Research Article

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Incidence and Characteristics of Infants with Retinopathy of Prematurity in Croatia

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Abstract

Purpose: To study the incidence of retinopathy of prematurity (ROP) in a neonatal intensive care unit in Croatia and obtain information on risk factors associated with ROP. There have been limited studies on ROP in Croatia where the screening for ROP and its treatment is still insufficient and not introduced in many intensive care units.

Material and methods: This retrospective study included 247 premature infants (birth weight (BW) $\leq$ 1500 g or gestational age (GA) $\leq$ 32 weeks; and BW between 1500 g and 2000 g or GA $>$ 32 weeks if there was an unstable clinical course), admitted to the neonatal intensive care unit of University Hospital Split, over a 5-year period between January 2012, and December 2016. The relationship between clinical risk factors and the development of ROP was analyzed..

Results: The overall incidence for ROP was 23.9% (59 infants), for Type 1 ROP was 9.3% (23 infants); for Type 2 ROP was 14.6% (36 infants). Median gestational age (GA) and birth weight (BW) were significantly lower among infants with ROP versus those without ROP (29: 23-34 vs. 31: 23-34, $p < 0.001$ and 1180: 630-2000 vs. 1485: 590-2000, $p < 0.001$ respectively). Multivariate analysis showed that only BW ($p = 0.029$) and small for gestational age (SGA) ($p = 0.045$) predicted the development of ROP.

Conclusion: Birth weight and small for gestational age were the most significant risk factors for developing ROP. In comparison with studies from highly developed countries, infants with a much wider range of gestational age and birth weights are developing type 1 ROP.

Keywords: ROP, Prematurity, Risk factor, Incidence

Introduction

Retinopathy of prematurity (ROP) is the most widely recognized cause of visual impairment after preterm birth. It is defined as a proliferative disorder of the developing retinal blood vessels in preterm infants which may lead to poor visual acuity or blindness [1]. Approximately 50 000 children worldwide are blind due to retinopathy of prematurity (ROP), and many more have significant visual disturbances [2]. The proportion of blindness as a result

of ROP varies greatly among countries, being influenced by level of neonatal care and by the availability of effective screening and treatment programs [3],[4].

Although knowledge about the pathogenesis of ROP has increased considerably, a lot is still unknown concerning the factors that initiate and promote the progression of this disease. The classic risk factors for ROP include low gestational age, low birth weight, and exposure to an oxygen rich environment [5],[6],[7].

Numerous epidemiologic studies have suggested additional risk factors for ROP disease and its severity [8],[9],[10]. These include multiple birth, maternal preeclampsia, intrauterine growth restriction, mechanical ventilation, need for blood transfusion, the presence of patent ductus arteriosus, intraventricular hemorrhage (IVH), pulmonary insufficiency and male gender among others [9],[10],[11],[12].

Prevalence varies by population, though it is estimated overall between 10–25% and incidence between approximately 50–70% in infants weighing less than 1500 grams at the time of birth [13],[14],[15],[16],[17]. In highly developed, industrialized countries (i.e. those ranked highly by the United Nations Development Programme [UNDP] on the basis of their Human Development Index [HDI]), the population of premature infants who are currently at risk for the advanced stages of ROP that requires treatment is extremely premature, with birth weights almost always < 1000 g [4],[18].

However, data from Latin America and the Former Socialist Economies of Eastern Europe showed that infants with much wider range of birth weights are developing severe ROP (ROP type 1), than in highly developed countries. Some of the possible reasons include increasing survival of premature infants, often with suboptimal standards of neonatal care and low implementation of screening and treatment of ROP [4].

Croatia is a former socialist country of southeastern Europe (i.e. with HDI ranking in the range 31–100). The screening and treatment of ROP are still insufficient and not introduced in many intensive care units in Croatia [19]. Although significant advances have been made in perinatal care, ROP remains a serious complication in prematurely born individuals. Information on the population of infants, who develop treatable ROP in Croatia, is required to develop screening programs that include all premature infants who are at risk.

The aim of the current retrospective study was to identify the incidence of ROP and the associated risk factors of ROP in the second largest Croatian neonatal intensive care unit (NICU) in southern Croatia, from January 2012 to December 2016.

Materials and Methods

Patients

This was a retrospective study in which we reviewed the

medical records of all infants born from 23- to 36-weeks of gestation who were admitted to the neonatal intensive care unit (NICU) of University Hospital Split from January 1, 2012 to December 30, 2016, which is a Level III nursery in southern Croatia. Ethical approval for this study was obtained from the University Hospital Split Ethics Committee (Nr.2181-147-01/06/M.S.-17-2).

Out of 1025 premature infants admitted during the 5-year study period, 247 (24, 1%) infants who met the criteria to enter screening for ROP by pediatric ophthalmologists were included in the study. Excluded were infants with major congenital malformation, chromosomal anomalies and infants who died before eye examinations could be performed.

Criteria for eye examination (ROP screening)

Eye examinations were performed on infants who met the following criteria recommended by the American Academy of Pediatrics (AAP): birth weight (BW) $\leq$ 1500 g or gestational age (GA) $\leq$ 32 weeks; and BW between 1500 g and 2000 g or GA $>$ 32 weeks if there was an unstable clinical course (as defined by the attending neonatologist), or/and need for cardio respiratory support. Infants born with gestational age of 27 weeks or less were first examined at 31 weeks postmenstrual age. Beyond 27 weeks gestational age, the first examination was performed 4 weeks after birth.

Eye examination methods

The examination was performed under topical anesthesia using 0.5% tetracaine eye drops and supportive oral sucrose. Pupils were dilated using a topical administration of one drop of 0.5% tropicamide ("Mydracyl", Alcon Laboratories, U.K limited) at 15 minutes intervals 3 times before examination. Indirect ophthalmoscopy was performed using a binocular indirect ophthalmoscope and 20 diopter lens. An infant speculum and scleral depressor were also used to perform the examination.

Follow-up and management of infants at risk for ROP

If no ROP was detected, eye examinations were continued every 2 weeks until vascularization had reached Zone 3. The follow-up examinations for infants with ROP were carried out every 1 to 2 weeks, but some infants were examined as frequently as twice per week depending on the zone and severity of ROP. ROP status was documented using the International Classification

of ROP, including stage, zone, and extent of disease and presence or absence of plus disease [20]. All infants requiring treatment were transferred to Department of Ophthalmology University Hospital Split for the procedure and most were readmitted to our department following treatment. Indication for treatment (type 1 ROP) was determined following the Early Treatment for Retinopathy of Prematurity Study criteria are: Zone I–any stage ROP with plus disease or Zone I–stage 3 ROP without plus disease, or Zone II–stage 2 or 3 ROP with plus disease [15].

During NICU care, all infants on supplemental oxygen were carefully monitored by continuous pulse oximetry in order to maintain oxygen saturation between 90% and 95%. No increase or decrease in target pulse oximetry has been performed after identification of any stage of ROP. No eye shields were used except when infants were receiving phototherapy.

For purposes of data analysis, the terms ‘type 1’ and ‘type 2’ ROP were used to differentiate eyes with significant changes of ROP that require treatment (type 1) from eyes with significant changes that do not require treatment but must be carefully monitored (type 2) [1].

Screened patients were divided into three groups: Group of infants without ROP, group with a mild form of ROP (Type 2 ROP) and group with a severe form of ROP treated with laser (Type 1 ROP). Infants were placed in one of five gestational age and birth weight groups for purposes of comparison. These groups were assigned based on the results of other studies for ease of comparison and to provide an even distribution of data obtained in this study.

Identification and definition of risk factors

The following perinatal-neonatal and demographic variables were extracted from the database for comparison of screened infants who had ROP and those who did not: gestational age (GA), birth weight (BW), small-for-GA (SGA) status, gender, Apgar score, intraventricular hemorrhage ≥ 2 , patent ductus arteriosus sepsis and severe apnea and blood transfusion. Respiratory data recorded included the presence of chronic lung disease, days on synchronized intermittent positive pressure mechanical ventilation (SIPPV), days on continuous positive airway pressure ventilation (CPAP) and the duration of oxygen therapy in days (SIPPV, CPAP and administration of supplemental oxygen to the patient in the incubator at the concentration greater than the air).

We also analyzed perinatal variables, including presence of multiple gestations, cesarean section delivery, maternal preeclampsia, maternal chorioamnionitis and maternal administration of steroids.

Definitions

Very low birth weight (VLBW) was defined as infants with birth weight < 1500 g; extremely low birth weight (ELBW) was defined as infants with birth weight < 1000 g. GA was ascertained based on last menstrual period and early ultrasonographic data. SGA was defined as a birth weight for GA below the 10th percentile [21]. IVH was graded according to Papile’s classification [22]. Sepsis was diagnosed based on a positive blood culture. Patent ductus arteriosus was diagnosed clinically and confirmed by 2-dimensional echography. Severe apnea was defined as absence of spontaneous breathing >20 seconds, associated with desaturation or bradycardia requiring more intervention than stimulation alone (temporary manual ventilation by Neopuff) [23]. Bronchopulmonary dysplasia (BPD) was defined as oxygen dependency beyond 36 weeks corrected age in association with chest radiographic findings of persistent hazy opacification or cyst-like pattern of density and lucency [24]. Chorioamnionitis was confirmed by pathohistological evidence of placental inflammation. Adequate antenatal administration of betamethasone was defined as the completion of 2 doses of betamethasone given 12 hours apart with the second dose administered more than 24 hours prior to delivery.

For each infant who had ROP, the age at which ROP was first detected, the maximum stage of ROP and laser therapy were recorded.

Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL). To assess the significance of proposed ROP risk factors univariate analysis was conducted with evaluation of odds ratios and confidence intervals and with appropriate significance of $P < 0.05$. To control all the variables of interest and estimate the independent risk factors for incidence of ROP, multivariate logistic regression was used.

Results

From July 2006 to July 2010, 247 infants fulfilled the inclusion criteria, having complete clinical data and were included in this analysis.

For the entire cohort, the overall incidence of any ROP was 23.9 % (59 of 247 infants), the incidence of Type 1 ROP was 9.3% (23 of 247 infants); the incidence of Type 2 ROP was 14.6% (36 of 247 infants). Among infants with ROP, type 1 was detected in 39% (23 of 59 infants), type 2 was detected in 61% (36 of 59 infants). In all infants with ROP both eyes were affected.

Distribution of ROP by gestational age

The median GA for the entire cohort was 31 weeks (range: 23-34 wk). Median GA age for infants without ROP was 31 weeks (range 23-34wk) and for those with any ROP was 29 weeks (range: 23-34 wk) ($p < 0.001$). The median age for infants with Type 1 ROP was 27 weeks (range 23-34 wk) and for type 2 ROP was 30 weeks (range 25-34) ($p=0.002$). Forty percent (25 of 62 infants) of extremely premature infants (≤ 28 GW) had any type of ROP. Thirty-two percent (19 of 59 infants) with ≥ 31 weeks of gestation had any type of ROP.

Distribution of incidence of ROP by gestational age is displayed in table 1.

Distribution of ROP by birth weight

The median overall birth weight (BW) was 1460 g (range: 590-2000 g). Median birth weight of infants without ROP was 1485g (range 590-2000) and median BW of infants with any Type of ROP was 1180 g (range: 630-2000 g) ($p < 0.001$). Median BW with type 1 ROP was 1020 g (range: 630-2000 g) and with type 2 ROP was 1395g (range 700-1920) ($p=0.043$). Forty-six percent (18 of 39 infants) with extremely low birth weight (≤ 1000 g) had any type of ROP. Twenty-nine percent (17 of 59 infants) with > 1500 grams had any type of ROP. Distribution of incidence of ROP by birth weight is displayed in table 2.

The median age at which ROP was detected was 34 weeks post-conception (range, 32 to 37 wk).

Results of univariate analysis of maternal and postnatal factors for ROP are shown in table 3.

Maternal Obstetric Factors

The incidence of maternal preeclampsia ($p=0.693$), multiple births ($p=0.906$), delivery by cesarean section ($p=0.315$), chorioamnionitis ($p=1.0$) and antenatal betamethasone use ($p=0.378$) were not significantly different between infants who developed any stage of ROP and those who had not developed ROP (Table 3).

Clinical characteristics of infants

Gender ($p=0.249$), intraventricular haemorrhage ($p=0.977$), sepsis ($p=0.644$) and blood transfusion ($p=0.907$) and BPD ($p= 0.073$) were not significantly different between infants who developed any stage of ROP and those who had no ROP. Twenty-five percent of the studied infants (62 of 247 infants) were SGA. There was no significant difference in the incidence of ROP between SGA and no SGA infants ($p=0.652$). The odds of developing ROP decrease by 1.7 times at each increase to a higher birth weight category ($p < 0.001$). Infants who developed any-stage ROP were more likely to have gestational age < 31 weeks ($p < 0.001$; OR 2.8), PDA ($p=0.034$; OR=2.5), severe apnea ($p=0.001$; OR=2.6) and 1 minute Apgar score < 6 ($p < 0.01$; OR=2.2) compared with infants without ROP.

Infants with ROP also were more likely to require supplemental oxygen > 14 days ($p < 0.001$; OR=3.2), > 1.5 days of invasive mechanical ventilation (SIPPV) ($p=0.004$; OR=2.4), > 2 days of non invasive ventilation (CPAP) ($p < 0.001$; OR=3) than infants without ROP.

Multiple logistic regression analysis

Results of the multiple logistic regression analysis are shown in table 4.

In these analysis birth weight (OR, 1.65; CI, 1.05-2.6) and SGA (OR, 2.45; CI1.02-5.9) were identified as factors associated with ROP. Independent variables were all those which were in univariate analysis significantly associated with ROP and those that were considered as clinically important independent variables (chorioamnionitis, sepsis, SGA and apnea).

Results of univariate analysis of maternal and postnatal factors for infants with type 1 ROP (Type 1, severe ROP requiring surgery) and type 2 ROP (Type 2, ROP not requiring any surgery) are shown in table 3.

Maternal Obstetric Factors

Seventy-nine percent (16 of 23) of infants with Type 1 ROP were born to mothers with chorioamnionitis, as opposed to 33% (12 of 36) infants with Type 2 ROP. Type 1 ROP was significantly associated with chorioamnionitis ($p=0.008$; OR=4.6). The incidences of maternal preeclampsia ($p=0.063$), multiple births ($p=0.097$), delivery by cesarean section ($p=0.938$), and antenatal betamethasone use ($p=0.894$) were not significantly different between infants who developed type 1 ROP and

Table 1: Distribution of ROP by Gestational Age

Gestational age (wk)	< 26	27-28	29-30	31-32	33-34	Total
N (%)	22	40	59	84	42	247
Any ROP	14(64%)	11(27%)	15(24%)	14(17%)	5 (12%)	59 (24%)
Type2 ROP	4	5	13	10	4	36 (15%)
Type 1 ROP	10	6	2	4	1	23 (9%)

ROP= retinopathy of prematurity; Type 2 ROP= ROP without surgery; Type 1 ROP= ROP with surgery

Table 2: Distribution of ROP by Birth Weight

Birth weight (g)	≤750	751-1000	1001-1250	1251-1500	1501-2000	Total
N (%)	14	25	42	63	103	247
Any ROP	9(64%)	9(36%)	14(33%)	10 (16%)	17 (16%)	59 (24%)
Type 2 ROP	5	3	7	8	13	36 (15%)
Type 1 ROP	4	6	7	2	4	23 (9%)

ROP= retinopathy of prematurity; Type 2 ROP= ROP not requiring surgery; Type 1 ROP= ROP requiring surgery

those who developed Type 2 ROP as is shown in table 5.

Clinical characteristics of infants

Infant gender ($p=0.347$), 1 minute Apgar score ($p=0.225$), IVH $\geq$ gr.2 ($p=0.781$), birth weight groups ($p=0.195$), intrauterine growth ($p=0.715$), blood transfusion ($p=0.907$) severe apnea ($p=0.379$), BPD ($p=0.130$), SIPPV ventilation >3 days ($p=0.373$) and CPAP ventilation >3 days ($p=0.718$), were not significantly associated with increasing the severity of ROP (Table 4). Infants who developed type 1 ROP were more likely to have had gestational age <29 weeks ($p < 0.001$; OR=6.8), sepsis ($p < 0.001$; OR=18), persistent ductus arteriosus ($p < 0.018$; OR=5.9) and prolonged need for oxygen support >29 days ($p < 0.039$; OR=3.2) compared with infants with type 2 ROP.

The odds of ROP 1 compared to non ROP was 4.4 times higher (95% CI: 1.6-12.2) ($p=0.005$) in infants with sepsis than in infants without sepsis. The odds of ROP 1 compared to non ROP was 2.6 times higher (95% CI: 1.02-6.6) ($p=0.045$) in infants from mothers with chorioamnionitis than in those without chorioamnionitis.

Discussion

This is the first population-based study in Croatia that evaluated both the incidence and risk factors of retinopathy of prematurity in a tertiary care unit.

Our results indicate that the overall incidence of any ROP for the entire cohort was 24% with 9% of infants having severe ROP requiring surgery. The incidence and

severity of ROP increased inversely to gestational age and birth weight. The reported incidence of ROP in the past decade has varied worldwide, ranging from 10% in highly developed countries such as Australia and New Zealand to 45.8% in moderately developed countries such as Taiwan [25],[26]. However, the incidence of ROP varies considerably not only in different populations and races but also among countries with similar neonatal intensive care facilities [27] leading to retinopathy of prematurity. Suppression of growth factors due to hyperoxia and loss of the maternal-fetal interaction result in an arrest of retinal vascularisation (phase 1]. Thus, in another Croatian study, ROP at any stage was reported in 23.3% and severe ROP in 8.8 % of infants with GA <32 weeks [19]. Other studies reported a wide variation in the incidence of ROP which might be partly due to differences in the proportions of infants at high risk of ROP who survive when born at an early gestational age [27] leading to retinopathy of prematurity. Suppression of growth factors due to hyperoxia and loss of the maternal-fetal interaction result in an arrest of retinal vascularisation (phase 1],[28],[29].

In our study severe ROP was found in more mature infants with birth weight >1500 grams and gestational age ≥ 31 weeks. The broader range of birth weight and gestational age make our study results more similar to data from moderately developed than to highly developed countries [2000 children are blind from retinopathy of prematurity (ROP)],[30].

The presence of severe ROP in larger, more mature

Table 3: Univariate analysis of risk factors (no ROP v. any ROP)

			ROP			OR	
		Total	No	Yes	P	(95% CI)	p†
		N=247	N=188	N= 59			
N (%)							
Multiple births		78 (32)	59 (31)	19 (32)	0,906		
Cesarean section		162 (66)	127 (68)	35 (59)	0,315		
Chorioamnionitis		116 (47)	88 (47)	28 (47)	1,0		
Preeclampsia		52 (21)	38(20)	14(24)	0,693		
Antenatal steroids		117(47)	92 (49)	25(42)	0,378		
Gender	Male	141 (57)	103 (55)	38 (64)	0,249*		
	Female	106 (43)	85 (45)	21 (36)			
Birth weight (g)	< 1000	37 (15)	19 (10,1)	18 (30,5)	< 0,001*	1,7 (1,3-2,2)	< 0,001
	1001-1250	43 (17,4)	30 (16)	13 (22)			
	1251-1500	60 (24,3)	49 (26,1)	11 (18,6)			
	>=1500 ‡	107 (43,3)	90 (47,9)	17 (28,8)			
Gestational age (wk)	< 31	121 (49)	81 (43)	40 (68)	0,002*	2,8 (1,5-5,1)	0,001
	≥31 ‡	126 (51)	107 (57)	19 (32)			
SGA		62 (25)	49 (26)	13 (22)	0,652*		
APGAR 1min,	< 6	106 (43)	72 (38)	34 (58)	0,014*	2,2 (1,2-4)	0,010
	≥6 ‡	141 (57)	116 (62)	25 (42)			
Severe Apnea		94 (38)	61 (32)	33 (56)	0,002*	2,6 (1,4-4,8)	0,001
CLD		28 (11)	17 (9)	11 (19)	0,073*	2,5(?)	
IVH ≥ gr2		52 (21)	39 (21)	13 (22)	0,977*		
PDA		27 (11)	16 (8,5)	11 (18,6)	0,053*	2,5(1,1-5,6)	0,034
Sepsis		109 (44)	85 (45)	24 (41)	0,644		
Blood transfusion		223 (90)	169 (90)	54 (91)	0,907*		
NCPAP (days)	≤2 ‡	156 (63)	131 (70)	25 (43)	< 0,001		< 0,001
	>2	90 (37)	57 (30)	33 (57)			
SIPPV (days)	≤1,5 ‡	124 (50)	104 (56)	20 (34)	0,006*	2,4(1,3-4,5)	0,004
	>1,5	122 (50)	83 (44)	39 (66)			
Duration of oxygen (days)	≤14 ‡	129 (52)	111 (59)	18 (31)	* < 0,001	3,2(1,7-6)	< 0,001
	>14	117 (48)	77 (41)	40 (69)			

*χ² test; †logistic regression, P < 0.05, significant ‡ level of reference

ROP= retinopathy of prematurity; SGA= Small for gestational age; BPD= Bronchopulmonary dysplasia; IVH= Intraventricular haemorrhage; PDA= patent ductus arteriosus; NCPAP= nasal continuous positive airway pressure; SIPPV: synchronised intermittent positive pressure ventilation; OR = odds ratio; CI = confidence interval

Table 4: Results of multivariate logistic regression analysis (no ROP v. any ROP)

		OR	95% CI	p
Birth weight (g)	< 1000	1,65	1,05-2,6	0,029
	1001-1250			
	1251-1500			
	>=1500**			
SGA	No*	2,45	1,02-5,9	0,045
	Yes			

SGA= small for gestational age; ROP = retinopathy of prematurity; OR = odds ratio; CI = confidence interval.

infants in our study can't be simply explained by the overuse of uncontrolled oxygen delivery. We are aware that neonatal care is compromised as a result of lack of medical resources and skilled personnel mostly due to financial constraints. Croatia is a moderately developed

former socialist economy of southeastern Europe, with a recent experience of war. During the war and the postwar period in Croatia, the share of preterm births increased. However, strict implementation of screening for ROP in NICU Split resulted in maximum yield of ROP diagnosis

Table 5: Univariate analysis of risk factors (Type 1 ROP v. Type 2 ROP)

			ROP			OR	
			TYPE 1	TYPE 2	P	(95% CI)	pt
			N= 23	N=36			
			N(%)				
Multiple births		19 (32)	4 (17)	15 (42)	0,097		
Cesarean section		35 (59)	13 (56)	22 (61)	0,938		
Chorioamnionitis		28 (47)	16 (79)	12 (33)	0,014	4,6 (1,5-14)	0,008
Preeclampsia		14 (24)	2 (9)	12 (33)	0,063		
Antenatal steroids		25 (42)	9 (39)	16 (44)	0,894		
Gender	Male	38 (64)	17 (74)	21 (58)	0,347*		
	Female	21 (36)	6 (26)	15 (42)			
Birth weight (g)	< 1000	18 (30,5)	10 (43,5)	8 (22,2)	0,195*		
	1001-1250	13 (22)	6 (26,1)	7 (19,4)			
	1251-1500	11 (18,6)	3 (13)	8 (22,2)			
	>=1500 ‡	17 (28,8)	4 (17,4)	13 (36,1)			
Gestational age (wk)	< 29	25 (42)	16 (70)	9 (25)	0,002‡	6,8 (2,1-22)	0,001
	≥29 ‡	34 (58)	7 (30)	27 (75)	0,002*		
SGA	Yes	13 (22)	4 (17)	9 (25)	0,715*		
APGAR 1min,	< 6	34 (58)	16 (70)	18 (50)	0,225*		
	≥6 ‡	25 (42)	7 (30)	18 (50)			
Severe Apnoea		33(56)	15 (65)	18 (50)	0,379*		
BPD		11 (18,6)	7 (30)	4 (11)	0,130*		
IVH ≥ gr2		13 (22)	6 (26)	7 (19)	0,781*		
PDA		11 (19)	8 (35)	3 (8)	0,028*	5,9 (1,4-25)	0,018
Sepsis		24 (41)	18 (78)	6 (17)	< 0,001	18 (4,8-67)	< 0,001
Blood transfusion		54 (91)	23 (100)	31 (86)			
NCPAP (days)	≤3 ‡	32 (55)	10 (45)	22 (61)	0,373‡		
	>3	26(45)	12 (55)	14(39)			
SIPPV (days)	≤3 ‡	31 (52)	10 (43)	21 (58)	0,397‡		
	>3	28(48)	13 (57)	15 (42)			
Duration of oxygen	≤29 ‡	30 (52)	8 (35)	22 (63)	0,068	3,2 (1,06-9,5)	0,039
(days)	>29	28 (48)	15 (65)	13 (37)			

*χ² test; †logistic regression, P < 0.05, significant ‡ level of reference

ROP= retinopathy of prematurity; SGA= Small for gestational age; BPD= Bronchopulmonary dysplasia IVH= Intraventricular haemorrhage; PDA= patent ductus arteriosus; NCPAP= nasal continuous positive airway pressure; SIPPV= synchronised intermittent positive pressure ventilation; OR = odds ratio; CI = confidence interval

at a median of 34 weeks postmenstrual age (range, 32 to 37). These findings were in accordance with a number of other reports that showed an age of onset of ROP between 32 and 35 weeks post-conception [6] risk factors and need for surgery for retinopathy of prematurity (ROP), [15].

There have been numerous investigators in the literature who studied the perinatal comorbidity risk factors for ROP. The factors which influenced the development of ROP in these studies have been recognized as connected to ROP in our study as well [6] risk factors and need for surgery for retinopathy of prematurity (ROP), [25], [30], [31]. Using the results of univariate analysis, we identified that gestational age, birth weight, patent ductus arteriosus, 1 minute Apgar score lower than 6, severe apnea, and number of days on invasive, non invasive mechanical ventilation and duration of oxygen therapy, were all associated with an increased risk of ROP. However, multivariate analysis identified that only birth weight and SGA were significant independent risk factors for ROP. Both factors are related to the extent of immaturity of retinal neural and vascular development at birth, and therefore the retinal vulnerability to insult [27] leading to retinopathy of prematurity. Suppression of growth factors due to hyperoxia and loss of the maternal-fetal interaction result in an arrest of retinal vascularisation (phase 1). Results of several studies have suggested that being small for gestational age at birth is associated with an increased risk for retinopathy of prematurity [25], [31], [32]. However, some other findings suggest that SGA increases the risk for retinopathy of prematurity only in infants older than 29 weeks gestational age at birth [33], [34], [35].

We found that severity of respiratory disease, evidenced by prolonged need for oxygen requirement (more than 14 days), need for synchronized intermittent positive pressure ventilation > 1.5 days, and continuous positive pressure ventilation > 2 days had significant impact on developing of any type of ROP ($p=0.004$; $p<0.001$; $p<0.001$ respectively). A number of previous reports clearly demonstrated the causal effects of these factors on the development of ROP even in more mature infants [6], [30], [36], [37]. In our study, infants who developed any ROP also had 1 minute Apgar score lower than 6 ($p=0.010$) and suffered from significantly more episodes of severe apnea than those without ROP ($p=0.002$). They also have statistically significantly higher rate of PDA, compared to those without ROP ($p=0.034$). The first minute Apgar score was detected as a factor that affects the occurrence

of ROP among premature infants in study of Khalesi et al. [38]. Multiple episodes of apneas requiring bag and mask ventilation may produce fluctuations in oxygen concentrations, which have previously been recognized as associated with risk of retinopathy of prematurity [39], [40]. However, there is paucity of literature regarding the effect of PDA, yielding different results. Higgins et al reported that infants with ROP of stage 2 or more were more likely to have RDS, BPD and PDA while Viejo et al., found that presence of PDA does not increase the risk of developing ROP or its severity [41], [42].

In the second part of the study we have expanded our research in order to identify risk factors associated with the development of type 1 ROP. By using univariate logistic regression analyses between the groups with type 1 ($n=23$) and type 2 ($n=36$) ROP, we found that presence of patent ductus arteriosus ($p=0.018$), gestational age lower than 29 weeks ($p=0.001$) and duration of oxygen therapy > 29 days ($p=0.039$) were significantly associated with severe ROP. However, in the multivariate analysis, no significant risk factors were associated with severe ROP. The present results emphasized the association of histologic chorioamnionitis ($p=0.008$) and infant septicemia ($p < 0.001$) with development of severe ROP. It is interesting that these factors were not found as important for predicting development of any type of ROP. But, subsequent univariate analysis between the groups with type 1 ROP ($n=23$) and non-ROP ($n=188$) revealed that infants with sepsis and histologic chorioamnionitis had a higher chance (4.4 and 2.6 more times respectively) of developing severe ROP than to remain without any type of ROP. Some of the studies investigating histologic chorioamnionitis as a risk factor for ROP also reported an association with ROP only in univariate analyses [3], [43]. The increased risk for ROP associated with chorioamnionitis and neonatal infection might be partly due to systemic inflammation, which could act synergistically with hyperoxia to mediate the effects of placenta infection [44], [45].

We acknowledge the limitations of our study. Our screening criteria were more inclusive than the recent American Academy of Pediatrics recommendation for screening at gestational age less than 30 weeks and birth weight less than 1500 g [46]. We are aware that this could have increased the number of unaffected patients and subsequently underestimated the incidence of ROP. However, our study results showed that more mature infants with birth weights > 1500 g and/or GA > 31 weeks developed threshold ROP. These findings confirm that

screening criteria for inclusion of patients should be created to suit the specificities of the region in which they are supposed to be implemented.

The infants who were affected by severe ROP in our study showed a mixture of first and second epidemic risk factors (inadequately monitored oxygen, and the extreme prematurity).

We showed that severe ROP has been diagnosed not only in ELBW, but also in more mature infants, who did not fall within widely acceptable criteria for screening in highly developed countries. The clinical implications of these findings indicate that the improvement of strategies for the treatment of premature infants and associated clinical conditions are needed so that the “third epidemic” can be avoided in the future.

Conflict of Interest Statement

None of the authors have potential conflicts or interest to be disclosed

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Author Contributions

- Mirjana Vucinovic is the leading researcher who designed and executed this study and wrote the manuscript.
- Ljubo Znaor participated in drafting the article and revising it critically.
- Vesna Capkun made a substantial contribution to analysis and interpretation of data.
- Ana Vucinovic made a substantial contribution in drafting and design of the article.
- Julijana Bandic participated in collecting the data and drafting the article.

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