse.

Review of medical records:

Includes a review of each child's current medical history, documents from birth or other medical facilities where the child has spent time. Potential risk factors were noted on a special form, as were the findings of the screening examination.

Course of the screening examination:

1. Placement of topical anesthesia and eye speculum;

All examinations were performed under topical anesthesia, accomplished by Proxymetacaine Hydrocloride (Alcaine 0.5%, Alcon), 30 - 60 seconds prior to the insertion of the pediatric eye speculum and the start of the examination.

2. Perform the examination by binocular indirect ophthalmoscopy (BIO);

BIO, which is considered the "gold standard" in ROP diagnosis, was used for all examinations. (5), (6) 20 D and 28 D power fundus lenses (Osular Instruments Inc.) were used, and the children's fundus was viewed using the "doll head phenomenon." Follow-up examinations were performed weekly until complete vascularization of the peripheral retina or regression of the disease if scarring was detected in two consecutive examinations.

3. RetCam examination;

Examinations using RetCam 3 were performed at the SBOBAL-Varna. The examination was performed after medicated mydriasis, topical anesthesia, placement of a pediatric blepharostat and contact gel (Corneregel). The aim of the examination was to acquire high-resolution images of the posterior pole (macular area and the

head of optic nerve), temporal, nasal, superior and inferior quadrants. After completion of the examination, the images were reviewed and those with the most significant informative value were selected. Through these images, it was possible to follow the children's condition dynamically, report the outcome after treatment, and perform a telemedicine consultation. Study technique:

Preliminary mydriasis and placement of eyelid speculum were required. A topical anaesthetic drop was placed. We tried to take a photograph of the iris in each case in order to demonstrate the size of the pupil before applying Dexpanthenol ocular gel (Corneregel 5% - Bausch and Lomb), which serves as a coupling agent between the probe and the cornea. Photographs of the ocular fundus or video are taken, and posterior pole photographs in all directions- superior, inferior, nasal, and temporal are mandatory.

After completion of the examination, it is mandatory to place a drop of antibiotic collyrium in the conjunctival sac.

The findings were noted in each patient's medical history as well as on a special form prepared for the study. Depending on the patient's condition, the next follow-up examination was determined or a treatment decision was made.

The treatment of the children was carried out by modern proven methods such as cryotherapy, laser therapy and intravitreal administration of anti-VEGF drugs.

The criteria used to determine which children should be treated were in accordance with the National Strategy for Screening and Treatment of ROP in Bulgaria (4).

The indications for treatment were:

- ROP in zone 1, any case with "plus"-disease, regardless of stage;

- ROP in zone 1, stage 3, whether or not "plus"-disease is present;

- ROP in zone 2, stage 3 with "plus"-disease.

It is extremely important to emphasize that in the presence of pre-threshold ROP, treatment should be administered within the first 48 h - 72 h of diagnosis. If aggressive posterior ROP is found, treatment should be given as soon as possible, within 48 h of diagnosis (5).

1. Laser therapy technique;

The procedure was performed in the neonatal intensive care unit, under general anesthesia, after maximal medical mydriasis. A diode laser, wavelength 814 nm, transpupillary was used. Laser radiation was administered through a binocular

indirect ophthalmoscope. Laser coagulants were applied to the avascular retina, avoiding the shaft. The laser radiation parameters were: power in the range 150 mW - 250 mW, exposure 100 ms. The aim was to obtain a grayish-white coagulum. Usually, an increase in power was necessary in the areas adjacent to the ridge. The number of coagulates varied between 1000-1500 coagulates in zone 2 and over 2000 coagulates in zone 1. The procedure usually began by forming a row of coagulates in front of the ridge and a row in front of the ora serrata and gradually filling the avascular zone. Antibiotic drops were prescribed after the manipulation. Follow-up was performed 7 days after the intervention, and follow-up continued weekly until regression was established.

Re-treatment with laser was warranted if there was persistent plus disease or evidence of progression. In these cases, the coagulate-free areas were treated.

2. Technique of cryotherapy;

It is performed under general anesthesia, again there is monitoring of basic vital signs by an anesthesiologist. After preparation of the operative field, cryocoagulation was performed transconjunctivally, the aim being to treat the entire avascular retina, avoiding cryoapplications to the shaft.

Postoperatively, topical antiobiotic and corticosteroid therapy was administered.

3. Technique of intravitreal administration of anti-VEGF medication.

Strict adherence to the rules of asepsis and antisepsis, the dose was half of the adult dose. Injection is performed 1 - 1.5 mm from the limbus, with the needle following a course parallel to the visual axis. It is necessary to take into account the anatomical features in premature infants. The lens is disproportionately large, the pars plana is underdeveloped. The risk of traumatic cataract is not small and it is considered an extremely serious condition. Performing cataract surgery in a premature infant with ROP could result in ischemia, neovascular re-proliferation and pupillary block.

Postoperatively, antibiotic collyr and unguent were prescribed

3.4 Statistical methods

Microsoft Office Excel (MS Office 2010) was used for data entry Statistical analysis was performed using specialized software- Statistical Package for Social Sciences (SPSS) for Windows, version 26.0 (SPSS Inc., Chicago, Illinois, USA).

3.4.1. Descriptive analysis - its methods are used to describe the parameters of the sample.

3.4.2. Comparative analysis

- Independent samples t-test (Student's t-test) - parametric method, compares the mean values of a quantitative indicator in two groups of cases;

- ANOVA - a parametric method that compares the means of a variable across more than two groups of cases;

- $\chi 2$ - test - It is a non-parametric method for the analysis of categorical attributes. We used it to determine whether a relationship between two variables was present. Where appropriate, we used Fisher's exact test.

- Linear regression analysis - its purpose is to examine the relationship between one or more independent variables and one dependent variable and to indicate the degree of dependence between them;

- According to the number of independent variables, single and multiple linear regression are distinguished. Single linear regression examines the effect of one independent variable on one dependent variable, while multiple linear regression analyzes the relationship between two or more independent variables and one dependent variable.

- According to the type of regression model, simultaneous, hierarchical and stepwise regression are distinguished. In the present study, we used stepwise regression to analyze the significant and independent risk factors for PH.

Microsoft Excel was used for graphical analysis and data illustration.

4. RESULTS

4.1 Analysis of incidence and ROP characteristics

In the present study, we covered a 4-year period (January 2017 - December 2020), in which 124 preterm infants from the Neonatal Intensive Care Unit at the Hospital "Prof. Stamatov" underwent eye screening for ROP, in accordance with the current screening program in Bulgaria. Data collection was divided into two periods:

– January 2017 – December 2018 – Retrospective data collection, data from medical histories and discharge summeries of children, treated in the Neonatal Intensive Care Unit for the respective period, were analyzed;

– January 2018 – December 2020 – Prospective follow-up of children enrolled in the eye screening program.

During the study period, signs of ROP were detected in 86 children (69.4% of all screened children), in 25 of them (20.2% of all screened children) the disease progressed to ROP requiring treatment. The remaining 61 children (49.2% of all children studied) showed signs of spontaneous disease regression. The distribution of cases according to the development and severity of ROP by year is presented in Table 5:

	2017	**2018**	**2019**	**2020**
Total number of children,underwent ROP screening (N)	39	31	36	18
ROP Incidence (N, %)	28 (71.8%)	17 (54.8%)	26 (72.2%)	11 (61.1%)
Mild ROP (N, %)	22 (78.6%)	14 (82.4%)	14 (53.9%)	7 (63.6%)
Severe ROP (N, %)	6 (21.4%)	3 (17.6%)	12 (46.1%)	4 (36.4%)

Tab. 1 Distribution of premature infants in calendar years, according to ROP development

Regarding the ROP severity, the distribution was as follows: stage I ROP was found in total of 34 children (27.4%), stage II in 28 children (22.6%), stage III in 20 children (16.1%), stage IVa in 2 children (1.6%), stage IVb in 2 children (1.6%). Stage V was not detected in any child. These results are presented in tabular form in Table 6.

Without ROP	Stage I	Stage II	Stage III	Stage IVa	Stage IVb	Stage V
38 (30.6%)	34 (27.4%)	28 (22.6%)	20 (16.1%)	2 (1.6%)	2 (1.6%)	0 (0%)

Tab. 2 Distribution of cases according ROP severity

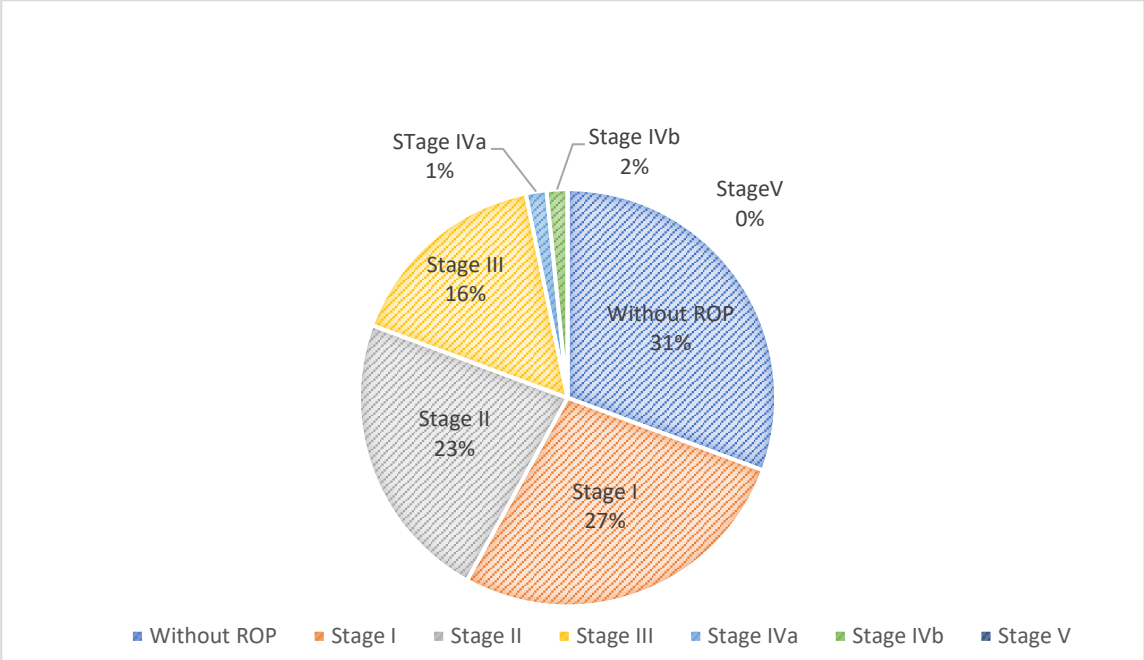


Fig.1 Distribution of cases according to ROP severity

According to the prevalence of ROP, the distribution in the group was as follows: zone 1 was found in 24 children (19.4%), zone 2 – 88 children (71.0%), zone 3 – 12 children (9.7%).

ROP	Zone 1	Zone 2	Zone 3
Without ROP	6 (4.8%)	28 (22.6%)	4 (3.2%)
ROP stage I	0 (0.0%)	27 (21.8%)	7 (5.6%)
ROP stage II	5 (4.0 %)	22 (17.7%)	1 (0.8%)
ROP stage III	10 (8.1%)	10 (8.1%)	0 (0.0%)
ROP stage IVA	2 (1.6%)	0 (0.0%)	0 (0.0%)
ROP stage IV B	1 (0.8%)	1 (0.8%)	0 (0.0%)
ROP stage V	0 (0.0%)	0 (0.0%)	0 (0.0%)

Tab.3 Distribution of cases according to severity and localization of the process

Plus-disease was detected among 27 children (21.8%), of which the process was localized in zone 1 in 16 cases (59.3%), in zone 2 in 10 cases (37.0%) and in zone 3 in 1 case (0.8%).

Treatment was necessary in 25 cases (20.2%), of which the process was localized in zone 1 in 14 children (56%), and zone 2 in 10 children (40%) , and zone 3 in 1 child (4%).

4.2 Analysis of the child's potential risk factors for the development and progression of ROP

We investigated 20 potential risk factors on the child's side: Birth weight, gestational age, sex, presence of multiple pregnancy, oxygen therapy, anemia of prematurity, two or more hemotransfusions, hyperbilirubinemia and phototherapy, and exogenous surfactant administration, intraventricular hemorrhage, persistent ductus arteriosus, neonatal pneumonia, intrauterine hypotrophy, respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, neonatal pneumonia, low APGAR score, intrauterine hypotrophy.

4.2.1. Birth weight

Birth weight is an indicator that is strongly associated with neonatal mortality and morbidity, and is considered an important predictor of future neonatal health status.(7) The mean birth weight for the entire cohort was 1106 g ± 254.4 g, with the smallest child having a birth weight of 580 g and the largest 2000 g. Table 4 shows the distribution of children by group according to BW and developmental ROP.

ROP	Group 1 BW < 999 g.	Group 2 BW (1000 g – 1250 g)	Group 3 BW (1251 g – 1500 g)	Group 4 TP > 1501 g
Without ROP	1 (0.8%)	20 (16.1%)	12 (9.7%)	5 (4.0%)
With ROP	41 (33.1%)	35 (28.2%)	10 (8.1%)	0 (0.0%)
ROP st. 1	11 (8.9%)	17 (13.7%)	6 (4.8%)	0 (0.0%)
ROP st. 2	14 (11.3%)	11 (8.9%)	3 (2.4%)	0 (0.0%)
ROP st.3	13 (10.5%)	6 (4.8%)	1 (0.8%)	0 (0.0%)
ROP st.4a	1 (0.8%)	1 (0.8%)	0 (0.0%)	0 (0.0%)
ROP st. 4b	2 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
ROP st. 5	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total:	42 (33.9%)	55 (44.4%)	22 (17.7%)	5 (4.0%)

Tab. 4 Distribution of cases in groups according to BW

The highest frequency of ROP was observed in the lowest weight groups. In the subsequent statistical processing of the data, we found a statistically significant difference between children with ROP and those without signs of the disease with respect to the trait BW ($p<0.001$).

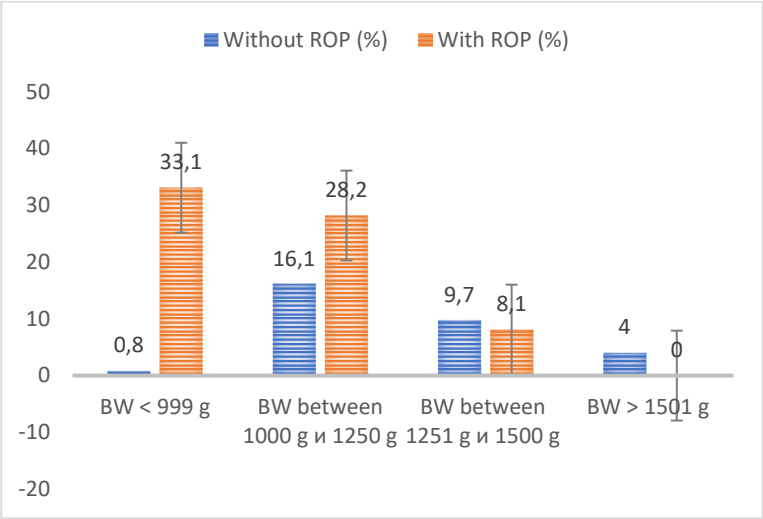


Fig.2 Comparison between ROP development and BW

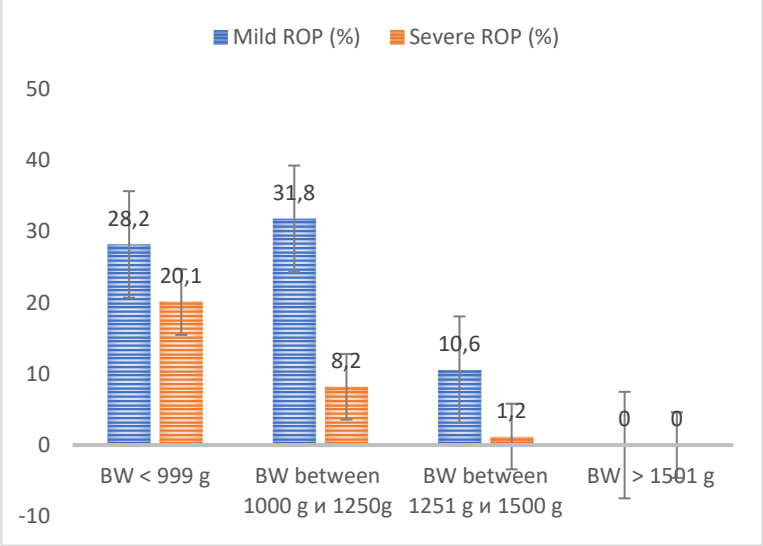


Fig.3 Comparisson between BW and severity of ROP

When comparing children with ROP (those with spontaneously regressed ROP and those with disease progression to the need for treatment), we again found a statistically significant difference ($p<0.001$) (Table 5).

ROP development	Number of cases	Median birth weight (g)	Standard deviation (g)	P-Value
Without ROP	38	1308.42	232.1	P< 0.001
With ROP	86	1017.8	209.8	
Mild ROP	61	1057.5	212.6	P = 0.006
Severe ROP	25	920.4	175.8	

Tab. 5 Comparison between BW and ROP development

4.2.2 Gestational age (GA)

Gestational age is the universal standard for discussing fetal maturity. The mean gestational age in the cases reviewed was 28.3 yr. ± 2.3 yrs. The youngest child was born at 24 yrs and the oldest at 34 yrs. Tab. 6 shows the distribution of the examined children by GA.

Gestational age (in weeks)	Number of children	Percentage:
24	5	4.0%
25	4	3.2%
26	16	12.9%
27	30	24.2%
28	19	15.3%
29	11	8.9%
30	16	12.9%
31	11	8.9%
32	7	5.6%
33	4	3.2%
34	1	0.8%
Общо:	124	100%

Tab. 6 Distribution of cases according to GA

In the subsequent statistical processing, we found a significant difference between group I (children without ROP) and group II (children who developed ROP) (p=0.001).

However, when comparing group IIA (children with spontaneously regressed ROP) and group IIB (children in whom ROP progressed to the stage requiring treatment), there was a statistically significant difference (p=0.007) (Table 7).

ROP	Count of cases	Mean GA	Standard deviation	P-value
Without ROP	38	29,3	2,1	P=0,001
With ROP	86	27,8	2,2	
Mild ROP	61	28,2	2,3	P = 0,007
Severe ROP	25	26,9	1,3	

Таб. 7 Development of ROP according to GA

4.2.3 Sex of screened children

In the study group, we found a predominance of the male sex – of the 124 children included, 74 (59.7%) were male and 50 (40.3%) were female. In the male children, 23 children (18.5%) were found to have no signs of ROP, 36 children (29.0%) developed mild ROP and 15 children (12.1%) developed severe ROP requiring treatment. In female children, the distribution was: 15 children (12.1%) without ROP, 25 (20.2%) with mild ROP and 10 (8.1%) with severe ROP requiring treatment.

In post-processing, we found no statistically significant difference between group I (patients without PH) and group II (patients with ROP) ($\chi 2$= 0.016 p= 0.898) , nor between group IIA (patients with spontaneously regressed ROP) and IIB (patients with ROP requiring treatment) ($\chi 2$= 0.007, p= 0.933).

4.2.4 Multiple gestation

Of the 124 children studied, 85 (68.5%) were born from singleton pregnancies and the remaining 39 (31.5%) from multiple pregnancies. Of all the multiple pregnancies, 3 were triplet pregnancies. Of the children born from multiple pregnancies, 16 (12.9%) did not develop signs of ROP, while 17 (13.7%) had mild ROP and 6 (4.8%) had severe ROP.

Subsequent statistical processing did not reveal a statistically significant difference between children born from singleton or multiple pregnancies in terms of the development of PH, a comparison between group I and group II ($\chi 2$=2.884; p=0.089), or the severity of disease when comparing group IIA or IIB ($\chi 2$=0.135, p=0.713).

4.2.5 Prolonged mechanical ventilation (invasive + non-invasive)

The role of oxygen in the pathogenesis of ROP was demonstrated more than 70 years ago. (8) The method of choice of oxygen therapy, its duration and the optimal level of oxygen concentration to maximally reduce the risk of developing ROP are still debated today. Different techniques of respiratory support are used in neonatal units: invasive mechanical ventilation, in which the child is intubated and the gas mixture is delivered by a mechanical ventilator; non-invasive mechanical ventilation, in which the gas mixture is again delivered by a mechanical ventilator, not by an intubation tube, but by special nasal cannulae. In children in a more stable condition, dosed oxygen therapy is administered via an oxygen tent or shower. In our study, the number of patients studied was relatively small, so we investigated a single parameter related to the administered respiratory support, namely the effect of prolonged cilorhodotherapy (with a duration equal to or exceeding 7 calendar days) on the development and progression of ROP. Prolonged oxygen therapy was administered to 52 children, in 50 (96.2%) of whom signs of ROP were detected. In the subsequent analysis, we found a statistically significant difference between group I and II ($\chi 2=35.217$, $p<0.001$) as well as between group IIA and IIB ($\chi 2=16.105$, $p=0.008$) regarding the factor prolonged oxygen therapy. (fig.4)

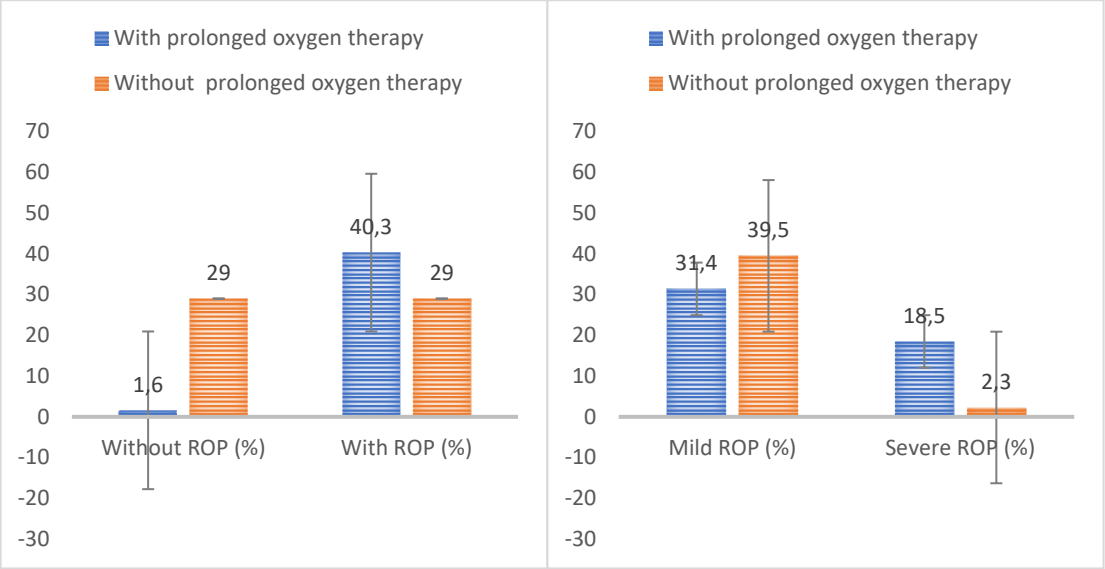


Fig. 4 *Comparison between the development and progression of ROP and prolonged oxygen therapy*

4.2.6 Neonatal anemia

In the study of 124 children, anaemia was found in 71 of them (57.3%), of which 40 children (56.3% of cases with anaemia) showed signs of mild ROP and 19 (26.8% of cases with anaemia) showed severe ROP requiring treatment.

In the subsequent processing, statistically significant differences were found between group I (patients without ROP) and group II (patients with signs of ROP) regarding

the sign of neonatal anemia ($\chi2=14.763$, $p<0.001$). A statistically significant difference was also noted when comparing groups IIA (cases with spontaneously regressed ROP) and IIB (cases with ROP requiring treatment) ($\chi2=4.274$, $p=0.039$) Neonatal anemia is a risk factor for the development of ROP as well as for disease progression to a stage requiring treatment. (fig. 5)

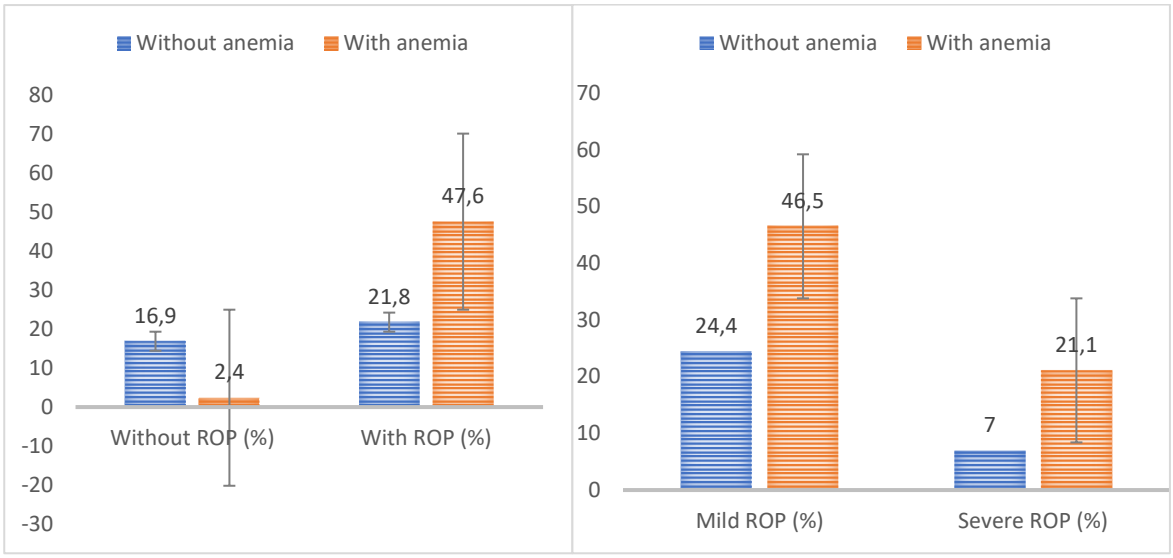


Fig.*5 Comparison between the development and progression of ROP and the factor "neonatal anemia"*

4.2.7 Hemotranfusions

Almost all children studied who were diagnosed with anemia had undergone hemotransfusions at some point during their hospitalization.

The distribution of children studied according to the number of haemotransfusions performed is shown in Fig. 6.

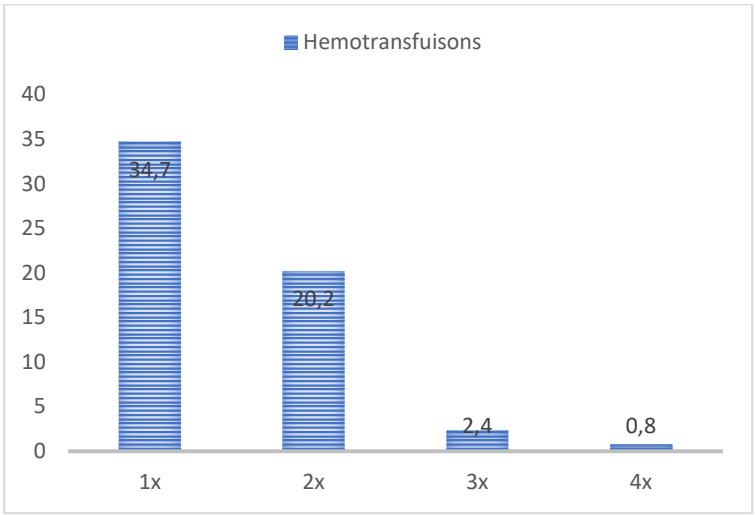


Fig.6 Distribution of cases according to number of hemotransfusions (%)

To assess the factor of hemotransfusions, we considered those cases in which ≥2 hemotransfusions were performed during the hospital stay.

In the post-processing, a statistically significant difference was found between group I and group II ($\chi2$=5.580, p=0.018), but not between group IIA and group IIB ($\chi2$=0.146, p=0.800) with respect to the hemotransfusions factor. In our study, the latter was a risk factor for the development of ROP but not for disease progression to a stage requiring treatment. (fig. 7)

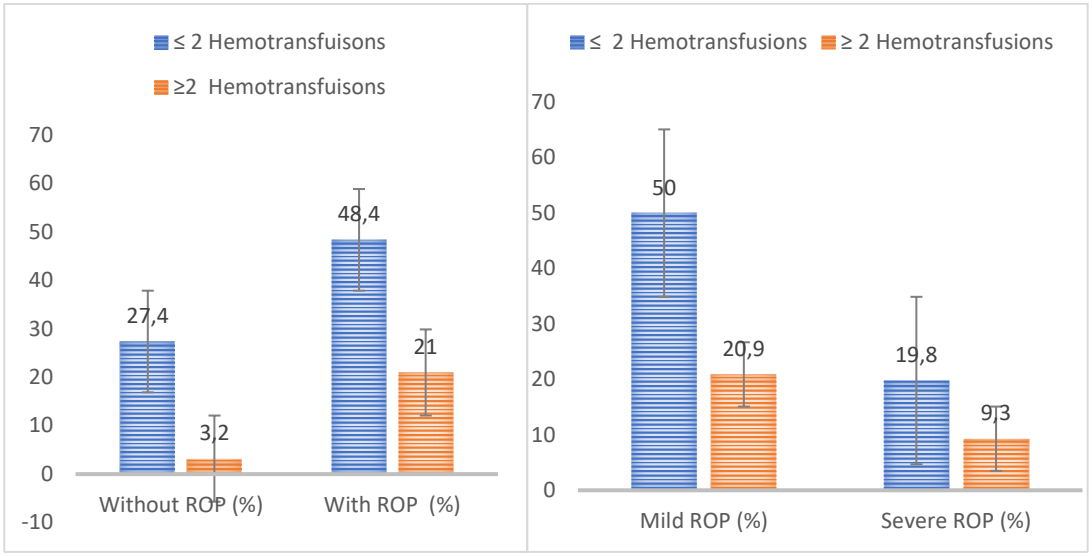


Fig.7 Comparison between the development and progression of ROP and the hemotransfusion factor

4.2.8 Persistent ductus arteriosus (PDA)

Of the preterm infants included in the study, 40 (32.3%) were found to have a persistent ductus arteriosus (PDA). The diagnosis within the present study was confirmed by a paediatric cardiologist after echocardiography. None of the cases required surgical treatment of the defect. Treatment with non-steroidal anti-inflammatory drugs (ibuprofen) and/or diuretic (furosemide) was undertaken in 6 (15% of all children diagnosed with PDA) of the cases. Signs of mild ROP were observed in 19 children (47.5% of all cases with PDA) and severe ROP in 9 children (22.5% of all cases with PDA).

In the subsequent statistical processing, we found no statistically significant difference between group I and group II regarding the presence of PDA ($\chi2$= 0.012;

p= 0.914), nor in the comparison between group IIA and IIB (χ2=0.190; p=0.663). (fig.8)

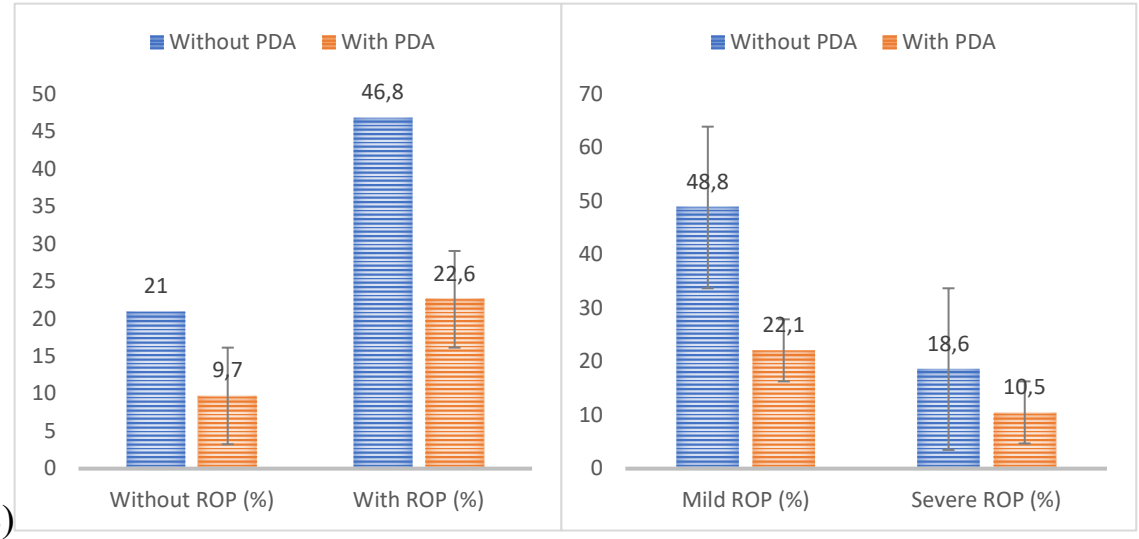


Fig.8 Comparison of the studied cases according to the development and progression of ROP with respect to the PDA factor

4.2.9 Necrotising enterocolitis (NEC)

In the study group, out of 124 preterm infants, only 2 children (1.6%) were found to have necrotizing enterocolitis. In both children, signs of ROP were detected, and in one the condition progressed to severe ROP, requiring treatment.

Subsequent statistical processing revealed no statistically significant difference between the groups with respect to the development of NEC. (χ2=0.488; p=1.000), (χ2=0.680; p=0.439)

4.2.10 Exogenous surfactant

Exogenous surfactant was administered to 101 children (81.5%) of the study group. In 18 of these children (17.8%) no signs of ROP were found, in 58 (57.4%) mild ROP was found, and in 25 (24.8%) ROP progressed to the point of needing treatment.

In the subsequent statistical processing, we found a statistically significant difference between group I and II (χ2=3.922; p=0.048), and between group IIA and IIB (χ2=5.716; p=0.016) regarding the administration of exogenous surfactant. (Fig.9)

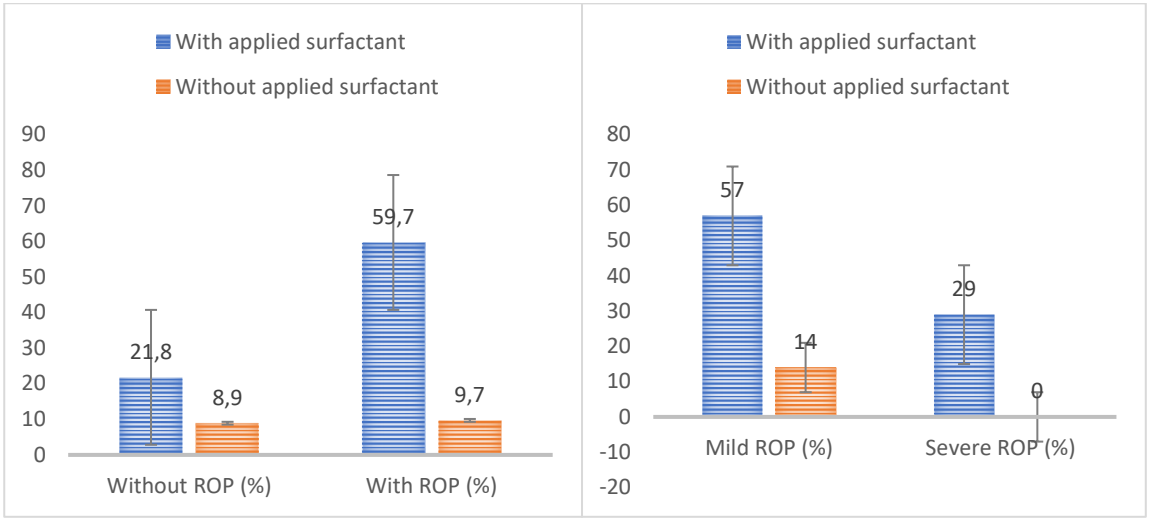
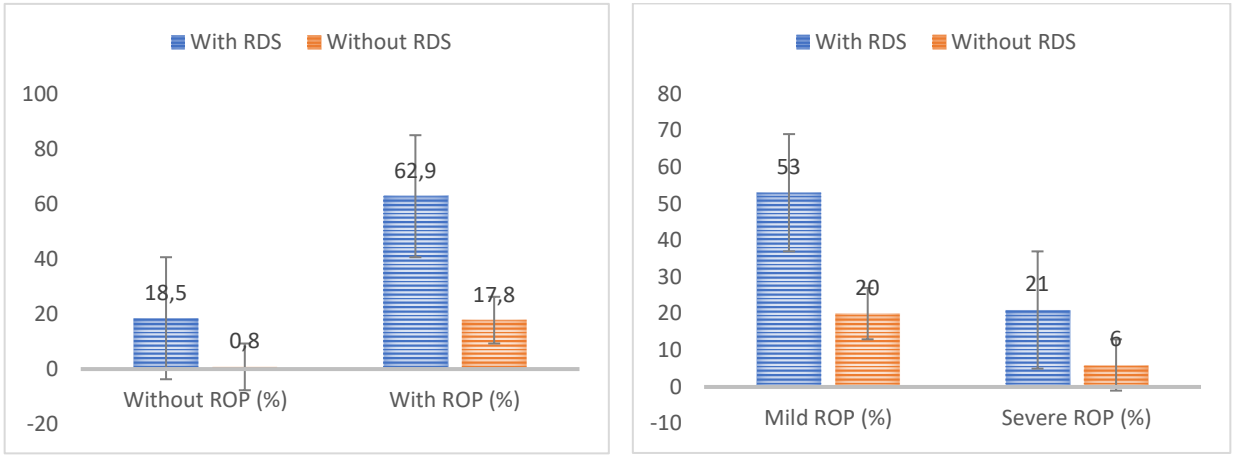


Fig.9 Comparison of ROP development and progression according to the factor "exogenous surfactant"

4.2.11 Respiratory distress syndrome (RDS)

Of the children included in the study, we found evidence of RDS in 99 children (79.8%). Of these, we found evidence of ROP in 72 children (58.1%) and evidence of severe ROP in 21 children (16.9%).

In the subsequent analysis, we found a statistically significant difference between groups I and II ($\chi 2=12.370$, $p<0.001$) and between groups IIA and IIB ($\chi 2=5.440$, $p=0.02$) with respect to the RDS factor.

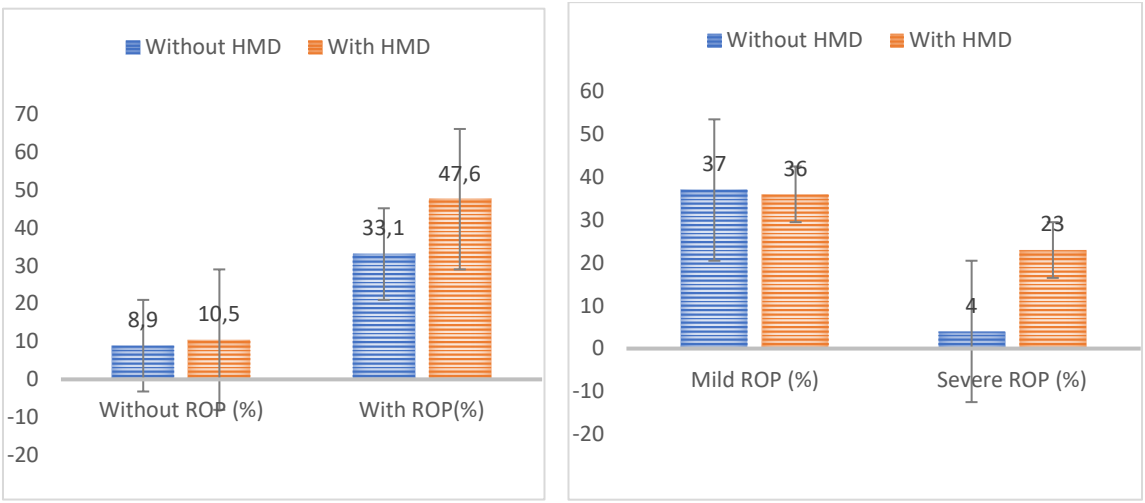


Фиг.10 Comparisson between ROP development and progression and the factor RDS

4.2.12 Hyaline membrane disease (HMD)

Of the children included in the study, 72 children (58.1%) were found to have evidence of hyaline membrane disease. Of these, 37 (51.4%) showed signs of mild ROP and 22 (30.6%) showed signs of severe ROP requiring treatment. Subsequent

analysis revealed a statistically significant difference between groups I (children without ROP) and II (children with ROP) ($\chi 2=10.206$, $p=0.006$), and between groups IIA (children with mild ROP) and IIB (children with severe ROP) ($\chi 2=10.100$, $p=0.002$). (fig.11)



Фиг.11 Comparison between ROP development and progression and the factor HMD

4.2.13 Neonatal pneumonia

Neonatal pneumonia is a serious respiratory disease whose etiology involves a large number of pathogens, and preterm birth and RDS are well-known risk factors for the development of this disease (9).

In 9 of the studied children (7.3%) signs of neonatal pneumonia were found. 5 of the same children (55.7% of the cases with neonatal pneumonia) showed signs of mild ROP and in 2 children (22.2% of the cases with neonatal pneumonia) severe ROP was found.

In the subsequent processing, we found no statistically significant difference between group I (children without signs of ROP) and group II (children with signs of ROP) ($\chi 2=0.324$, $p=0.569$) , nor between group IIA (children with mild ROP) and IIB (children with severe ROP) ($\chi 2=0.001$, $p=0.976$).

4.2.14 Bronchopulmonary dysplasia

Of the group of premature infants studied, 32 children (25.8%) were found to have signs of bronchopulmonary dysplasia. Of these, 8 children (25% of the BPD group) were found to have no signs of ROP, 15 (46.9% of the BPD group) were found to have mild ROP, and 9 (28.1% of the BPD group) progressed to severe ROP requiring treatment. In the subsequent analysis, we did not find a statistically significant difference between group I and II ($\chi 2=0.647$, $p=0.421$), nor between group IIA and

IIB ($\chi 2$=1.147, p=0.284) regarding the presence of bronchopulmonary dysplasia. (fig.12)

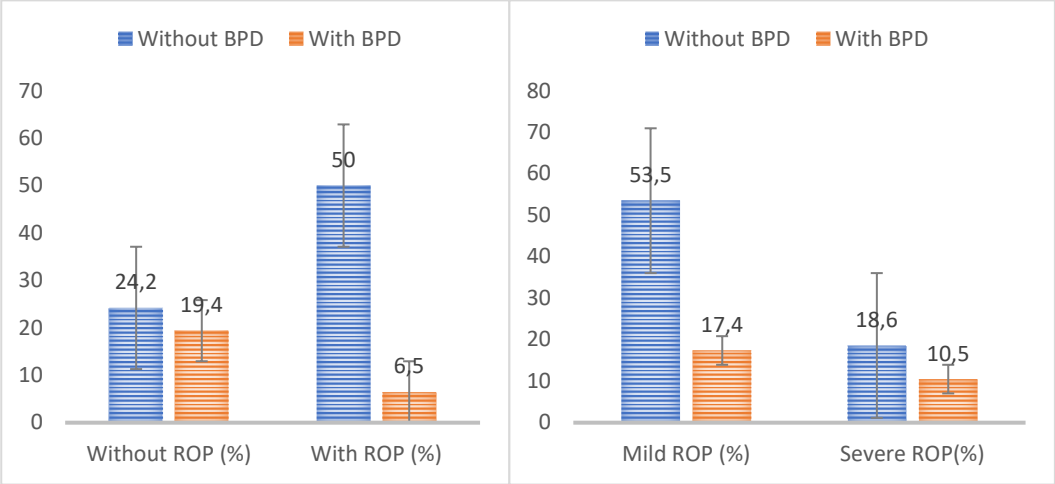


Fig.12 Comparison between the factor BPD and the development and progression of ROP

4.2.15 Hyperbilirubinemia and phototherapy

Hyperbilirubinemia was recorded in 17 preterm infants (13.7%) of the study group. Of these, 9 children (52.9% of the hyperbilirubinemia group) showed no signs of ROP, 5 children (29.4% of the hyperbilirubinemia group) had mild ROP, and 3 children (17.6% of the hyperbilirubinemia group) had severe ROP requiring treatment. In the post-processing, we found no statistically significant difference between group I and group II ($\chi 2$=1.327, p=0.317), and between group IIA and IIB with respect to the hyperbilirubinemia trait ($\chi 2$=0.304, p=0.581). Phototherapy was administered to 14 children (11.3% of all children included in the study) to normalize bilirubin levels, with a mean treatment duration of 3.2±1.1 days. There was also no statistical difference between the groups in terms of phototherapy administered, either in terms of ROP development ($\chi 2$=1.107, p=0.293) or disease progression ($\chi 2$=1.874, p=0.171).

4.2.16 Intraventricular hemorrhage (IVH)

Of the children studied, 42 (33.9%) were found not to have IVH, 12 (9.7%) had grade I IVH, 46 (37.1%) had grade II IVH 19 (15.3%) had grade III IVH, and 5 (4.0%) had grade IV IVH (10). (fig.13)

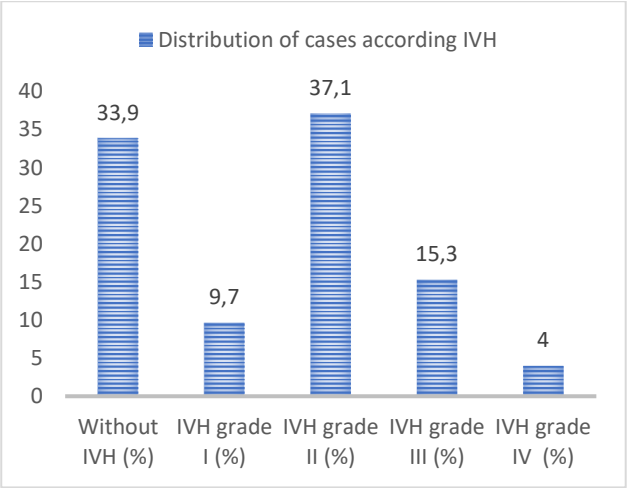
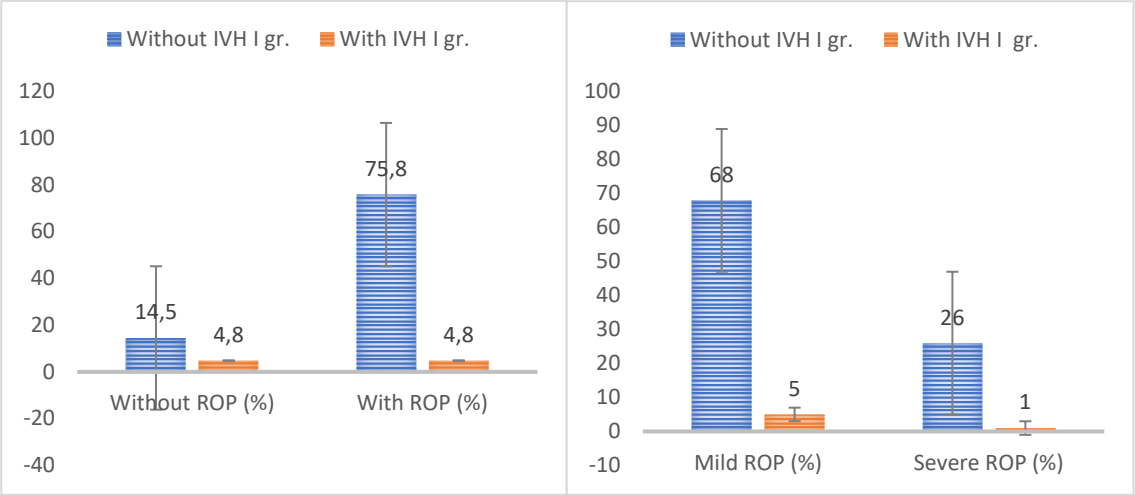


Fig.13 Distribution of cases according to the degree of IVH

In subsequent statistical processing, we found a statistically significant difference in the presence of stage I IVH ($\chi2=7.994$, $p=0.012$) and stage III IVH ($\chi2=5.385$, $p=0.023$) in terms of the development of ROP (comparison between group I and group II)



Фиг. 14 Comparison between the presence of grade I IVH and the development and progression of ROP

When comparing between group IIA (with mild ROP) and group IIB (with severe ROP), we found a statistically significant difference in terms of grade III IVH ($\chi2=15.559$, $p<0.001$). (fig 15)

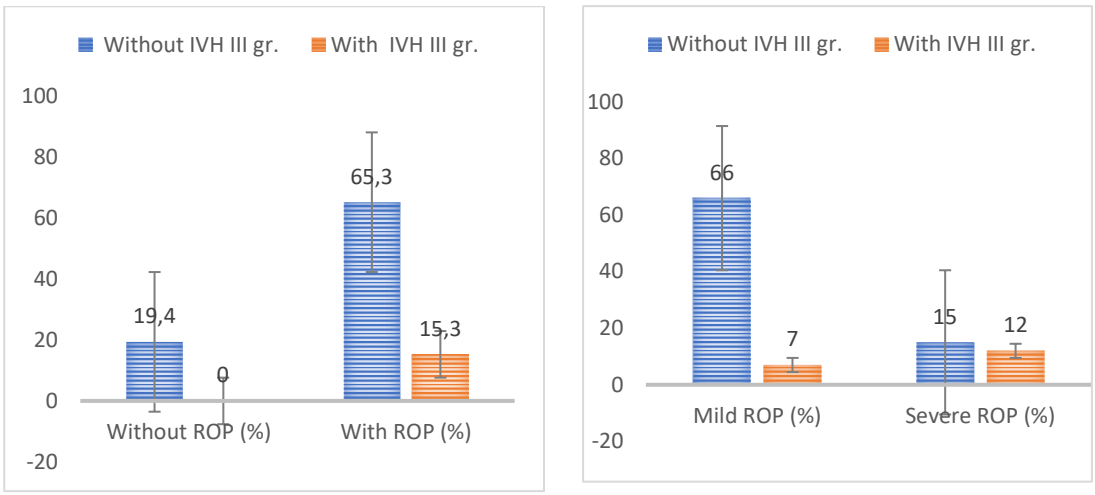


Fig. 15 Comparison between presence of IVH III grade and ROP progression

4.2.17 Posthemorrhagic hydrocephalus

Of the preterm infants included in the study, 7 (5.6%) developed hydrocephalus. Of these, 2 children (28.6% of children with hydrocephalus) showed signs of mild ROP and 3 (42.9% of children with hydrocephalus) showed signs of severe ROP requiring treatment. In statistical processing, we did not find a statistically significant difference between group I (without ROP) and group II (with ROP) ($\chi 2=0.015$; $p=0.902$), nor between group IIA (children with ROP who underwent spontaneous regression) and group IIB (children with severe ROP, with the need for treatment) ($\chi 2=2.463$; $p=0.117$) with respect to the sign of hydrocephalus (Fig. 16)

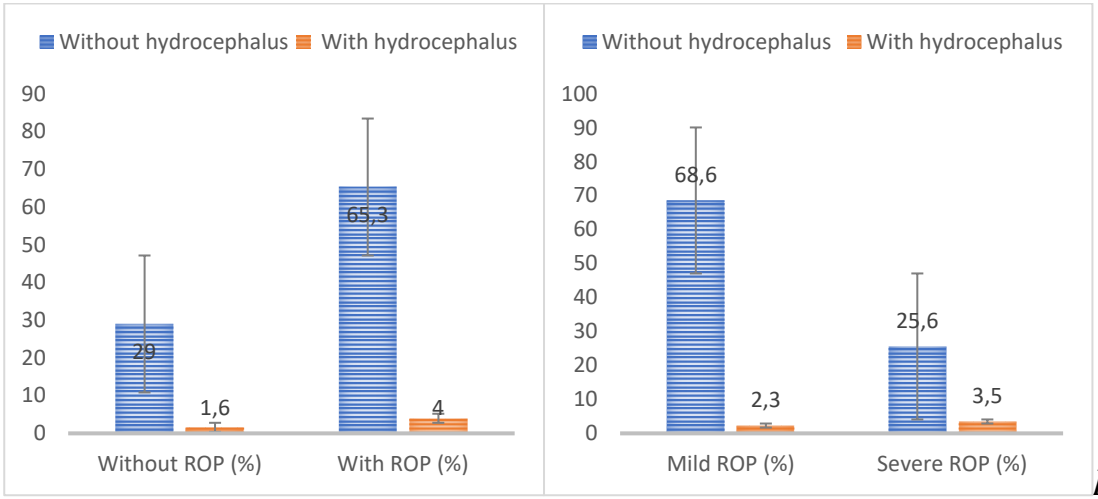


Fig.16 Comparison between the factor "hydrocephalus" and ROP development and progression

4.2.18 Periventricular leukomalacia (PVLM)

Of the children studied, 59 (47.6%) showed signs of periventricular leukomalacia. Of these, 53 children (89.8%) showed signs of ROP and 20 (33.9%) showed signs of severe ROP. In the subsequent statistical processing, we found a statistically

significant role of the periventricular leukomalacia factor both between group I (without ROP) and II (with ROP) in terms of disease progression ($\chi 2=6.084$, $p=0.02$) and between group IIA (with mild ROP) and IIB (with severe ROP) in terms of progression of the already developed disease to a stage requiring treatment ($\chi 2=6.594$, $p=0.013$). (fig. 17)

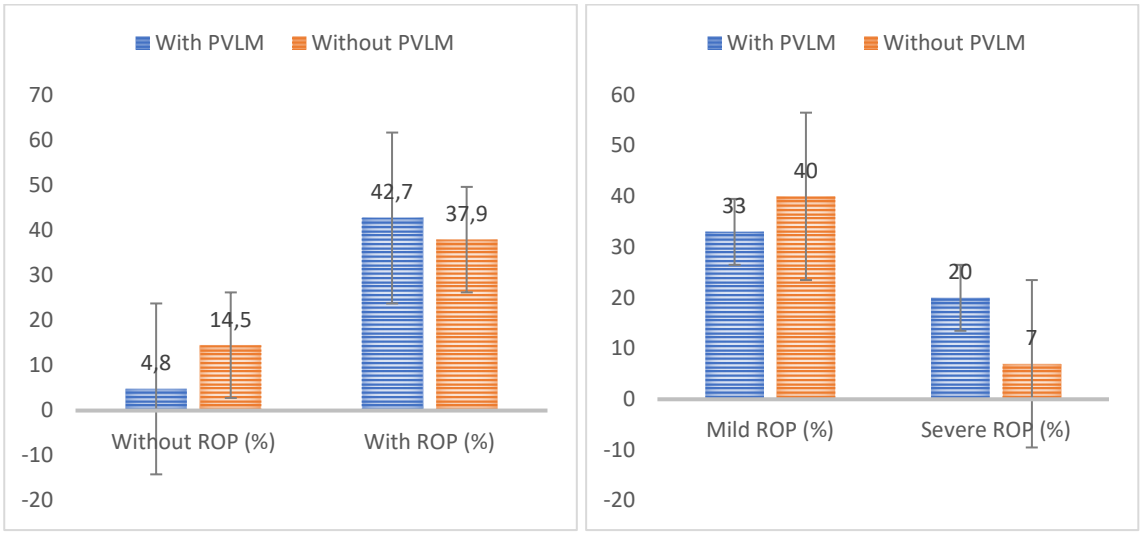


Fig.17 Comparison of cases according to the PVLM factor regarding the development and progression of ROP.

4.2.19 Low APGAR score

There is a suggestion that low APGAR score, as a general indicator of poor neonatal health, may be associated with a higher incidence of ROP. The mean APGAR values at the 1st minute for the study group were 2.59±1.60 and at the 5th minute were 3.94±1.93. In the initial statistical processing, we found a statistically significant difference between in terms of APGAR values at the 1st minute both between group I (without ROP) and group II (with ROP) ($F=1.505$, $p=0.041$) and between group IIA (with mild ROP) and group IIB (with severe ROP) ($F=8.123$, $p=0.002$). Regarding the APGAR values at the 5th minute, we did not find a statistically significant difference between group I and group II ($F=1.250$, $p=0.989$), but one was found between group IIA and IIB ($F=6.087$, $p=0.002$).

4.2.20 Intrauterine growth restriction (IUGR)

Of the group of preterm infants studied, 5 children (4.0%) were found to have intrauterine growth restriction. Of these, 2 children (40% of the IUGR group) showed signs of ROP, and the other 3 (60% of the IUGR group) showed no signs of ROP. In the subsequent statistical processing, we found no statistically significant difference between group I and group II children with respect to the sign of intrauterine growth restriction. ($\chi 2=2.112$, $p=0.146$) When comparing group IIA and IIB children who

developed the disease, we also found no statistically significant difference. ($\chi 2$=0.839, p=0.360) (Fig.18).

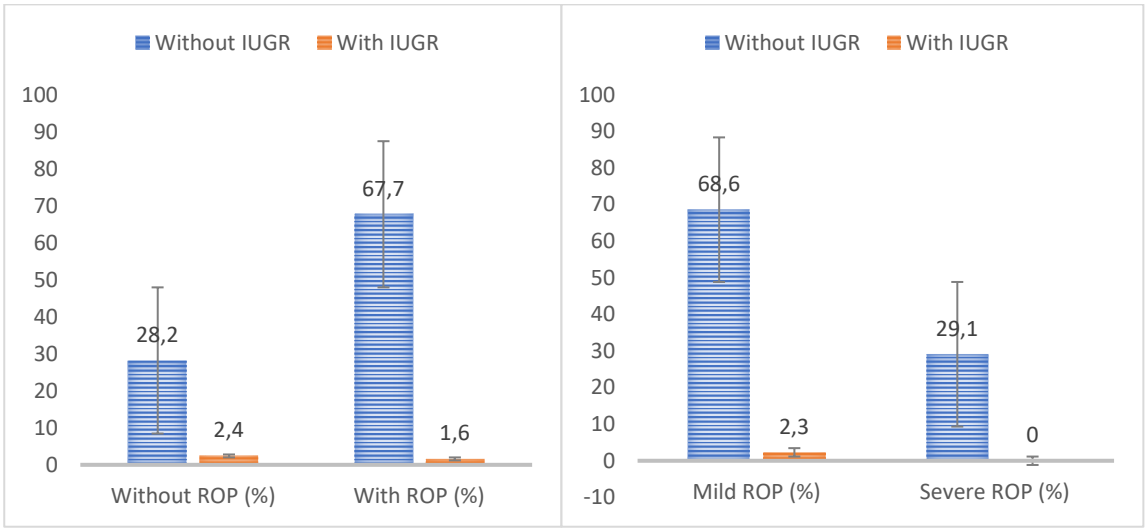


Fig. 18 Comparison of cases according to the IUGR factor regarding the development and progression of ROP.

The summary results of the analysis of fetal risk factors are presented in Table 15:

Risk factor	ROP developement		ROP progression	
	$\chi 2$ / F-value	P-value	$\chi 2$ / F-value	P-value
Low birthweight	0,098	<0,001	3,420	<0,001
Small gestational age	0,627	0,001	12,377	0,007
Sex	0,016	0,898	0,007	0,933
Multipe gestation	2,884	0,089	0,135	0,713
Prolonged mechanical ventilation ≥ 7 days	35,217	<0,001	47,360	<0,001
Neonatal anemia	14,763	<0,001	4,274	0,039
Hemotransfusions	5,580	0,018	0,052	0,819
Persistent ductus arteriosus	0,012	0,914	0,190	0,663
Necrotising enterocolitis	0,488	1,000	0,680	0,439
Exogenous surfactant	3,922	0,048	5,716	0,016
Hyaline-membrane disease	10,206	0,006	10,100	0,002
Respiratory distress syndrome	12,370	<0,001	5,440	0,02
Bronchopulmonary dysplasia	0,647	0,421	1,147	0,284
Hyperbilirubinemia	1,377	0,317	0,304	0,581
Phototherapy	1,107	0,293	1,874	0,171
IVH I grade	7,994	0,012	0,346	0,680
IVH II grade	0,181	0,815	0,118	0,818

Tab. 8 continues on page 34

Continuation of tab.8

IVH III grade	5,385	0,023	15,559	< 0,001
IVH IV grade	1,423	0,248	1,144	0,561
Posthemorrhagic hydrocephalus	0,015	0,902	2,463	0,117
Periventricular leukomalacia	6,084	0,02	6,594	0,013
Low APGAR score 1st minute	1,505	0,041	8,123	0,002
Low APGAR score 5th minute	1,250	0,989	6,087	0,001
Intrauterine growth restriction	2,112	0,146	0,939	0,360

Tab. 8 Summary results of univariate analysis regarding fetal risk factors

Factors that were shown to be significant in the univariate analysis were subjected to multivariate analysis by stepwise regression. In this way, significant and independent risk factors for both the ROP development and its progression were determined.

After regression analysis, only low birth weight, invasive ventilatory support, neonatal anemia, and grade III intraventricular hemorrhage were confirmed as significant and independent factors for the development of ROP.

Risk factors	**OR (95% CI)**	**P-value**
Low birthweight	3,463 (3,022 – 3,905)	0,009
Prolonged mechanical ventilation	3,037 (1,753 – 2,938)	0,011
Neonatal anemia	3,007 (2,934 – 4,288)	<0,001
IVH III grade	3,118 (2,447 – 3,788)	0,032

Tab. 9 Significant results from the multivariate regression model regarding the development of ROP

Risk factors associated with disease progression that proved significant in univariate analysis were also subjected to multivariate regression analysis, and low birth weight and the presence of grade III IVH were confirmed as significant and independent.

Risk factor	**OR (95% CI)**	**P-Value**
Low birthweight	3,715 (3,269 – 4,164)	<0,001
IVH III grade	3,370 (2,728 – 4,013)	0,001

Tab.10 Significant results of the multivariate regression model on ROP progression

4.3 Analysis of potential maternal risk factors associated with disease development and progression.

4.3.1. In vitro fertilisation (IVF)

In the study group, 23 (18.5%) preterm children were conceived after IVF (In Vitro Fertilization). In 7 (30.4% of the IVF group) of them there were signs of mild ROP, and in 5 (21.7% of the IVF group) children ROP progressed to severe ROP with the need for treatment.

Subsequent statistical processing revealed a statistically significant difference between group I (children with no ROP) and group II (children with ROP) ($\chi 2=3.922$; $p=0.048$), but no such difference was found between group IIA (children with spontaneously regressed ROP) and IIB (children with ROP requiring treatment) ($\chi 2=1.073$; $p=0.300$) with respect to mode of conception. (fig. 19)

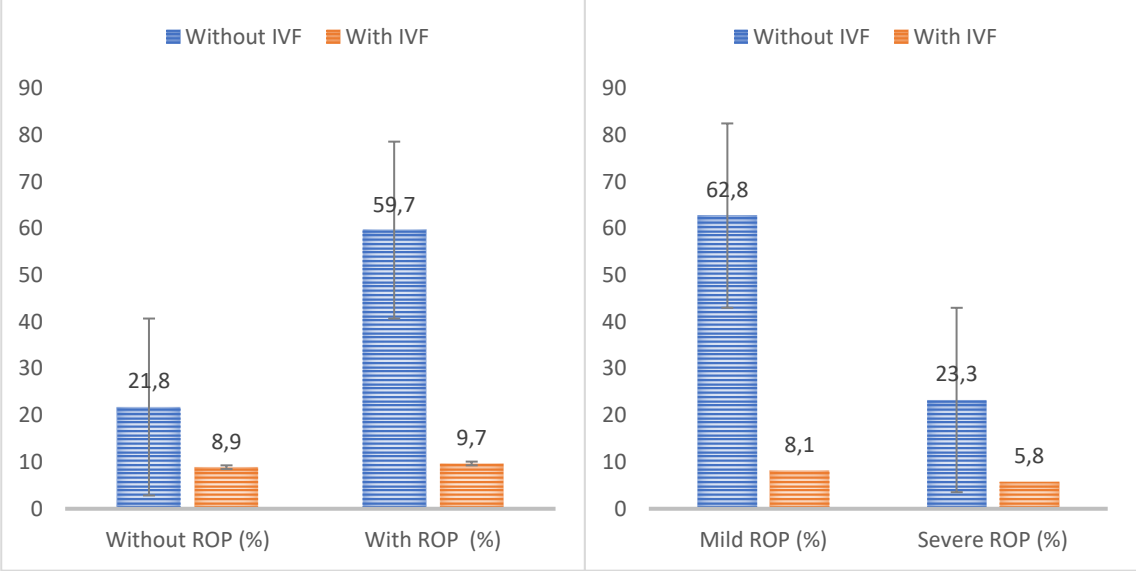


Fig.19 Distribution of cases according to the factor "IVF" and the development and progression of ROP

4.3.2. Mode of delivery

In the studied group of children, the predominant number of deliveries was performed by cesarean section – 84 (67.7%), and the remaining 40 cases (32.3%) – by normal mechanism (per vias naturalis).

Among the children born by operative delivery, 26 (31% of the group of children born with SC) showed no signs of ROP, 41 (48.8% of the group of children born with SC) showed mild ROP, and 17 (20.2% of the group of children born with SC) showed signs of severe ROP.

Among children delivered per vias naturalis, 9 (22.5% of the group of children born PVN) showed no signs of ROP, 23 (57.5% of the group of children born PVN) showed mild ROP, and 8 (20.0% of the group of children delivered PVN) showed severe ROP.

In subsequent statistical processing, we found no statistically significant difference between group I and II ($\chi 2$=0.012; p=0.914), nor between group IIA and IIB in terms of mode of delivery ($\chi 2$=0.005; p=0.944) (fig. 20)

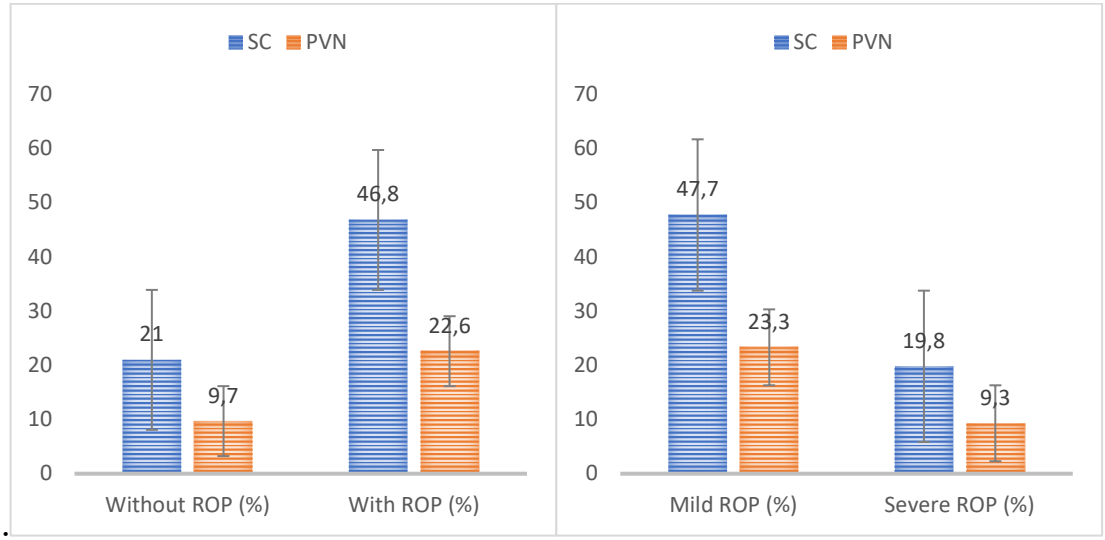


Fig.20 Comparison of cases according to the factor "mode of delivery" and the RPO development and progression

4.3.3. Mother's age

The mean age of the mothers of children who developed ROP was 29.57 ± 6.32 years, and the mean age of those whose children did not develop ROP was 29.25 ± 4.92 years. The youngest mother included in the study was 15 years old and the oldest was 43 years old. In the subsequent statistical processing, we found no statistically significant difference between group I and group II (F=6.694, p=0.856) , nor between group IIA and IIB (F=0.199, p=0.878) in terms of maternal age.

4.3.4. Hypertensive conditions during pregnancy

In the cases reviewed, the mothers of 20 of the children (16.1%) were diagnosed with hypertensive conditions. In the majority of cases (18 cases), it was preeclampsia. In the remaining two cases, there was evidence of gestational hypertension. In 4 (20.0% of the group with this disorder) of these children no signs of ROP were found, in 11 (55.0% of the group with this disorder) mild ROP was found and in 5 (25.0% of the group with this disorder) severe ROP was found. In the subsequent statistical processing, we did not find a statistically significant difference between groups I (without ROP) and II (with ROP) ($\chi 2$=1.271; p=0.260), nor between group IIA (mild

ROP) and IIB (severe ROP) ($\chi 2=0.045$; $p=0.831$) with respect to the factor "hypertensive conditions during pregnancy". (fig.21)

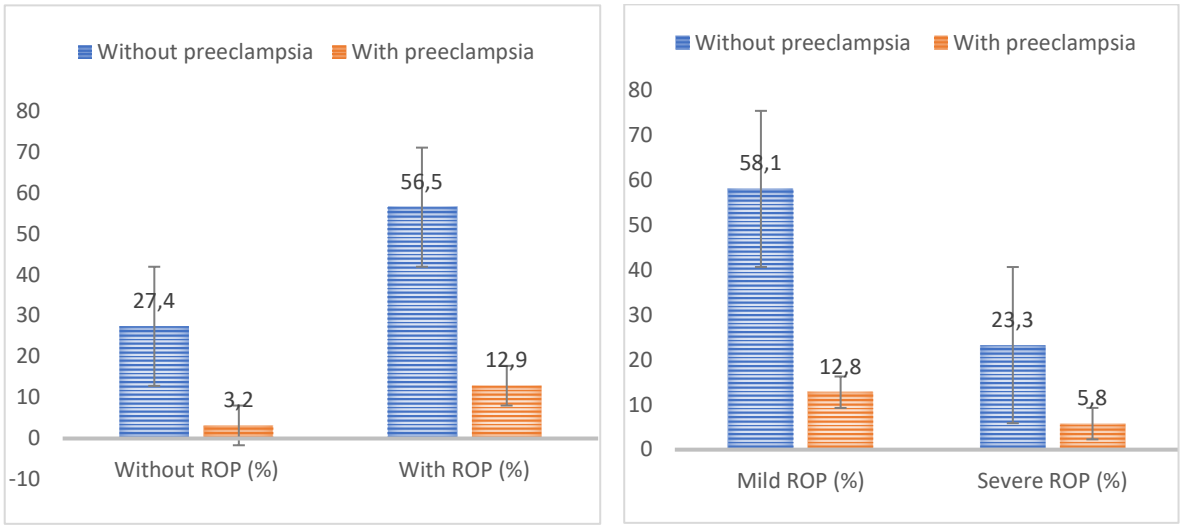


Fig.21 Comparison of cases according to the factor "hypertensive states during pregnancy" and the development and progression of ROP

4.3.5 Placental abruption

Placental abruption is an extremely serious late pregnancy complication that threatens both lifes of the mother and the baby and requires urgent treatment. Eighteen (14.5%) of the children studied were found to have evidence of placental abruption during pregnancy. In 8 (44.4% of the group in whom the factor "placental abruption" was recorded) children no signs of ROP were found, in 8 (44.4% of the same group) – mild ROP, and in 2 (11.1% of the same group) – severe PH. In the subsequent statistical processing, we found no statistically significant difference between group I and group II ($\chi 2=1.887$; $p=0.170$) , and between group IIA and IIB ($\chi 2=0.451$; $p=0.502$) in terms of placental abruption.

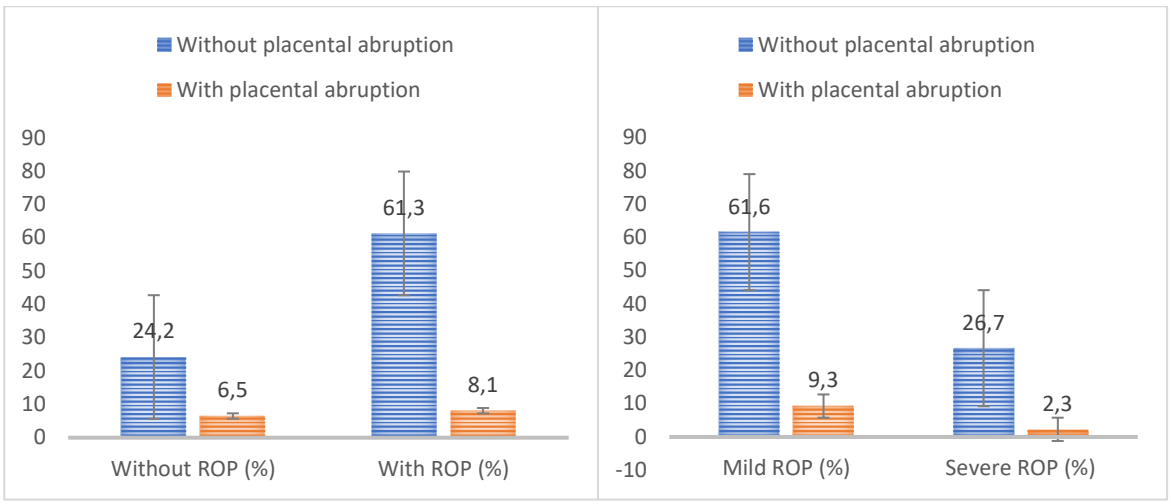


Fig.22 Cases distribution according the factor „Placental abruption“

4.3.6. Premature rupture of the amniotic sac (PRAS)

Among the studied group of preterm infants, premature spontaneous rupture of the amniotic sac (more than 12 h) was observed in 20 cases (16.1%). In 8 (40.0% of the PRAS group) of these cases, no signs of ROP were detected, in 10 cases mild ROP (50.0% of the PRAS group), and in 2 (10.0 % of the PRAS group) severe ROP. In the subsequent statistical processing, we found no statistically significant difference between group I (without ROP) and group II (with ROP) ($\chi 2$=0.982; p=0.322), and between group IIA (with mild ROP) and group IIB (with severe ROP) ($\chi 2$=1.040; p=0.308) in terms of PRAS. (fig.23)

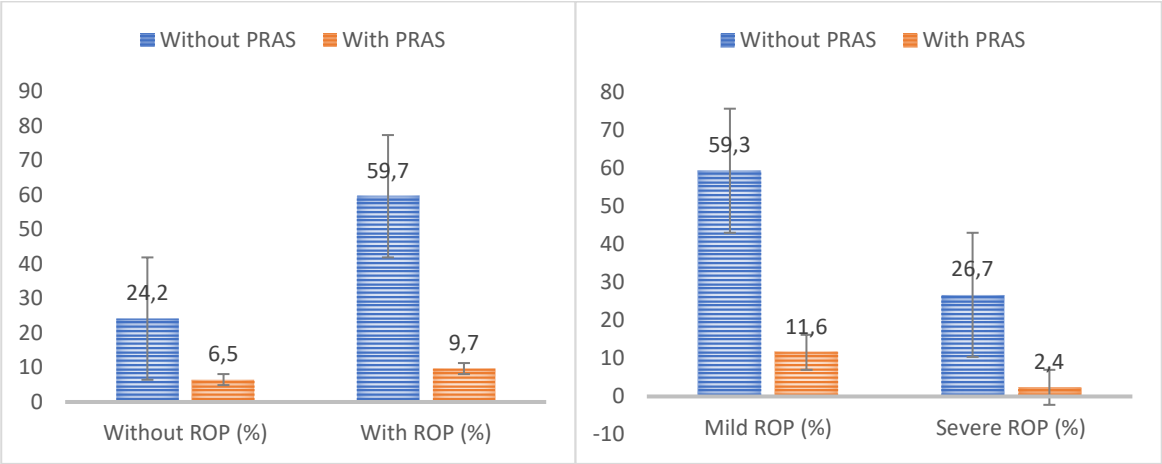


Fig.23 Distribution of cases according to the factor "premature rupture of the amniotic sac"

4.3.7. Surgical interventions on the cervix

Surgical interventions on the cervix can be a serious risk factor for preterm birth.

In 7 (5.6%) mothers of preterm infants in the study group, surgical intervention on the cervix was performed during pregnancy, particularly cerclage. In 3 of the children (42.9% of the cerclage group), signs of mild ROP were found, and in the remaining 4 (57.1% of the cerclage group), no signs of the disease were found.

Subsequent statistical processing revealed no statistically significant difference between groups I and II ($\chi 2$=2.451; p=0.117), and between group IIA and IIB ($\chi 2$=1.274; p=0.259) with respect to this parameter.

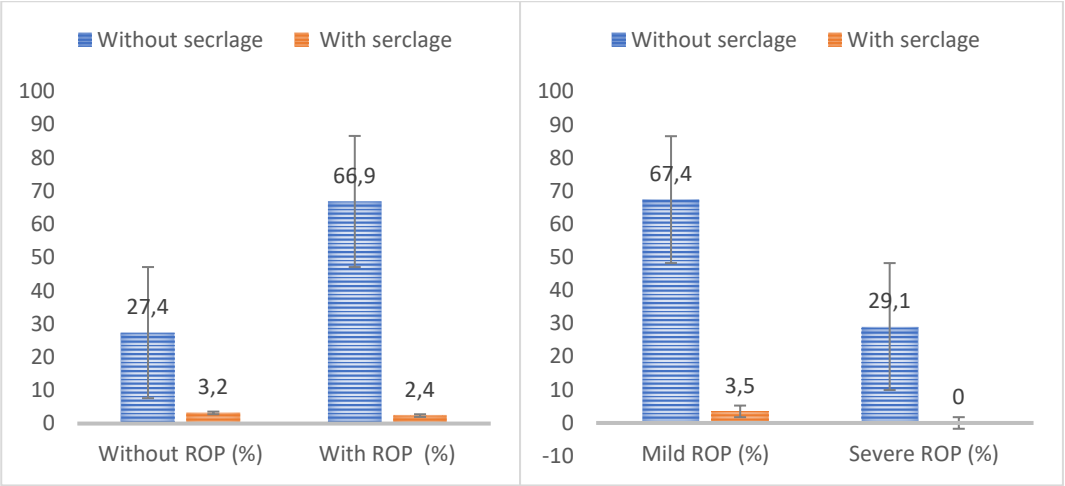
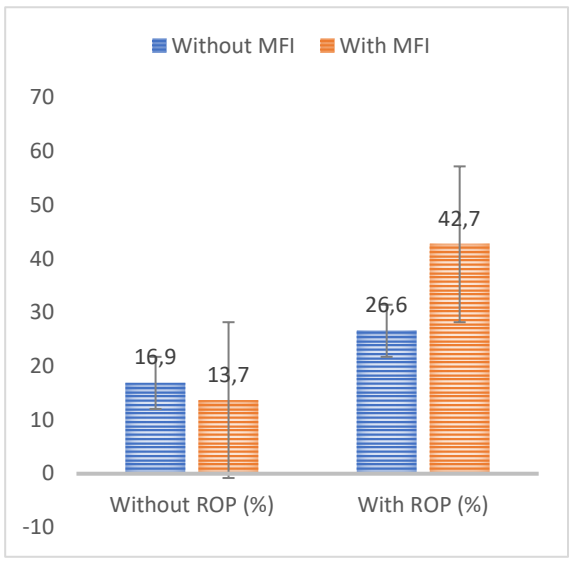


Fig.24 Distribution of cases according to the factor "cervical cerclage"

4.3.8 Maternal-fetal infections (MFI) and intraamniotic infections (IAI)

Of the study group, we found data on maternal-fetal infections (MFI) in 70 children (56.5% of the whole group) and intra-amniotic infections in 14 children (11.4% of the whole group). Of the children with MFI data, 17 (24.3% of the MFI group) had no evidence of ROP, 34 (48.6% of the MFI group) had mild ROP, and 19 (27.1% of the MFI group) had evidence of severe ROP requiring treatment. Subsequent processing of these results revealed no statistically significant difference between group I and II ($\chi2=3.059$, $p=0.080$), nor between group IIA and group IIB ($\chi2=3.079$, $p=0.079$) with respect to the MFI factor. Of the children with evidence of IAI, 6 children (42.9% of the children with IAI) had no signs of ROP, 7 children (50% of the group with IAI) were described as having mild ROP, and 1 child (7.1% of the children with IAI) had severe ROP requiring treatment. In post-processing, there was no difference between group I and II ($\chi2=1.107$, $p=0.293$), nor between group IIA and IIB ($\chi2=1.175$, $p=0.278$) with respect to the IAI factor.



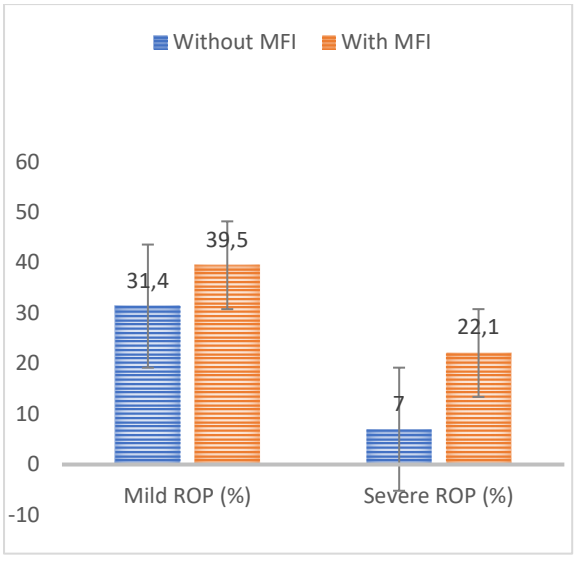
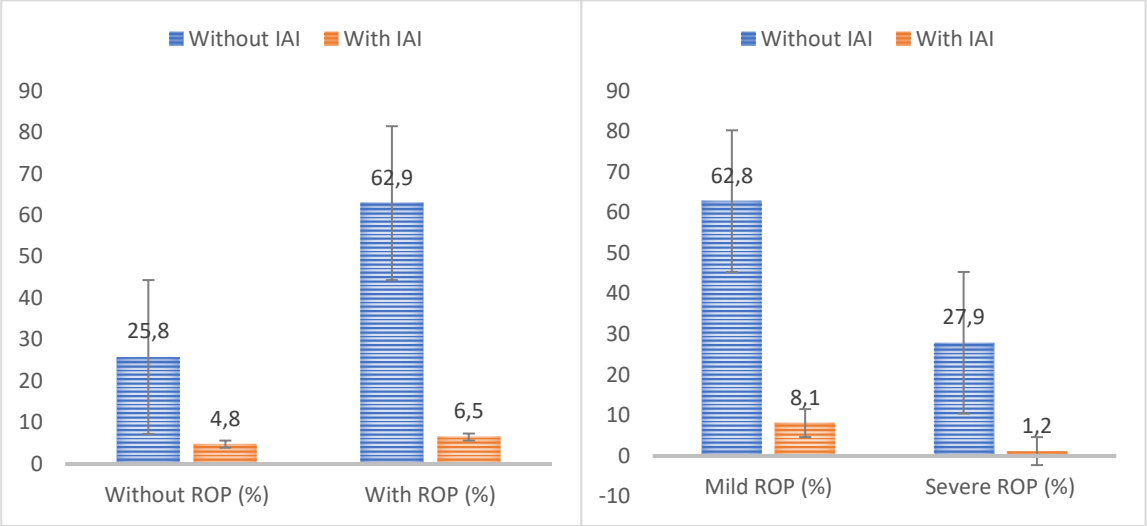


Fig.25 Comparison of cases regarding the MFI factor and the development and progression of ROP.



Φиг.26 Comparison of cases according to the IAI factor and the development and progression of PH.

4.3.9 Gestational diabetes

In the sample studied, evidence of diabetes was found among 4 (3.2%) of the mothers. Of the children born, 1 (25% of the group with gestational diabetes) was found to have no evidence of ROP, 2 (50% with gestational diabetes) had mild ROP, and 1 (25% with gestational diabetes) had severe ROP requiring treatment.

In subsequent statistical processing, we found no statistically significant difference between group I vs group II ($\chi2=0.006$, $p=0.939$), and between group IIA and IIB ($\chi2=0.005$, $p=1.000$).

4.3.10 Antenatal Corticosteroid Prophylaxis

The goal of antenatal corticosteroid prophylaxis is to prevent respiratory distress syndrome, intraventricular hemorrhage and necrotizing enterocolitis without adversely affecting pre-existing maternal and fetal morbidities. Its effect on ROP remains controversial. (11) Of the preterm infants enrolled in the study, 94 (75.8% of all infants enrolled in the study) had evidence of antenatal corticosteroid prophylaxis, and of these, 31 (33% of the CS prophylaxis group) infants had no evidence of ROP, 50 (53.2% of the same group) had mild ROP, and 13 (13.8% of the same group) had severe ROP requiring treatment. In subsequent statistical processing, we found no statistically significant difference between group I and II ($\chi2=0.995$, $p=0.318$) in terms of corticosteroid prophylaxis administered, nor between group IIA and IIB ($\chi2=4.332$; $p=0.115$)

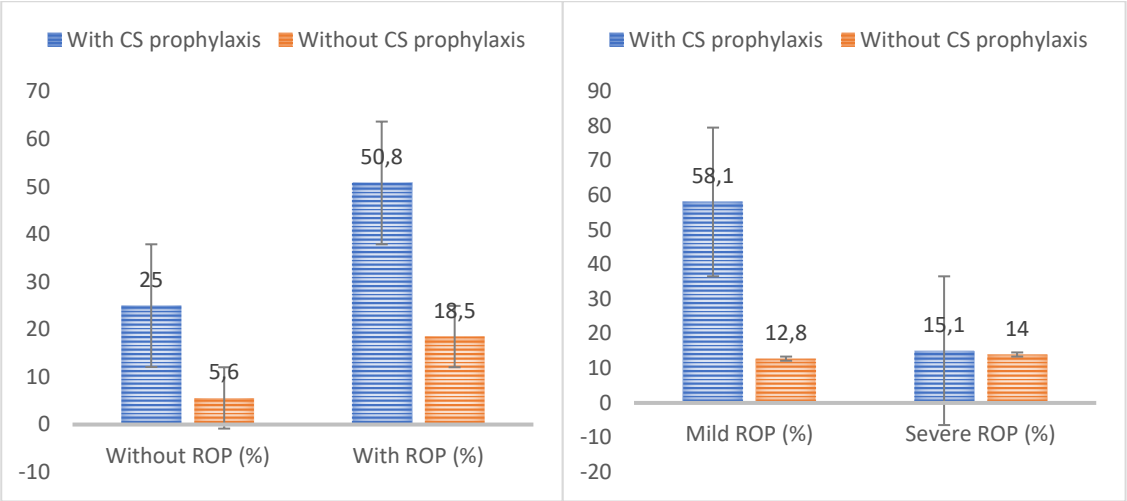


Fig.27 Distribution of cases according to the factor "Antenatal corticosteroid prophylaxis"

The summary results of the assessment of potential maternal risk factors are presented in Tab. 17 on the following page.

Risk factor	ROP developement		ROP progression	
	$\chi 2$ /F-value	P-value	$\chi 2$ /F-value	P-value
IVF fertilisation	3,922	0,048	1,073	0,300
Mode of delivery	0,012	0,914	0,005	0,944
Mother's age	6,694	0,856	0,199	0,878
Hypertonic conditions during pregnancy	1,271	0,260	0,045	0,831
Placental abruption	1,887	0,170	0,451	0,502
Premature ruptures of membranes	0,982	0,322	1,040	0,308
Surgical interventions of cervix	2,451	0,117	1,274	0,259
Mother-fetal infections	3,059	0,080	3,079	0,079
Intraamniotic infection	1,107	0,293	1,175	0,278
Gestational diabetes	0,006	0,939	0,005	1,000
Antenatal corticosteroid prophylaxis	0,995	0,318	4,332	0,115

Tab.11 Summary results of univariate analysis regarding potential maternal risk factors for the ROP development and progression

Of the risk factors examined, we subjected the one with established significance (IVF) to logistic regression analysis to determine whether it was an independent risk factor for the development of ROP. The result revealed IVF as an independent significant factor for the development of ROP (OR 1.056; CI 95% 0.795 - 1.317)

4.4 Evaluation of the early posttherapeutic effect of intravitreal administration of anti-VEGF drugs

This is a relatively new modality for the treatment of ROP that has gained momentum in the last two decades as a rapid and atraumatic technique.

Of the 124 children included in the study, 27 children (21.8%) progressed to a stage requiring treatment. One of these children (3.7%) was referred directly for pars plana vitrectomy to a clinic abroad. One child (3.7%) was treated with laser therapy, and another with cryotherapy (3.7%). The remaining 24 children were treated with anti-VEGF medication, of whom 4 children (14.8%) were treated with combined laser therapy and 1 (3.7%) with cryotherapy. Monotherapy with anti-VEGF medication was given to 19 children (70.4%) - 36 eyes. Two anti-VEGF medications were used, Bevacizumab (Avastin) in 12 eyes and Aflibercept (Eylea) in 14 eyes. Subsequent statistical processing found no statistical difference in terms of the medication used and the early anatomical outcome achieved after treatment (p=1.000). Application of anti-VEGF medication was performed in the operating room, with full compliance with asepsis and antisepsis, after careful preparation of the operative field. Injection of the drug was performed at a distance of 1-1.5 mm from the limbus, with the needle following a course parallel to the visual axis. Postoperatively, antibiotic collyrium and antibiotic ointment were administered for 5 days.

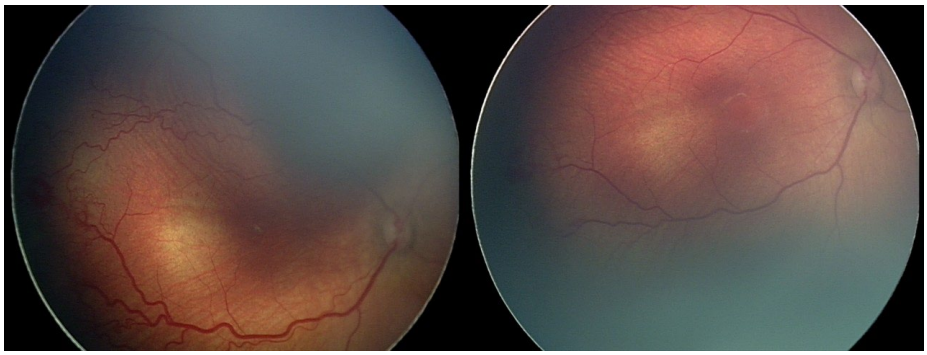


Fig.28 Early post-treatment effect (7 days) after treatment with Aflibercept (Eylea) OD. Left - before drug administration, right - after.

In our study, monotherapy with anti-VEGF medication was administered in 19 children (36 eyes in total). In 5 children (10 eyes), treatment was performed that combined intravitreal administration of anti-VEGF medication with ablative therapy (8 eyes were treated with laser therapy and 2 eyes with cryotherapy). In two of the treated children (4 eyes) the process progressed to stage 4b and they were referred for pars plana vitrectomy. Regression of the ophthalmoscopic finding was observed in

the remaining 22 children (42 eyes), representing 91.7% of the children who received anti-VEGF drug therapy and 81.5% of all children treated.

In 1 child, transient bradypnea was observed immediately after the injection of the medicament, which resolved spontaneously.

All children treated with anti-VEGF medication were followed up to 60 weeks postconceptional age, and no reactivation of retinopathy was observed in any of them.

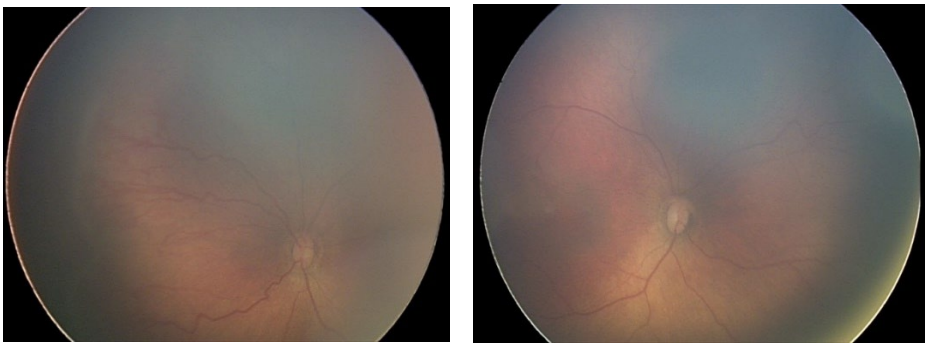


Fig.29 Early post-treatment effect (7 days): left - before treatment, right - after administration of Eylea OS

4.5 Evaluation of ROP as a cause of severe visual impairment and blindness among the students in "Prof. Dr. Ivan Shishmanov" school in Varna, Bulgaria The study was conducted in the period August 2022 - November 2022 among the students of the school for children with visual impairment "Prof. Dr. Ivan Shishmanov" in the town of. Varna. The present study included a sample of 143 individuals, of whom 88 were boys (61.5%) and 55 (38.5%) were girls. The students were aged between 6 and 20 years, and the mean age for the whole group was 12.5 ± 3.7 years. In all age groups, a higher prevalence of males was found.

Table 12 shows the etiological causes of visual impairment according to the anatomical localization of the impairment. (Table 2) It shows the leading cause of severe visual impairment was optic nerve atrophy (74.8%). Retinopathy of prematurity was the second most common disease (8.6%).

Tab 13 shows the details of the students with visual impairment due to PH, which are 12 in total. The mean birth weight was 1033 g (range 610 - 1750 g) and the mean gestational age was 34.4 weeks (range 31 - 36 g). The ratio of boys: girls was 2:1. The majority of cases were treated for retinopathy (83.3%) with cryotherapy (41.7%) and surgery (41.7%). Almost all children (91.7%) were found to have neuro-psychiatric retardation, and hearing impairment was diagnosed in 41.7%.

We found that retinopathy of prematurity ranked second among the causes of visual impairment and blindness among the students studied, after optic nerve atrophy, accounting for a total of 8.6% of vision loss.

Anatomical localization	**4-8 years**	**8-12 years**	**12-16 years**	**16-20 years**	**Total**
Anomalies of the globe(microphthalmos, anophthlamos)	0 (0%)	0 (0%)	0 (0%)	1 (0,7%)	1 (0,7%)
Cornea	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lens (cataract, ectopy)	0 (0%)	1 (0,7%)	1 (0,7%)	1 (0,7%)	3 (2,1%)
Uvea (aniridia,coloboma, uveitis)	0 (0%)	1 (0,7%)	0 (0%)	0 (0%)	1 (0,7%)
ROP	1 (0,7%)	2 (1,4%)	6 (4,2%)	3 (2,1%)	12 (8,4%)
Retinoblastoma	0 (0%)	1 (0.7%)	0 (0%)	0 (0%)	1 (0.7%)
Glaucoma	0 (0%)	1 (0,7%)	1 (0,7%)	0 (0%)	2 (1,4%)
Optic nerve atrophy	24 (16,8%)	23 (16,1%)	33 (23,1%)	27 (18,9%)	107 (74,8%)
Cortical visual impairment	2 (1,4%)	2 (1,4%)	3 (2,1%)	1 (0,7%)	8 (5,6%)
Others	0 (0,0%)	2 (1,4%)	3 (2,1%)	3 (2,1%)	8 (5,6%)
Total	27 (18,9%)	33 (23,1%)	47 (32,9%)	36 (25,1%)	143 (100%)

Table 12 Distribution of cases according to the anatomical localization of the lesion

Birth Weight (g)	1033 ± 352
Gestational age (weeks)	34,4 ± 1,44
Sex: – Boys (N, %) – Girls (N, %)	 8 (66,7%) 4 (33,3%)
Treatment: – No – Yes: Cryotherapy Lasertherapy Anti-VEGF medications Surgical treatment	 2 (16,7%) 10 (83,3%) 5 (41,7%) 0 (0%) 0 (0%) 4 (41,7%)
Degree of visual impariment: – Severe visual impairment – Blindness	 6 (50,0%) 5 (50,0%)
Others: – Delay in neuromotor developement (N, %) – Hearing abnormalities (N, %)	 11 (91,7 %) 5 (41,7%)

Tab. 13 Details about students with severe visual impairment due to ROP

5. DISCUSSION

5.1 Epidemiology of ROP in Bulgaria and worldwide

The assessment of epidemiological processes affecting ROP is extremely difficult due to the heterogeneity of data across countries. This can be explained by different inclusion criteria in studies, differences in diagnostic methods used, concomitant risk factors, infant survival rates and heterogeneous standards of health care worldwide. All of these make it currently impossible to construct a unified global screening protocol for ROP and make interpretation of the results of published data difficult.

We are now considered to be in the so-called 'third epidemic' of ROP, which mainly affects emerging countries in Latin America, South-East Asia and former socialist countries in Eastern Europe. It began in the 1990s and its causes are complex. On the one hand is the trend towards increased incidence of preterm births, as well as improved neonatal care leading to increased survival of preterm infants. On the other hand are the varying levels of oxygen monitoring in neonatal units, difficulties in providing screening examinations and timely treatment to children at risk (12).

In recent years, data regarding the incidence of ROP has also been accumulating in some countries in Africa, which until recently was considered a ROP-free zone. (13) Published data from Egypt, Kenya, Sudan, Nigeria, South Africa, Ghana and Rwanda report ROP incidence rates ranging from 13.7% to 69.4%. (14)

In our study, we found a significantly higher prevalence of ROP at 69.4%. We compared our results with a similar study conducted in the same neonatal intensive care unit more than 15 years ago. (15) In this study, 686 preterm infants were screened and the total number of children diagnosed with ROP was 143 (20.85%). The incidence of the disease was found to be significantly lower compared to our study. One possible explanation is that the mean BW and GW in this previous study were significantly higher compared with ours, with values of 1319.5 (± 323) and 30 (± 2) yr, respectively.(15) Improved neonatal care in recent years has resulted in the survival of many more high-risk children, increasing the chance of developing ROP. (16) Our results confirm that global trend. The data we obtained for the Varna region exceeds the frequency reported by other Bulgarian authors in different regions of Bulgaria – 30.3% for the Plovdiv region (17), 22.8% for the Sofia region (18) and 5.4% for the Stara Zagora region (19).

Grozeva's dissertation included 1029 children treated in the Newborn Children's Unit (NICU) at the Multiprofile Hospital for Active Treatment "Prof. C. Kirkovich" and in the NICU of the Medical-Social Care Home for Children, Stara Zagora, for the

period from March 2002 to August 2010, but the inclusion criteria for the study are significantly broader than ours - BP < 2500 g and BW < 36 y.o., as well as children with low birth weight < 2500 g and BW > 36 y.o. This fact, in our opinion, explains the significantly lower incidence of the disease found by the author (19).

The demonstrated heterogeneity, as well as the lack of data from a number of other districts, are a sign that a multicentre nationwide study covering each administrative district is necessary to build a clear vision of the epidemiological characteristics of this disease in our country.

We compared our data with the two largest therapeutic trials to date -The Multicenter Study of Cryotherapy of ROP (CRYO-ROP), and The Early Treatment of Retinopathy of Prematurity trial (ETROP).

The CRYO-ROP trial, which included over 4000 preterm infants with a BW < 1251 g, reported an incidence of ROP regardless of stage of 65.8%. The highest prevalence was reported among the children with the lowest BW, 90% for those with TP < 750 g, 78% for those with TP between 751 g and 1000 g, and 47% for those with BW between 1001 g and 1250 g (20).

A decade later, the ETROP study reported a similar incidence of 68% (21).

We find that the incidence of ROP we found is comparable to the data from these two large-scale studies. Because of their age (over 30 years from the CRYO-ROP study and almost 20 years from the ETROP study), we decided to look for more recent data regarding the epidemiology of ROP.

We identified a significant number of population-based studies from the last 10 years in highly developed countries in Europe and worldwide analyzing the incidence of ROP and severe ROP.

Reports from Sweden (22), Turkey (23), and South Korea (24) have reported an incidence of ROP around 30%, whereas countries such as the Netherlands and Switzerland report even lower incidence rates of 21.9% (25) and 9.3% (26), respectively. In 2019, data from the Swedish SWEDROP register were published for a 10-year period. 7249 children were covered, of which 31.9% were diagnosed with ROP and 6.1% were treated (22).

Two population-based studies have been conducted in Turkey concerning the epidemiology and risk factors of ROP, the so-called TR-ROP study. The first of these was published in 2015 and included 11,803 preterm infants, from 49 neonatal intensive care units, followed up between 2011 and 2013. The incidence of ROP was found to be 30.0% and of severe ROP 5.0%.(27) A more recent study published in

2018 and covering the cases of 6115 preterm infants reported a 27.0% incidence of ROP and 6.7% of severe ROP requiring treatment.(23)

A report from the United Kingdom, resulting from studying the incidence of ROP over 22 years (1990 to 2011), reported that the incidence of ROP increased tenfold over the study period: from 1.3% in 1990 to 12.5% in 2011. The trend was similar for ROP requiring treatment with cryotherapy or laser, from 0.17% in 1990 to 1.48% in 2011.(28) The authors attribute this increase since 2005 to the publication of the ETROP study results in 2003, which demonstrated that early treatment reduced adverse functional outcomes from 19.5% to 14.5% and adverse structural outcomes from 15.6% to 9.1%.(29)

A large-scale study conducted in 29 hospitals in the US and Canada (G-ROP Study) covering 7483 preterm infants (w/o 2006 and 2011) reported an incidence of 43.1% ROP and 6.1% type 1 ROP (30).

We found a report published at the end of 2022 that examined the incidence of ROP in Poland between 2012 and 2021 and found an incidence of 15.3% ROP and 6.1% ROP requiring treatment.It is important to note that the screening criteria in Poland are broader than those adopted in Bulgaria: BW ≤ 1800 g and GA ≤ 33 weeks (31).

In 2021, a study from Romania was published, conducted between 2017 and 2019 in a hospital in the city of Gdansk. The study was conducted in 2019 and 2019 from 2019 to 2019. 247 children were included in the study and the prevalence of PH was found to be 66.4% (164 children). (32) The data in the study are similar to our results, from which we can conclude that the health systems in Romania and Bulgaria share similarities and some common challenges related to ROP disease. Currently, the national screening criteria match with ours, i.e. GA ≤ 32 yrs and BW ≤ 1500 g, and older children with risk factors (hypoxia at birth, mechanical ventilation, neonatal sepsis, blood transfusion, IVC, neonatal shock treated with dopamine). Laser therapy in Romania was started in 2004 and anti-VEGF drug treatment in 2010.(33) A longitudinal study of 1783 children showed an incidence of ROP of 55.0%, with a need for treatment in 15.2%.(34) We also found a 2017 publication from the Filatov Institute in Ukraine that retrospectively studied 2682 preterm infants followed between 2009 and 2016. The incidence of ROP was found to be 24.7% and ROP requiring treatment was 4.9%. The authors noted a trend of increasing incidence from 10.0% in 2009 to 27.1% in 2016. Children with BW < 2500 g and GA < 35 weeks were included in the study. We believe that these broader screening criteria, which differ significantly from ours, account for the observed lower incidence of ROP and ROP in need of treatment (35).

GonÇalves et al. reported an incidence of ROP of 44.5% among 110 children at a university hospital in Montis Claros, Brazil, and treatment was necessary in only 1.8% (2 children) of cases (36).

Authors from Hospital México-City performed a retrospective study from October 2011 to March 2015, including 261 patients, and reported an incidence of ROP of 83.0%, and 33.0% ROP requiring treatment (37).

From the data reviewed, we can conclude that in highly developed countries in Western Europe, North America, and some parts of Asia, well-functioning ROP screening programs are observed, aiming to maximize efficiency with minimal waste of resources. The incidence of ROP requiring treatment is low, less than 10% of all children diagnosed. In these countries, the "third epidemic" of ROP appears to be under control due to economic and technological resources as well as staffing.

In a number of emerging countries, including Bulgaria, the situation is less optimistic. There are heterogeneous data reporting low incidence of ROP, similar to well-developed countries, and those demonstrating significantly higher incidence.

Most studies originate from single large university centres, while data from smaller units (e.g. in smaller regional cities of Bulgaria) are lacking. The lack of databases reporting incidence at the national level contributes to an inaccurate picture of the epidemiology of ROP. To summarize, the explanation of the high incidence of PH found in our study lies in the following several factors:

- The study was conducted at the Hospital "Prof. Dimitar Stamatov", which is the largest medical center for specialized obstetric-gynecological care in Northeastern Bulgaria, and the neonatology department is the only one in the region that fully meets the Medical Standard of Neonatology for level III competence for intensive care, special care and primary resuscitation for neonates at risk. A large proportion of preterm infants were brought from other hospitals in the region in severe general condition and comorbidities, which automatically puts the studied patients at higher risk of developing ROP and severe ROP requiring treatment, as they are in a more damaged condition compared to the general population of preterm infants;

- Data were missing for children who were live-born and at risk of ROP but died before reaching the age of 4 weeks post-conceptual age at which the ROP screening programme starts;

- Data are missing for children who are live births, eligible for ROP screening, but are brought to other facilities for further treatment (or discharged) before reaching the screening age;

- There has been a downward trend in neonatal mortality in Bulgaria over the past 20+ years, from 13.3/1000 in 2000 to 5.6/1000 in 2021 (according to the National Institute of Statistics), allowing for the survival of increasingly immature children, with extremely low birth weights (the smallest child in the study weighed only 580 g), who are at highest risk of developing the disease;

Determining the incidence of ROP and ROP in need of treatment in the population of newborn preterm infants is just one stroke in studying the epidemiology of this disease. One of the most important questions we have tried to answer is related to the incidence of adverse outcomes and its proportion among causes of vision loss in later life.

In the countries studied, where there are clearly regulated registries of people with visual impairment, the answer to this question is relatively straightforward. Since Bulgaria is not yet among them, we chose to use the approach of examining the incidence of school-age children with visual impairment.

The subject of the study were the students attending the "Prof. Dr. Ivan Shishmanov" School of Visual Impairment in Bulgaria. Varna. We found that retinopathy of prematurity was the second cause of visual impairment and blindness, after optic nerve atrophy, responsible for a total of 8.6% of vision loss. Several foreign publications investigating the same problem were found in the literature. Rohrschneider et al. conducted a study in a school for visually impaired children in the German city of Ilvesheim and found that the leading causes of vision loss were optic nerve atrophy (37.3%), followed by inherited retinal diseases (24.6%) and retinopathy of prematurity (20.0%). (38) Mezer et al. reported optic nerve atrophy, retinitis pigmentosa, and ROP as the leading causes of childhood blindness. Their data came from the National Registry of the Blind in Israel, operational since 1999, and the etiological causes of blindness were included in 2003 (39).

A study by Zepeda-Romero et al. conducted in two schools for the blind in Guadalajara, Mexico, identified ROP as the leading cause of severe visual impairment and blindness in 34.7% of cases (50 children out of 144 included in the study), followed by optic nerve lesions (17.4%) and glaucoma (14.6%). The authors also reported the mean BW and GA of children who lost vision due to ROP to be 1200 g (range 700 g to 1980 g) and 28 yr (range 25 yr to 34 yr), respectively, and these values were lower than our results. (40) It is known that in many emerging countries there is heterogeneity in the development of ROP and indicators of BW and GA, with older and more mature infants being diagnosed with it. (41)

In highly industrialized countries, cerebral visual impairment (CVI) is the leading cause of vision loss in childhood, with hypoxic ischemic encephalopathy (HIE) being

the main cause of its development. (42) In our study, we found this condition to be the third most common, at 5.6%.

In Israel, until 2009, the term "cerebral visual impairment" was not used in the context of childhood blindness and these cases were designated as bilateral optic nerve atrophy. (39) We do not exclude the possibility that a similar situation exists in Bulgaria - that there is an overlap between the two nosological entities and that the actual incidence of CVI is significantly higher. Elucidation of the root cause of the numerous cases of optic nerve atrophy, supported by brain imaging studies, would aid diagnostis and refine future epidemiological studies. Diseases of the anterior segment of the eye are a more common cause of vision loss in Third World countries, with cataracts (43) and corneal diseases being among the most common causes. (44) Our results lacked cases of corneal blindness, and lens-related visual impairment was found in only 2.1% of cases.

From what has been said so far, we can conclude that diseases of the optic nerve and retina are the main cause of severe visual impairment among the students at the school for children with visual impairment "Prof. Dr. Ivan Shishmanov" in Sofia. Varna. Retinopathy of prematurity continues to be an important cause of blindness among students attending schools for visually impaired children, both in Bulgaria and in other countries.

5.2 Analysis of fetal risk factors for the development and progression of PH

Hundreds, if not thousands, of studies investigating the role of potential risk factors on the development and course of ROP can be found in the world literature. In the Bulgarian literature, these data are scarce, and the last known data are from 2016, presented by Mladenov in his dissertation, with a focus on screening for ROP in hospitals in the Sofia region.(18) For this reason, we conducted our study among premature infants undergoing mandatory ophthalmic screening and treated in the Intensive Neonatology Unit at the Hospital "Prof. Dimitar Stamatov" in Varna. We examined 22 potential fetal and 11 maternal risk factors.

5.2.1 Birth weight (BW) and gestational age (GA)

The two most important and widely discussed risk factors in the literature are undoubtedly low BW and low GA of preterm infants, and they are the main criteria on which any screening programme for ROP is based.

The data from our univariate analysis confirmed BW as a statistically significant factor in both the development of retinopathy and its progression to stages requiring

treatment, and multivariate regression analysis confirmed the role of BW as an independent and significant factor in the development and progression of ROP.

Our results are in support of what has been shown by other Bulgarian authors (17), (18) , (15).

A number of foreign publications prove the undeniable role of BW as a risk factor for ROP.(32), (45), (46), (47), (48),(49) The mean BW of preterm infants included in our study was 1106 g. ± 254 g, which is lower than that found by collectives in other countries with evolving iconomics – In Romania, Borteca et al. found an average BW of 1234 g. ± 373 g (32) ; in Turkey, Akkoyun et al. found 1365 g. ± 421 g (48); and in Egypt Gaber et al. found an average BW of 1955 g (48). ± 692 g. (50) These results confirm one of the characteristics of the "third epidemic" of ROP in emerging countries, namely the heterogeneity of children developing ROP in terms of BW and GA indices, with the disease being found among more mature children with higher BW and greater GA compared with developed countries in Western Europe and North America.

Gestational age is another key vital sign to discuss fetal maturity. Our results demonstrated a statistically significant difference between group I (patients with no signs of ROP) and group II (patients who developed ROP), and between group IIA (patients who developed ROP that underwent spontaneous regression) and group IIB (patients with ROP that progressed to a stage requiring treatment) with respect to the GA factor. According to our interpretation, there is an inverse relationship between GA and severity of ROP: the lower the GA, the higher the risk of disease progression to a stage requiring treatment.

In the multivariate analysis performed, GA has dropped as a significant and independent factor for the development and progression of ROP. One possible explanation lies in the small number of cases in which other statistically significant factors may have displaced the GA factor. Similar results are reported by Marinov in his dissertation. (17) On the other hand, there is an opinion in our available Bulgarian literature that defines low gestational age as an independent risk factor for both the development of ROP and the progression of already developed disease to stages requiring treatment. (18)

From our available foreign language literature, we found studies that reach similar results to ours. Borteca et al. studied 247 preterm infants with GA < 32 yr and they found a significant role of GA in the development of ROP in univariate analysis, but it dropped as a risk factor in their regression model. The authors attributed these results to the fact that all children included in their study had low GA and other factors with stronger predictive value regarding the development of ROP (BW and

mechanical ventilation in their results) displaced the GA factor in the regression model. (32) Akkoyun et al. studied 88 preterm infants, dividing them into three groups (no ROP, mild ROP, and severe ROP), and found that low GA had no predictive value regarding the severity of ROP. The authors found a marked difference between the groups in the development of ROP and the GA score, but not for its progression to a stage needing treatment (48).

We also found multiple publications that identified BW as an independent risk factor for PH.(36), (45), (46), (47), (49), (1), (51), (52)

5.2.2 Sex and multiple pregnancy

There is a presuption in the scientific literature that the male gender could be considered a risk factor for the development of ROP. (25), (53).

Analyzing our data, we found no conclusive association between gender and the development of ROP. These results are supported by other authors. (36), (17), (18), (350), (52), (54), (55)

In recent years, an increase in multiple pregnancies has been reported, which is related to the trend towards increasing the age of women to give birth to their first child, the widespread use of hormonal preparations and the development of technologies for the treatment of infertility.

It is a known fact that multiple pregnancy is associated with an increased risk of complications and a greater chance of preterm birth, but its role as a risk factor for the development of ROP is a matter of debate.(56) The potential association between multiple pregnancy and the risk of ROP was first reported almost 70 years ago, when Kinsey reported a threefold increased risk of developing cicatricial ROP among children born from multiple pregnancies. There are more recent studies that support this possibility (51), (57), (58).

In our study, there was no statistically significant association regarding the development and progression of ROP versus the factor of multiple gestation. (36), (18), (53), (59) On the other hand, Marinov found a higher risk of developing ROP for children of multiple pregnancies.

At this time, we believe that there is no convincing evidence for a direct association of multiple gestation with ROP, but rather an indirect effect due to the increased risk of preterm birth, as well as the lower GA and BW of children born from such pregnancies.

5.2.3 Oxygen therapy

The main aim of oxygen therapy is to maintain adequate oxygenation of the neonatal tissues, especially the CNS and heart, and to support the incomplete haemodynamic adaptation to an extrauterine lifestyle demonstrated by the persistent ductus arteriosus (PAC) and increased resistance of the pulmonary vessels. At the same time, however, it is necessary to minimize the toxic effects of oxygen on the eyes, brain, and other organs. (60) The immaturity of the preterm infant's antioxidant systems makes it susceptible to oxygen-induced damage, and the association between oxygen therapy and the development of PH was demonstrated decades ago. Since then, debates have continued regarding the optimal oxygen saturation, the method of choice of oxygen therapy and its duration.(61), (62), (63)

Because almost all preterm infants treated in the Neonatal Intensive Care Unit required a respiratory support at some point during their hospitalization, we decided to investigate only the impact of prolonged ventilatory support – that are the cases in which ventilatory support lasting ≥7 days– on the development and progression of ROP. In investigating this parameter, we did not focus on the method of choice of ventilator or the mode used, but only on its duration in days.

The analysis of data from our study demonstrated the role of prolonged ventilatory support as an independent significant risk factor for the development of ROP, but not for its progression to stages requiring treatment. These results support the findings of other Bulgarian authors. (17), (18), (15) Marinov established the index of total duration of oxygen supplementation (AV + 02) as an independent risk factor for the development of ROP, but did not associate it with disease progression to stages requiring treatment. (17) Mladenov reached a similar conclusion in his dissertation, who, after conducting a multiple logistic regression analysis, found that the indices of invasive and noninvasive ventilatory support are independent risk factors for the development of ROP, but not for the progression of already developed disease to stages requiring treatment (18).

A number of foreign authors have investigated the influence of oxygen therapy on the course of ROP. Choi et al. published a study in which they concluded that prolonged mechanical ventilation (more than 14 days) was associated with an increased risk of developing ROP requiring treatment, as well as other comorbidities such as periventricular leukomalacia, bronchopulmonary dysplasia, and pulmonary hypertension.(64) Hadi et al. studied 152 preterm infants in Alexandria (Egypt) and found that invasive mechanical ventilation and oxygen therapy via nCPAP were risk factors for the development of ROP. (54) Borteca et al. also identified mechanical ventilation as a significant predictor of ROP. (32) In contrast, Tandon et al. in India

failed to find a statistically significant result with respect to administered oxygen therapy. (47)

5.2.4 Anaemia and haemotransfusions

Often premature infants develop anaemia, especially children born before the age of 28 weeks. (65)

According to our results, anaemia of prematurity is an independent risk factor for the development of ROP, but not for the progression of pre-existing disease.

Tandon et al. studied 105 preterm infants and found in univariate analysis that anemia correlated with the incidence of ROP. But in multivariate logistic regression, neonatal anemia dropped out as an independent risk factor.(47) A similar result was shared by Ramirez et al. who studied 458 preterm infants and found neonatal anemia to be a risk factor for the development and progression of ROP, but not an independent risk factor.(49)

In his dissertation, Mladenov demonstrated the role of neonatal anemia as an independent risk factor for both the development of ROP and disease progression to stages requiring treatment (47).

There are also publications that deny the role of anemia as a risk factor for ROP. Englert et al. studied 292 children and found that anemia of prematurity was a notable risk factor for ROP. (66) Bossi et al. found no association between hemoglobin levels and the development of ROP in their sample of 639 children. (67)

Another factor closely associated with anemia in preterm infants is the performance of hemotransfusions. Regarding this indicator, there is an opinion that, due to the lower affinity of adult haemoglobin (used for haemotransfusions) for oxygen, haemotransfusions may lead to hyperoxia of tissues and organs, including the retina, and hence accelerate the development of ROP. (36) Another possible explanation is related to the increased free iron ion load from transfusions, which amplifies the oxidative stress leading to ROP. (68)

As part of the work for this dissertation, we examined only those children who underwent two or more hematotransfusions during their hospitalization and found significance with respect to the development of ROP but not for disease progression to stages requiring treatment. In a multivariable regression model, hemotransfusions were dropped as an independent risk factor regarding the development of ROP. A similar result was reached by another Bulgarian author in his thesis (17).

In our available foreign literature, a number of authors confirm the role of hemotransfusions as a risk factor for PH.(36), (51), (52), (54), (69) while others express the opposite opinion.(45), (47)

Lust et al. studied 1635 preterm infants at St. Louis University Hospital, USA, and found a nearly fourfold increase in the risk of developing severe PH requiring treatment when hemotransfusions were performed during the first 10 days of the infants' lives. The authors explained their results by hypothesizing that decreased fetal hemoglobin (HbF) levels cause a rightward shift of the hemoglobin dissociation curve, increasing the oxygen concentration reaching the developing retina (70).

From what has been said so far, we believe that the presence of anemia and the need to correct it by hemotransfusions are factors that mark an aggravated general condition of the premature infant and are advisable to be taken into account by the screening ophthalmologist.

5.2.5 Persistent Ductus Arteriosus (PDA)

Persistent arterial ductus arteriosus is a common problem among preterm infants, and its incidence is directly related to the degree of prematurity.(71),(72) It occurs in about 10% of preterm infants born between 30 and 37 yr, but in younger infants born between 25 and 28 yr, its incidence rises to nearly 80%.(73)

Analysis of our data revealed no statistical significance with respect to the PDA factor in the development and progression of PH.

We found publications by other authors who, like us, found no association between PDA and ROP.(36), (47), (52)

Mladenov found that PDA increased the risk of ROP but not of progression to stages requiring treatment (18).

Ramirez et al. found that PDA was a significant risk factor for the development and progression of ROP. (49) A study of 487 preterm infants in Turkey found that PDA is an independent risk factor for severe ROP. (74)

Despite the result, we believe that the presence of PDA, especially in cases where treatment is necessary, is a complication of prematurity that requires the attention of the screening ophthalmologist.

5.2.6 Necrotizing enterocolitis (NEC)

NEC is an inflammatory intestinal disease in premature infants, especially those born before 32 gestational weeks of age, which can be life-threatening and furthermore increases the risk of growth impairment and neuro-psychiatric developmental delay.

(56) Its role as an independent risk factor for the development and progression of ROP is still debatable.

In our study, the number of children with this condition was very small and we could not find a statistical relationship between the NEC factor and ROP, which is confirmed by other authors (18).

Fundora et al. performed a retrospective analysis of data from the G-ROP study involving 7483 children and found a statistically significant association between NEC requiring surgical treatment and the development of ROP, but found no effect on the course of retinopathy (75).

In contrast to the publications listed above, Seiberth et al. reported that children with NEC developed ROP less frequently than children without this condition. (46) Yau et al. found a detectable role for NEC in the univariate model but not in the regression analysis. (51) Other authors reached similar results. (49)

We believe that NEC is a life-threatening complication of prematurity and, if present, needs to be considered by the screening ophthalmologist.

5.2.7 Pulmonary impairment and ROP (hyaline-membrane disease, respiratory dystress syndrome, neonatal pneumonia and bronchopulmonary dysplasia)

The presence of lung injury in the preterm infant is associated with increased oxygen supplementation requirements, possible fluctuations in oxygen concentration, and episodes of intermittent hypoxia, factors whose role in the pathogenesis of ROP has been demonstrated (76).

Analyzing our data, HMB proved to be a statistically significant factor in the development and progression of ROP in the univariate analysis applied. After logistic regression analysis, HMB has dropped as an independent risk factor for ROP.

Gupta et al. reported HMB as a risk factor for the development of ROP. (77) Carranza et al. studied 216 preterm infants in Peru and found HMB to be an independent risk factor for the development and progression of ROP. (78) Freitas et al. demonstrated that the major risk factors for the development of preterm type 1 ROP were a BW < 1000 g and the presence of pulmonary pathology. (76) Regarding the RDS factor, our results show a significant association at the univariate analysis level between RDS and the development of ROP. We found no significance with respect to progression of already developed disease to stages requiring treatment.

In his dissertation, Mladenov found that the presence of RDS was an independent risk factor for the development of ROP, but not for disease progression.(18) In contrast, Marinov found no statistically significant difference with respect to the presence of

ROP either between children who developed ROP and those who did not, or between spontaneously regressed and treated children.

We found several studies in the foreign language literature that reported an increased risk of developing ROP in children with RDS. Lin et al. performed a large retrospective study in Taiwan including children born before age 37 for the period 2000-2009, (79) Akkoyun et al. also considered RDS to be an independent risk factor for the development of ROP but also for progression to stage 3. (48) An earlier study in Sweden involving 202 children found a twofold increased risk of ROP for children with RDS. (80) Reyes et al. also reported an association between RDS and the development of ROP. (81)

We also studied cases in which evidence of neonatal pneumonia was available. This condition is potentially life-threatening.(9)

We found no statistically significant result regarding this factor and the development and progression of ROP.

In his dissertation, Mladenov found a statistically significant difference between children with ROP and those without signs of the disease with respect to neonatal pneumonia, but not when comparing spontaneously regressed and treated cases.(18)

There are publications in the foreign literature that neonatal pneumonia is not an independent risk factor for ROP (confirming our results) (69), as well as those that take the opposite view (82), (83).

We believe that the development of neonatal pneumonia should be considered comprehensively, together with other lung diseases associated with prematurity.

The current approach to preterm infants consists of a complex of measures including antenatal corticosteroid therapy, administration of exogenous surfactant after birth, and advanced oxygen supplementation techniques, with the aim of avoiding cases of pulmonary complications seen decades ago in such children. However, oxygen therapy continues to affect normal lung development and in some cases causes a pathology known as bronchopulmonary dysplasia. It is a chronic lung disease characterized by remodeling and enlargement of the alveoli, as well as disturbances in the microvascular circulation of the immature lungs of the premature infant.(84) The major risk factor for its development is prolonged mechanical ventilation (more than 28 days).(56)

Our results did not prove statistical significance of the factor BPD in relation to the development and progression of ROP, which confirms the findings of other authors.

(47) However, in the scientific literature available to us, we found publications that identify BPD as a risk factor for the development of ROP. (36), (18), (49), (1), (85)

5.2.8 Exogenous surfactant administration

There is a suggestion that the exogenous surfactant use is associated with an increased risk of developing ROP, and the likely mechanism being associated with increased oxygen partial pressure and possible hyperoxaemia after surfactant administration.(86) Analysing our data, we found this factor to be significant in terms of ROP development and progression, but this factor dropped out as an independent factor in our regression model.

Other authors have found that exogenous surfactant administration is a risk factor for the development of ROP, but not for the progression of disease already present to stages requiring treatment.(17),(18)

We support the view of other authors (87), (18) that surfactant administration among preterm infants cannot be considered in isolation, as it is associated with respiratory immaturity and the need for oxygen therapy.

5.2.9 Hyperbilirubinemia and phototherapy

Unconjugated bilirubin is a breakdown product of haemoglobin. Since children born prematurely may have a more protracted and severe course of hyperbilirubinemia, we investigated the influence of this factor on ROP.

Analyzing our data, we found no statistically significant association between the hyperbilirubinemia factors and the administration of phototherapy and the development and progression of ROP. Our results are consistent with those of other Bulgarian authors (17), (18).

In our available foreign language literature, we also found studies that denied the association between hyperbilirubinemia and the development of ROP. (47), (54)

There is a view that bilirubin has a protective role in the development of ROP due to its physiological antioxidant action. (52), (53), (88)

5.2.10 Risk factors from intraventricular hemorrhage

Intraventricular hemorrhage (IVH) is an important complication of prematurity that is often associated with a negative impact on the child's neuro-mental development (56).

The incidence of IVH is about 10 - 20% in preterm infants born before GA of 30 weeks, with an increase to 35 - 45% among children with BW < 750 g (89).

Our results showed that stage III IVH is an independent risk factor for both the development of ROP and disease progression to stages requiring treatment. From the univariate analysis of the results, we also found significance of IVH stage I on the development of ROP, but it dropped out in the regression model.

In the Bulgarian literature available to us, we found similar results in Mladenov (18), who found that IVH III st. is an independent risk factor for the development of ROP, whereas stage II IVH is an independent risk factor for progression of already developed disease to stages requiring treatment. On the other hand, Marinov found no significant difference in IVH between the groups studied. (17) Tsvetkova et al. also demonstrated the role of IVH as a risk factor for ROP. (15)

There are foreign publications that identify the presence of IVH as a risk factor for ROP. (1), (54) Chang et al. report that IVH is a significant factor in both the development of ROP and the progression of disease to a stage requiring treatment. (1) Yucel et al. (55) and Shah et al. (90) demonstrate an association between IVH and ROP in univariate analysis, but there is no detectable significance in the multiple regression model.

Approximately one third of children with IVH develop posthemorrhagic hydrocephalus, which is defined as ventricular dilatation above the 97th percentile for the respective gestational age. (91) Analyzing our data, we found no significant role of the posthemorrhagic hydrocephalus factor either in terms of disease development or progression. Our results are consistent with those found by other authors. (18) From the foreign language literature available to us, Halimic et al. rejected the role of hydrocephalus as a factor influencing the development of active ROP. (45) The same view is supported by Akkawi et al. (52)

In an analysis of risk factors for ROP among 162 preterm infants, Chang et al. found that hydrocephalus was associated with an increased risk for ROP in univariate analysis, but this factor dropped out as significant in their multivariate regression model (1).

Another complication on the CNS side and directly related to prematurity that we considered is the condition periventricular leukomalacia (PVL). It is hypoxia-induced damage to the white matter of the cerebrum.(92) This condition is the most common etiologic cause of cortical visual impairment (CVI), the adverse outcome of which, affecting visual function, is now known. (92) In recent years, the term "cortical visual impairment" has been replaced by "cerebral visual impairment," as it is not uncommon for cerebral white matter damage to also affect subcortical structures (e.g., radiation optica) (92).

We believe that abnormalities of the central nervous system (IVH, hydrocephalus, and periventricular leukomalacia) can be considered as a risk factor for ROP and should be taken into account by the screening ophthalmologist.

5.2.11 Low APGAR as a risk factor for ROP

In the univariate analysis of our data, we found that low APGAR at birth was a risk factor for both the development of ROP and disease progression to stages requiring treatment. In the regression model, however, this parameter dropped out as an independent risk factor.

Alajbegovic-Halimic et al. reported lower 1st and 5th minute APGAR values in children who developed active ROP compared with those in whom no signs of disease were detected.(45) Abrishami et al. reached a similar conclusion.(53)

Goncalves et al. also reported significance of APGAR < 7 in univariate analysis, but it dropped out in the multiple regression model (36).

Although APGAR cannot be used as a predictor of future neonatal morbidity, we believe it is advisable for the screening ophthalmologist to pay attention to these values.

5.2.12 Intrauterine hypotrophy (IUH)

Intrauterine hypotrophy is defined as a fetal growth disturbance above 2 standard deviations below the 10th percentile for the respective GW. In the foreign literature, these children are defined as "small for gestational age" (SGA) (93).

In our data analysis, we were unable to demonstrate a significant association between IUH and the development and progression of ROP.

We found conflicting opinions in the available literature regarding the role of IUH as a risk factor for ROP. Thakre et al. reported a higher incidence of ROP among children with IUH compared with those with normal fetal development, without finding a difference in other risk factors and severity of retinopathy progression. (94) A large-scale meta-analysis by Razak et al. from 2019 found that preterm infants with IUH were at greater risk of developing severe, treatment-requiring ROP compared with those with normal anthropometric indices according to GA. (93) An analysis by Filho et al. among a cohort of 345 preterm infants, rejected IUH as a discernible risk factor for ROP and found no significant differences in risk factors between children with IUH and those not diagnosed with the condition. (95) On the other hand, there is evidence that children with IUH are at lower risk of developing ROP. (96)

5.3 Analysis of maternal risk factors for progression and development of ROP

5.3.1 Assisted reproductive technologies (ART)

Assisted reproductive technology is associated with an increased incidence of preterm births and multiple pregnancies, which often result in the birth of smaller and more immature children. (97) This raises the question of whether ART, and in vitro fertilisation (IVF) in particular, can be considered an independent risk factor for ROP.

Analyzing our data, we found a significant association between the IVF factor and the development of ROP, but not for progression of already developed disease. Our results support the findings of other national and international authors, although the role of assisted reproductive technologies in the development of ROP is still a matter of debate (97) (98).

We believe that the data we have collected and analyzed are not sufficient to conclude definitively that IVF is a risk factor for ROP, but it has been shown and accepted to be a risk factor for preterm birth, (58), (99) and as such deserves consideration by screening ophthalmologists**.**

5.3.2 Mode of delivery

Analyzing our results, we found no significance regarding the mode of delivery (by cesarean section or vaginal delivery) and the development and progression of ROP. Our results confirm what has been shown by other Bulgarian (17) and foreign authors (49), (53), (100), (101).

There is still a debate in the available literature about the potential role of the mode of delivery as a risk factor for ROP, and which mode of delivery is associated with an increased risk of ROP.

Some authors point to delivery by normal mechanism (per vias naturalis) as such. (18) Manzoni et al. studied 174 children with extremely low birth weight (<1000 g) and found that vaginal delivery was an independent risk factor for the development of threshold ROP. The authors explained their result by the mechanism and dynamics of birth by a normal mechanism that could potentially have an adverse effect on cerebral vessels, leading to ischemia and/or oxidative stress, whose role in the pathogenesis of ROP is considered well established. In addition, passage through the birth canal poses a greater risk for infections of the newborn (e.g., from agents such as Ureaplasma urealyticum, Candida spp.), which are themselves a potential risk factor for ROP (102).

We believe that the lack of definitive data regarding the risk factor under consideration (mechanism of delivery) is due to the variety of maternal and fetal

comorbidities associated with preterm birth and the particular situation in each case requiring appropriate obstetric management.

5.3.3 Maternal age

There is debate about the influence of maternal age on the development and progression of PH. It is known that maternal age below 20 years and above 40 years are considered risk factors for preterm birth (103).

We were interested to investigate the influence of maternal age on the development and progression of ROP, but were unable to find statistical significance of this factor with respect to ROP.

From the available foreign language literature we found publications that support our results.(49), (104)

According to some authors, older maternal age correlates with an increase in the risk of ROP, (105) while others suggest an inverse relationship, (106)

5.3.4 Hypertensive conditions during pregnancy

Hypertensive conditions during pregnancy include a wide range of disorders, which are generally characterised as arterial blood pressure above 140/90 mmHg, in the presence or absence of oedema and proteinuria. (107) These disorders are classified as chronic hypertension, gestational hypertension, preeclampsia, eclampsia, or unspecified hypertension. (108) The conditions listed are associated with increased maternal and perinatal morbidity and mortality, but their possible protective role with respect to ROP disease is also discussed.(56)

Analyzing our data, we could not find a significant association between the factor "hypertensive states during pregnancy" and ROP.

Similar results have been found by other Bulgarian (17), (18) and foreign authors.(49), (51), (109)

Two meta-analyses by Chan et al. (110) and Zhu et al. (107) failed to demonstrate a significant association between ROP and hypertensive states during pregnancy.

5.3.5 Placental abruption

Placental abruption is a serious obstetric complication that threatens the life of the mother and the child. It is associated with a cascade of pathophysiological mechanisms, the end result of which is the separation of the placenta from the uterine wall before delivery. Pregnancies complicated in this way are characterized by

increased incidence of preterm birth, low birth weight, and increased perinatal mortality (111).

There is little evidence of work examining the potential relationship between the placental abruption factor and ROP. Analyzing our results, we were unable to detect a discernible role for this factor in the development and progression of ROP.

Our results confirm those of other Bulgarian authors (17), (18).

From the available foreign literature, we found publications confirming our data. (112), (113)

5.3.6 Preterm premature rupture of the membranes (PPROM)

Preterm premature rupture of the membranes is defined as a spontaneous rupture of the amniotic sac occurring before 37 g.w.. It occurs among about 3% of pregnancies, and this condition is thought to be responsible for about 1/3 of all preterm births. PPROM is associated with significant perinatal mortality and morbidity, with the main causes related to the short period between rupture and delivery, increased risk of infection, and umbilical cord compression as a result of amniotic fluid reduction. The development of amnionitis and clinical placental abruption are commonly associated with PPROM, and on the fetal side, described complications include IVH, necrotizing enterocolitis, and sepsis (114).

Analyzing this factor, we could not find a statistically significant association regarding the development and progression of ROP. Similar results have been found by other Bulgarian (17), (18) and foreign authors.(49)

Lee et al. found that children born after PPROM were at lower risk of developing plus disease and threshold disease. (115) Lynch et al. reached a similar conclusion, and a possible explanation according to the authors lies in the therapeutic interventions in cases with PPROM (corticosteroid therapy, broad-spectrum antibiotics, intensive medical monitoring), which probably suppress inflamatory processes in the immature retina and thus have an inhibitory effect on the development of ROP. (116)

5.3.7 Cervical cerclage

Cervical insufficiency is a clinical diagnosis characterized by recurrent, painless dilatation and shortening of the cervix. Introduced in the 1950s, cerclage is an obstetric procedure designed to compensate for this condition and reduce the risk of preterm birth (117).

Data on the impact of cervical surgery on ROP are scarce, but we did find a few publications that investigated the influence of this factor.

Analyzing our data, we found no statistically significant difference with respect to the factor "cervical cerclage" in the development and progression of ROP. These results are confirmed by other Bulgarian (17), (18) and foreign authors (53).

5.3.8. Maternal-fetal infection and intraamniotic infections

The roles of maternal and fetal inflammatory responses in the development of ROP have been the subject of increased interest by researchers in recent years. The "prenatal phase" model of ROP has been proposed, according to which the systemic inflammatory response is involved in disease development by two main mechanisms. The first is indirect and concerns its role as a factor in the induction of preterm birth and any adverse consequences for the child associated with it. The second is direct and examines the influence of the inflammatory response on angiogenesis.(118) Maternal-fetal infection is thought to induce the production of proinflammatory cytokines such as interleukins (IL-1, IL-6, IL-8) and tumor necrosis factor α (TNF-α). (119) Inflammatory mediators via the fetal circulation reach the neonatal retinal and choroidal vessels and induce a local inflammatory response, inducing phase 1 of ROP, and postnatal hyperoxia appears to be a potentiating rather than initiating factor of ROP. (118) There is evidence that the systemic inflammatory response is associated with decreased levels of insulin-like growth factor-1, which in turn also increases the risk of developing ROP.

We also reviewed cases in which the diagnosis of intra-amniotic infection (chorioamnionitis) was made. Analyzing our data, we found no significance regarding MFI and the development and progression of ROP. We also came to an improved result with respect to IAI.

Our results support what other authors have found. Marinov found no difference between children who developed/ did not develop signs of ROP, and between spontaneously regressed/progressed patients (group IIA and group IIB) with respect to the presence of maternal infections (17).

A meta-analysis by Mitra et al. comprising 27 studies and 10,590 preterm infants found a significantly higher risk of developing ROP among children exposed to intraamniotic infections (chorioamnionitis), including for severe ROP (≥ stage 3). However, after further analysis using only those studies in which data were adjusted for gestational age (8 in number), the authors found no significant association between the presence of chorioamnionitis and the development of ROP. (120) Ahn et

al. also reported an association between intra-amniotic infections and ROP and aggressive ROP.(121)

A more recent extended meta-analysis by Villamor-Martinez et al. covering 50 studies and 38956 children confirms the results of Mitra et al. It concluded that intramamniotic infections are associated with an increased risk for the development of ROP, but that some of the effects on the pathogenesis of ROP may be mediated by its role as an etiologic factor for preterm birth and hence for all the sequelae associated with it.(122)

We believe that the presence of perinatal inflammation should be interpreted with caution by screening ophthalmologists.

5.3.9 Gestational diabetes

Gestational diabetes is thought to influence the development and course of ROP due to increased retinal VEGF levels as a consequence of hyperglycemia (56).

Analyzing our data, we could not find a statistically significant result regarding the factor "gestational diabetes" in the development and progression of ROP.

Our results are supported by Bulgarian (17) and foreign authors (47), (49), (1), (51), (69).

A retrospective study in Turkey among children with a BP ≥ 1500 g compared data from 78 preterm infants born to diabetic mothers with 258 control children and reported a 6.3-fold increased risk of developing type 1 ROP among children of diabetic mothers.(123) Oraga et al. also reported that maternal diabetes increased the risk of severe ROP 3.5-fold. (124)

We believe that maternal diabetes is a factor that should be considered by the screening ophthalmologist.

5.3.10 Antenatal corticosteroid prophylaxis

Antenatal corticosteroid prophylaxis is recommended for all women at risk of preterm birth between 24 and 34 years of age. There is evidence of the benefits of antenatal CS prophylaxis in reducing neonatal morbidity and mortality, including RDS, IVH, necrotizing enterocolitis, etc. The relationship between this factor and ROP is still debated (11).

Analyzing our results, we could not find a significant relationship between ROP and the factor "antenatal CS prophylaxis".

A meta-analysis by Yim et al. comprising 434 studies and 20 731 preterm infants reported a decreased risk of developing and progressing ROP after antenatal corticosteroid prophylaxis was administered. (11) One possible explanation for these results lies precisely in the theory of the inflammatory genesis of ROP, which we discussed above. It is possible that the potent anti-inflammatory effect of CS medications exerts a beneficial effect by suppressing some of the pathological mechanisms associated with ROP.

5.4 Early postherapeutic effect after intravitreal application of anti-VEGF medications.

In our study, monotherapy with anti-VEGF medication was administered in 19 children (36 eyes in total). In 5 children (10 eyes), treatment was performed that combined intravitreal administration of anti-VEGF medication with ablative therapy (8 eyes were treated with laser therapy and 2 eyes with cryotherapy).

In two of the treated children (4 eyes) the process progressed to stage 4b and they were referred for pars plana vitrectomy.

Regression of the ophthalmoscopic finding was observed in the remaining 22 children (42 eyes), representing 91.7% of the children who received anti-VEGF drug therapy and 81.5% of all children treated.

From our available Bulgarian literature, Mladenov reported a good early structural outcome after intravitreal administration of Bevacizumab (Avastin) among 12 children (24 eyes).

Monotherapy with anti-VEGF medication was elegant, specific, and not associated with destruction. Anterior segment ischemia of the iris and lens was avoided. It allows preservation of the visual field, especially in zone 1. The procedure is short, there is no need for special equipment, it can be performed under topical anaesthesia without the need for sedation and general anaesthesia. Treatment with anti-VEGF medication is associated with a lower rate of refractive abnormalities (myopia and astigmatism) compared with laser therapy (125), (126).

On the other hand, this treatment modality is also associated with some question marks. One of them is the duration of the therapeutic effect and the possibility of recurrence of PH. The use of bevacizumab was associated with a higher incidence of ROP recurrence compared to ablative laser therapy. (127) In the BEAT-ROP trial, ROP recurrence was observed at a mean of 19.2 weeks after IVB, compared to 6.4 weeks after laser therapy in zone 1. (128) In a subsequent report by Mintz- Hittner et al. found reactivation of ROP in 7.2% of treated eyes (mean 16.2 weeks posttreatment, 51.2 weeks postconceptual age). (129) There are publications that

report late reactivation of ROP up to 3 years after the administered treatment. (130), (131) These results lead to the conclusion that prolonged follow-up of children treated with anti-VEGF medications is necessary.

In addition, anti-VEGF therapy is inevitably associated with some complications - both local and systemic. Local complications are associated with the risks of intravitreal injections - endophthalmitis, retinal haemorrhage, cataract and retinal detachment (132), (133).

Regarding adverse systemic effects, there are concerns about negative effects on the neuropsychiatric development of children treated with anti-VEGF medications. A retrospective study conducted in Canada noted an increased incidence of motor defects at 18 months of age in children treated with intravitreally administered Bevacizumab for ROP. The authors reported a threefold increase in risk in children with neurodevelopmental impairment compared with those treated with laser therapy.(134) On the other hand, a study by Lien et al. found no difference in neurodevelopmental impairment between children treated with laser therapy and those treated with anti-VEGF medication. However, the authors found an increased incidence of psychomotor disorders, in children treated with combined bevacizumab and laser therapy. One possible explanation is the impaired blood-retinal barrier from laser treatment, which contributes to higher levels of anti-VEGF in the systemic circulation. (135) A meta-analysis of 13 studies found a significantly increased risk of moderate cognitive impairment in preterm infants with severe ROP treated with bevacizumab compared with children treated with laser or cryotherapy. (136) Another meta-analysis published almost simultaneously with the above found no association between bevacizumab treatment and neurodevelopmental impairment. (137)

It is worth noting here that the association between severe ROP and impaired psychomotor development is already known. (138) By presumption, children with type 1 retinopathy and aggressive ROP are smaller, less mature, and at greater risk of systemic complications as a consequence of prematurity. The conflicting opinions in the literature indicate that it is currently extremely difficult to draw a line of demarcation between these complications and the side effects of the treatment performed for ROP.

5.5 Creation of a web-based information portal dedicated to ocular pathology in the preterm infant

The main goal we set ourselves is to improve awareness of parents and medical professionals at a national level, summarizing the facts and presenting them in an adapted form suitable for a mass audience. We believe that the availability of such a

website is a necessity, given the limited time available to ophthalmologists in neonatal clinics to verbally inform parents not only about the current status of their premature child, but also about the nature of the disease in general. The creation of a digital information portal in Bulgarian is a state-of-the-art solution that aims at visibility and easy access to systematized information throughout the country, not only in the major university centers. This option is also extremely convenient for the specialists who develop and maintain it, as it allows easy management of information, its supplementation and expansion. We believe that the information portal will facilitate parents and all professionals involved in the care of premature babies. In the website we have included data concerning the epidemiology, pathogenesis, diagnosis and current therapeutic options for the treatment of ROP.

We believe that promotion of this portal would allow for the inclusion of other medical professionals who have dedicated their careers to premature infants, hopefully facilitating the entire process of ongoing care and rehabilitation of our youngest and most vulnerable patients.

6. CONCLUSIONS

6.1 Conclusions:

6.1.1 Incidence of ROP among premature infants treated in the Neonatology Intensive Care Unit at the Hospital "Prof. Dimitar Stamatov" - Sofia, Bulgaria. Varna and screened for the disease is high - 69,4%.

6.1.2 The incidence of ROP in the Varna region is significantly higher than in the Plovdiv region (30.3%), the Sofia region (22.8%) and the Stara Zagora region (5.4%).

6.1.3 The incidence of preterm infants with ROP in the Varna region needing treatment is 20.2%, which again significantly exceeds the previously reported incidence, for example in the Sofia region - 7.4%.

6.1.4 Low birth weight, invasive ventilatory support, grade III IVH, and the presence of neonatal anemia are significant and independent risk factors for the development of ROP.

6.1.5 Low birth weight and grade III IVH are significant and independent risk factors for disease progression.

6.1.6 Small GA, presence of HMB; performing more than 2 hemotransfusions; exogenous surfactant administration; grade I IVH, low 1-min APGAR were statistically significant but not independent risk factors for developing ROP.

6.1.7 Low GA, presence of neonatal anemia, HMD, exogenous surfactant administration; low APGAR at 1st and 5th minute are statistically significant but not independent risk factors for progression of ROP to stages requiring treatment

6.1.8 Of the maternal risk factors examined, we found IVF to be significant for the development of ROP but not for disease progression. In regression analysis, IVF was dropped as a significant risk factor for the development of ROP.

6.1.9 The study of the etiological causes of vision loss among the students of the school for children with visual impairment "Prof. Dr. Ivan Shishmanov" Varna, shows that diseases of the optic nerve and retina are the leading causes of severe visual impairment and blindness, and among the studied students ROP takes second place after optic nerve atrophy as the etiological cause of vision loss.

6.1.10 Timely treatment with intravitreal administration of an anti-VEGF drug for type 1 pre-treshold disease has a good early anatomic outcome.

6.2 In finale:

Retinopathy of prematurity continues to be a leading cause of irreversible vision loss worldwide. Knowledge of the disease, its pathogenesis and natural course continues to improve, and technological advances provide opportunities for early diagnosis and telemedicine consultations.

Retinopathy of prematurity is a condition whose consequences can be devastating for the child, his or her loved ones and society. In order to avoid them, it is necessary to ensure the highest quality of neonatal care, to strictly follow the recommendations of the National Strategy for the Screening and Treatment of ROP, to improve the awareness of parents and medical professionals involved in the care of premature infants, and to work towards correcting the problems in this regard of the health system in Bulgaria.

7. CONTRIBUTIONS

7.1 Contributions with cognitive nature

7.1.1 An in-depth literature review on the prevalence, pathogenesis and risk factors of ROP was conducted;

7.1.2 Current trends concerning the diagnosis, treatment and follow-up of children with ROP were updated.;

7.2 Contributions with scientific nature

7.2.1 For the first time in Northeastern Bulgaria, a study of the clinical and epidemiological characteristics of ROP was conducted, involving 124 preterm infants;

7.2.2 The long-term effects of ROP were analysed using a survey of 145 students attending a specialised school for visually impaired children;

7.2.3 An in-depth analysis of maternal and fetal risk factors involved in the development and progression of ROP has been carried out, which confirmed the findings of other researchers in major university centres in Sofia and Plovdiv;

7.2.4 Evidence for the beneficial early post-treatment effect of intravitreal administration of anti-VEGF drugs has been collected;

7.2.5 For the first time in Bulgaria, an online-based information portal dedicated to ROP has been proposed and implemented to support parents and medical professionals.

7.3 Contributions with confirmatory nature

7.3.1 The effectiveness of the screening programme for PH adopted and operating in the territory of the Republic of Bulgaria has been confirmed;

7.3.2 The role of risk factors in the development and progression of ROP has been confirmed;

7.2.3. Efficacy of anti-VEGF drugs used to treat confirmed.

8. PUBLICATIONS AND SCIENTIFIC COMMUNICATIONS RELATED TO THE DISSERTATION WORK

Publications, related to the dissertation work:

1. Ilieva, A., Manolova, Y. (2022) Incidence of retinopathy of prematurity in Varna region, Bulgaria, and evaluation of perinatal risk factors. Scripta Scientifica Medica, 54(4), 36-43.
2. Илиева-Кръстева А., Манолова Я. Opportunities for integration of artificial intelligence and smart technologies in retinopathy of prematurity screening programs Medinfo 2023 г. issue 2 (30-33)
3. Ilieva A, Manolova Y. Ophthalmology conditions in the premature child GPNews 2023 issue 6 (45-49)
4. Ilieva, A., Manolova Y. Retinopathy of prematurity as a cause of severe visual impairment and blindness among students from the Prof. Dr. Ivan Shishmanov Specialized School Students with Visual Impairment in Varna, Bulgarian review of ophthalmology 2023 issue 2

Scientific communications, related to the dissertation work:

1. Ilieva A, Manolova Y., Manolova T. Childhood blindness in a school for visually impaired children in City of Varna, Digital poster XVth Congress of the Bulgarian ophthalmology society “Light matters”, 28.09-01.10.2023 Albena

Abstract:

Introduction

Protecting children's ocular health is a task that presents many challenges. One of them is a disease called retinopathy of prematurity (ROP), a multifactorial vasoproliferative disease that results from abnormal processes occurring in the immature retina. Retinopathy of prematurity is a disease that was documented in the first half of the 20th century when the foundations of modern neonatal care were being laid. In recent decades, science and technology have made rapid advances that have influenced methods of diagnosis and treatment of this disease. However, ROP continues to be the leading cause of preventable childhood blindness worldwide, and the reasons for this are complex: a trend towards increased incidence of preterm births, increased survival of extremely preterm infants, differences in standards of neonatal care across countries, and different levels of functioning of screening programs.

Objective

To determine the epidemiological and clinical characteristics of ROP among preterm infants in the Varna region according to the current screening program for ROP adopted in the Republic of Bulgaria, and to propose a platform to improve the awareness of parents and medical professionals about this disease.

Materials and methods

The clinical material includes the premature children who were screened for ROP in the Specialized Hospital of Obstetrics and Gynecology "Prof. Dimitar Stamatov" – Varna and followed up in the University Hospital for Active treatment in Ophthalmology-Varna for the period from January 2017 to December 2020. The inclusion criteria for the study were consistent with the standard adopted at the National Workshop on Screening and Treatment of ROP in Bulgaria in 2009. The systematic course of the study included: identification of the preterm children to be screened; examination of medical records - current medical history, discharge summary from birth, as well as from neonatology departments where the child was treated; provision of maximum medical mydriasis; placement of topical anesthesia and eye speculum; examination by binocular indirect ophthalmoscopy at the "Prof. Dr. Dimitar Stamatov" and/or photodocumentation of the clinical findings with RETCAM at University Hospital for Active treatment in Ophthalmology-Varna ; determination of management- follow-up or treatment. In the study concerning the incidence of ROP in the School for visually impaired children "Prof. Dr. Ivan Shishmanov". 145 children were included. Data were collected on the age, gender

and cause of visual impairment according to the available medical information of the visually impaired students attending the school in the school year 2022/2023.

Results and discussion

During the study period, signs of ROP were detected in 86 children (69.4% of all children studied), and in 25 of them (20.2% of all children studied) the disease progressed to ROP requiring treatment. The remaining 61 children (49.2% of all children studied) showed signs of spontaneous disease regression. We compared our results with a similar study conducted in the same neonatal intensive care unit more than 15 years ago. In that study, 686 preterm infants were screened and the total number of children diagnosed with ROP was 143 (20.85%). The incidence of the disease was found to be significantly lower compared to our study. Despite the high incidence of ROP diagnosed in our study, most of the cases were found to have mild form of the disease in which spontaneous regression was observed. One possible explanation is that the mean BW and GA in this previous study were significantly higher compared to ours, with values of 1319.5 (± 323) g and 30 (± 2) yr, respectively. In our study, the mean BW of preterm infants was 1106 (± 254.4) g and the mean BW was 28.3 (± 2.3) g.w. Improved neonatal care in recent years has resulted in the survival of many more high-risk infants, increasing the chance of developing ROP. Our results confirm this global trend.

Hundreds, if not thousands, of studies investigating the role of potential risk factors on the development and course of ROP are found in the world literature. In the Bulgarian literature, these data are scarce; therefore, we investigated 22 potential risk factors on the fetal side and 11 factors on the maternal side. The two most important and widely discussed risk factors in the literature are undoubtedly the low BW and the low GA of preterm infants, and these are the main criteria on which any screening program for ROP is based. From univariate analysis, we found the following risk factors to play a significant role: low BW, small GA, prolonged mechanical ventilation, hyaline membrane disease and respiratory distress syndrome, anemia, more than 2 hemotransfusions, exogenous surfactant intake, intraventricular hemorrhage stages I and III, and low APGAR. With respect to the factors sex, multiple gestation, persistent ductus arteriosus, necrotizing enterocolitis, neonatal pneumonia, hyperbilirubinemia, phototherapy, bronchopulmonary dysplasia, intrauterine hypotrophy, posthemorrhagic hydrocephalus, and periventricular leukomalacia, we found no discernible role for either the development of ROP or disease progression. Multivariate analysis confirmed the role of low BW, prolonged ventilatory support, neonatal anemia, and stage III intraventricular hemorrhage

as independent factors in the development of ROP, and low birth weight and stage III IVH as independent factors in disease progression.

Regarding maternal risk factors, we found a significant association between the factor in vitro fertilization and the development of ROP, but not for the progression of the already developed disease. Assisted reproduction is associated with an increased incidence of preterm births and multiple pregnancies, which often result in the birth of smaller and more immature children. With respect to the remaining 10 risk factors-mechanism of delivery (by cesarean section or vaginal delivery), maternal age, hypertensive conditions during pregnancy, placental abruption, premature rupture of the amniotic sac, cervical surgery, chorioamnionitis, maternal-fetal infections, gestational diabetes, and antenatal corticosteroid prophylaxis-we found no significant association for the development and progression of ROP.

These data do not contradict the results found so far by researchers at home and abroad. We believe that it is highly advisable for screening ophthalmologists to become familiar with the comorbidities of premature infants and the treatment administered, which again emphasizes the need for a multidisciplinary approach to this disease.

We investigated the early posttherapeutic effect of anti-VEGF medications used to treat ROP. We found good efficacy of the treatment administered, achieving regression of ophthalmologic findings in more than 80% of the children. Monotherapy with anti-VEGF medications was elegant, specific and not associated with destruction. On the other hand, this treatment modality is also associated with some question marks. These include the duration of the therapeutic effect, the possibility of ROP recurrence, the unclear effect of anti-VEGF medication on neuropsychiatric development, and the risk of local complications (endophthalmitis, cataract, retinal detachment).

We searched for the place of ROP as a cause of severe visual impairment and blindness among students in the School for visually impaired children "Prof. Dr. Ivan Shishmanov". We found that the leading cause of visual impairment among them was diseases of the posterior segment of the eye, with optic nerve atrophy being the leading cause and ROP the second. Retinopathy of prematurity continues to be an important cause of blindness among students attending schools for visually impaired children, both locally and in other countries.

We set out to improve awareness among parents and medical professionals nationally by summarizing the facts and presenting them in an adapted form suitable for a mass audience. We have accomplished this task by developing a web-based information portal at www.rop-info.com. We believe that the availability of such a site is a

necessity, given the limited time available to ophthalmologists in neonatal clinics to verbally inform parents not only about the current status of their premature infant, but also about the nature of the disease in general.

Conclusion:

Retinopathy of prematurity continues to be a leading cause of irreversible vision loss worldwide. Knowledge of the disease, its pathogenesis and natural course continues to improve, and technological advances provide opportunities for early diagnosis and telemedicine consultation. Hopes are pinned on relatively new treatment modalities, but more studies and results are needed to answer questions concerning the safety and long-term effectiveness of these drugs.

Retinopathy of prematurity is a condition whose consequences can be devastating for the child, his or her relatives and society. In order to avoid them, it is necessary to ensure the highest quality of neonatal care, to strictly follow the recommendations of the National Strategy for the Screening and Treatment of ROP, to improve the awareness of parents and medical professionals involved in the care of premature infants and to work towards eliminating the problems in this respect of the health system in Bulgaria.