

## GENETIC POLYMORPHISMS AND RETINOPATHY OF PREMATURITY

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### Abstract

Retinopathy of Prematurity (ROP) represents a major health issue in the modern society, being the main cause for blinding in children all over the world.

We analyzed the results of a retrospective study performed on a 6-year period (2009-2014), within the Neonatal Unit of the County Emergency Hospital from Timisoara. We assessed 1948 premature infants with GA < 35 weeks and BW < 1500 grams (GA = gestational age; BW = birth weight). Incidence of retinopathy in the study group was 48.55% (945 infants). Laser therapy was applied to 155 of these (7.95%). The disease evolution was favourable in 143 infants (86.5%). The unfavourable evolution in one eye, yet favourable in the other eye, was noticed in 14 of the assessed children (9%), and the severe loss of the visual acuity / blindness was noted in 7 cases (4.5%).

The screening of ROP is essential for a timely diagnosis, allowing the application of a therapy at the right moment. The examination is performed by the ophthalmologist, upon request from the neonatologist. Finding an alternative screening method (telemedicine, the study of genetic polymorphisms), which is much easier to perform, would allow a more efficient selection of infants at a risk for severe ROP.

**Key words:** retinopathy of prematurity (ROP), screening, genetic polymorphisms

### Introduction

Retinopathy of prematurity (ROP) is a proliferative disease that affects retinal vasculature in the eyes of premature babies. It can lead to severe visual impairment or even to blindness. The quality improvement in the medical care of premature infants has allowed the survival of children with extremely low gestational age and birth weight. However, because of premature birth, the normal development of retinal vasculature (process starting in the 16th week of pregnancy) stops. The later fibrovascular proliferation results in haemorrhages, traction on the retina and, finally, retinal detachment [1].

The international classification of the retinopathy of prematurity was established in 1984, when the disease

was described as an active process focused on two aspects: staging and localization [2].

Thus, 5 stages of the disease were described:

- stage 1 (demarcation line): a thin, well-defined, silvery-white structure, present in the retina; this structure separates the posterior vascular retina from the anterior avascular retina;
- stage 2 (ridge): the line in stage 1 engorges both in height and in width and it occupies an expanded volume outside the plane of the retina; neovascularization may be present in the posterior area of the retina.
- stage 3 (fibrovascular ridge): extraretinal fibrovascular proliferation (neovascularization) adds to the anterior aspect and retinal haemorrhage may be often present;
- stage 4: partial retinal detachment, with or without the involvement of the macular area;
- stage 5: complete retinal detachment;

The severe tortuosity of blood vessels indicates an apparent vascular incompetence. This is described as "plus disease", with the following features: arterial and venous engorgement of the posterior pole, iris vascular engorgement, vitreous haze and pupil rigidity. In the posterior aggressive form of the ROP, although there are small vascular changes at the edges of the retina, the vascular tortuosity is important, the developed vascular shunts are numerous and the disease evolves fast towards stage 5.

The disease location is done by dividing the retina into three zones:

- zone 1: it is the zone centred on the optic nerve, the radius of which extends twice the distance from the optic nerve to the centre of the macula;
- zone 2: the circular zone surrounding zone 1, with the radius equal to the distance from the optic nerve to the nasal ora serrata;
- zone 3: represents the residual temporal crescent of the retina, outside zone 2.

The circumferential extent of the disease is described in 12 equal segments, similar to the hours on the face of a clock (their sum represents the extent of the disease).

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The main risk factor for the development of retinopathy of prematurity is, first of all, the premature birth. The earlier an infant is born and the less birth weight it has, the higher the possibility to develop this disease later in life. Other factors include: oxygen therapy, mechanical ventilation, sepsis, anaemia, blood transfusion, respiratory distress, hypoxia, apnoea, pulmonary interstitial emphysema, bronchopulmonary dysplasia, surfactant administration, vitamin E deficit, patent ductus arteriosus, indomethacin administration, acidosis, bradycardia, hypotension, intraventricular haemorrhage, ulcerative hemorrhagic enterocolitis, belonging to the white race etc. The newborns with systemic conditions and an unstable clinical evolution have a higher probability of developing severe forms of the disease.

The most efficient treatment for these cases is the laser therapy. This is applied in the severe forms of the disease (stage 3, threshold/pre-threshold, or the posterior aggressive form). In stages 4 and 5, the laser therapy is applied at the same time with vitrectomy surgery.

### Objectives

In this paper, we analyzed the results of a retrospective study performed on a 6-year period (2009-2014). We have assessed the incidence of severe forms of retinopathy of prematurity in infants belonging to a risk group from the western part of Romania (screening and laser therapy). The authors have analyzed the opportunity of improving the means for diagnostic and monitoring of the ROP, by the study of the VEGF (vascular endothelial growth factor) genetic polymorphisms, starting from the hypothesis that the genetic polymorphisms can be associated to the disease severity and development. Last but not least, we followed the correlation between the precocious visual stimulation and the functional result in the treated children from the study group.

### Methods and material

During 2009-2014 (including September 2014), within the Neonatal Unit of the County Emergency Hospital from Timisoara, we assessed 1948 premature infants and we applied laser therapy to 155 of these (Table 1).

Table 1. Distribution per years of the assessed and treated infants.

year	No. of assessed children	No. of children where laser therapy was applied
2009	322	15
2010	292	21
2011	340	37
2012	355	33
2013	366	26
2014 including September	273	23

The inclusion criteria in the study group are as follows:

- history of premature birth (GA < 35 weeks and BW < 1500 grams),
- unstable clinical evolution,
- associated risk factors (regardless of GA and BW),
- patients with a history of ROP transferred to our clinic for monitoring.

Exclusion criteria:

- full-term birth,
- existing opacities in the visual axis (e.g., congenital cataract),

- presence of major eye malformations (e.g. anophthalmia)

The first examination (screening start) was done at 4-6 weeks of chronologic age (post-birth) or at 31-33 weeks of post-conceptual age (post-menstrual age), and the classification was done in agreement with the ICROP (localization, disease expansion and development stages). The interval between 2 successive examinations varied between a few days and three weeks (Table 2). The screening is ended when the visualization of the retina is complete, the retinopathy of prematurity is completely in remission or when the laser therapy is applied.

Table 2. The rhythm of examination of premature infants.

Time period	Disease stage
a few days	- stage 1, in zone 1 - stage 2, in zone 1 - stage 3 in zone 2 with/without PD which does not yet require ablation
after one week	- zone 1 without ROP, but with immature vascularisation - stage 2, in zone 2
after 2 weeks	- stage 1, in zone 2 - ROP in remission from zone 2
after 3 weeks	- zone 2 without ROP, but with immature vascularisation - stage 1, in zone 3 - stage 2, in zone 3 - ROP in remission from zone 3

The examination was performed by an ophthalmologist, experienced in the management of this disease in the premature infant, by indirect ophthalmoscopy. The examination was performed one hour after feeding, in mydriasis induced with (cyclopentolate 0.5%, tropicamide 0.5% or phenylephrine 2.5%). For topical anaesthesia, propacaine drops 0.5% were administered. A blepharostat, scleral depressor and a 28-diopter lens were used.

The laser therapy performed in the Neonatal Unit was applied in severe cases of the disease (stage 3 in zone 1 or 2 for 5 continuous or 8 cumulated clock hours, with or without PD or aggressive posterior forms). This was performed within the first 48-72 hours from the diagnostic, in order to reduce neovascularization and retina damage, and prevent retina detachment and the severe loss of the visual acuity. The indirect ophthalmoscope diode laser (810 nm) was used. Peripheral retinal ablation anterior to the fibrovascular ridge of ROP was done using scatter pattern photocoagulation, with impulses placed one half width apart, resulting in moderate white laser lesions on the entire peripheral avascular retina. The set laser power varied between 150 and 500 mW, and the exposure time was of 0.2 seconds. The number of impulses placed varied between 400 and 200 for a treatment session.

The results were assessed one week from the treatment session. Disease remission involves the disappearance of fibrovascular proliferation and the vascularisation of the retina beyond the ridge, in the previously avascular area. The complete involution is defined as the absence of the active neovascular tissue, absence of vessel engorgement and tortuosity at the level of the posterior pole and absence of retina detachment [3]. The unfavourable results consist in the disease progression and the occurrence of retina detachment.

Functional results were assessed at the age of 9 to 12 months. The Cardiff Acuity tests were used. The refraction was measured with a paediatric auto-refractometer. The amblyopia and anisometropia treatment was started (where applicable), and the refraction errors (hypermetropia, myopia, astigmatism) were corrected.

Early visual stimulation in children where laser therapy was applied improves the retina functional response. This is done by introducing the child in an environment rich in visual stimuli of different sizes and intensities (visual stimulation activities using various light and non-light stimuli, e.g. torches, toys with/without lights, colour stimuli), presented in a variety of situations, provided by: the luminosity of the environment, the distance between the object and the child, presentation position, background). The child's responses were followed and recorded. The choice of visual stimuli was done according to the remaining visual function and the child's functional level, as well in consideration of any neurological conditions (e.g. epilepsy); the increase in the attention span was done by changing stimuli within the same activity; black light activities or the use of visual stimulation software may be alternatives to the moving objects following activities (vertical/horizontal movement, or within the near or farther visual field).

**Results**

The incidence of retinopathy within the study group (regardless of stage) was of 48.55% (945 infants). The laser therapy was applied in 155 of the infants (7.95%). The distribution on years of the assessed/treated children is presented in Figure 1.

The infants included in the study were weighed less than 2,000 grams at birth and their gestational age was under 35 weeks. Premature infants not complying with these criteria were also assessed, when the neonatologist deemed this examination necessary due to the unstable clinical evolution of the infants and the multiple associated risk factors.

When therapy was recommended, the disease stages were as follows: stage 3 in 112 infants (72%) - in zone 1, there were 14 cases, while in zone 2 there were 98 cases; stage 4 in 6 infants (4%) and the aggressive posterior form of the disease in 37 infants (24%) (Figure 2). The children were 37 weeks old on average when the laser therapy was applied.

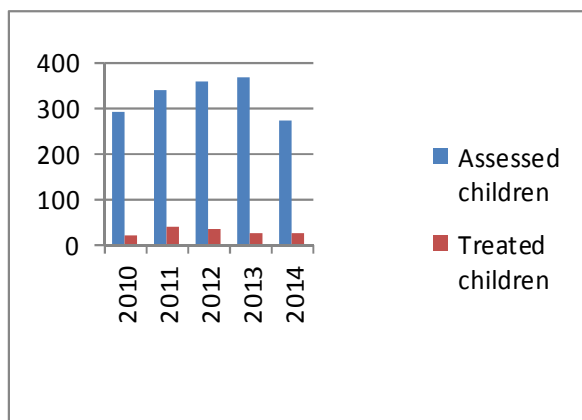


Figure 1. The distribution of the assessed and treated children according to the year of study.

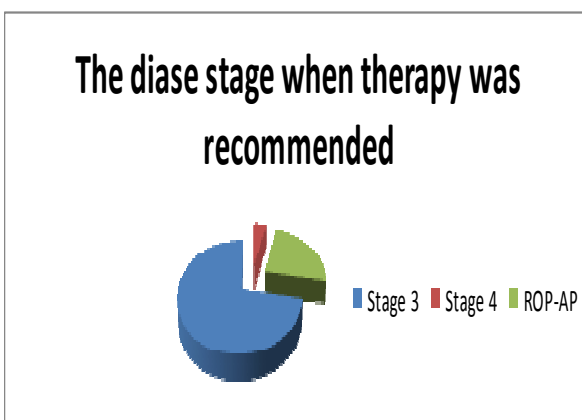


Figure 2. The disease stage when therapy was recommended.

Disease remission and the disappearance of extraretinal fibrovascular proliferation was noted in 143 infants (86.5%). Unfavourable evolution, with fibrous retinal tractions and retinal detachment in one eye, yet favourable in the other eye, was seen in 14 of the assessed children (9%). Unfavourable evolution, with bilateral retinal detachment and loss of visual acuity / blindness was seen in 7 cases (4.5%).

We have studied the connection between the child's age when laser therapy was applied and the favourable post-surgery evolution (Figure 3). There is no close connection between the child's age upon therapy recommendation and the favourable post-surgery evolution, although we would have expected this correlation to be a strong one, given the fact that retina vascularisation is a process which continues after premature birth until week 45 (post-corrected age).

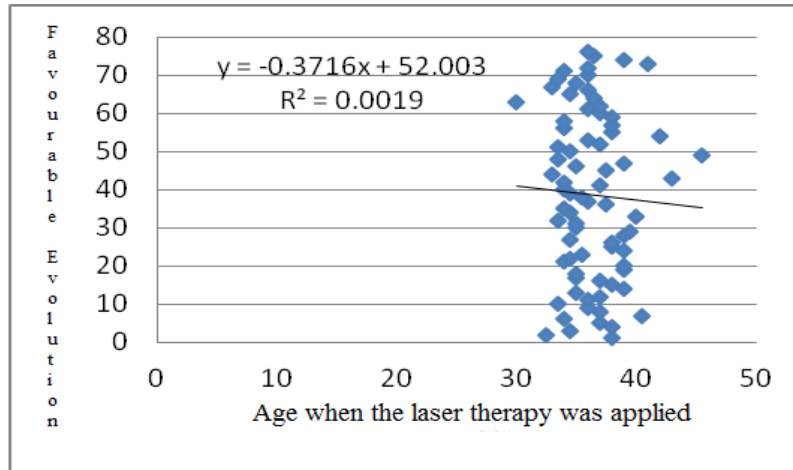


Figure 3. Correlation between the favourable post-surgery evolution and the age when the laser therapy was applied.

On the other hand, the correlation is positive and very significant from the statistical point between the favourable post-surgery evolution and stage 3 of the disease at the moment the laser therapy was applied (Figure 4);

there is also a positive correlation between the unfavourable post-surgery evolution and the presence of the posterior aggressive form of the disease when the laser therapy was applied (Figure 5).

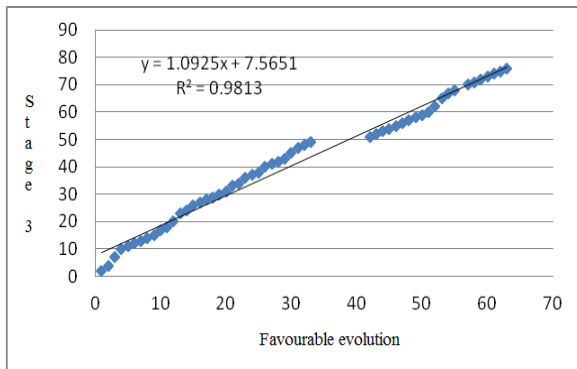


Figure 4. The correlation between the disease stage when the laser therapy was applied and the favourable post-surgery evolution.

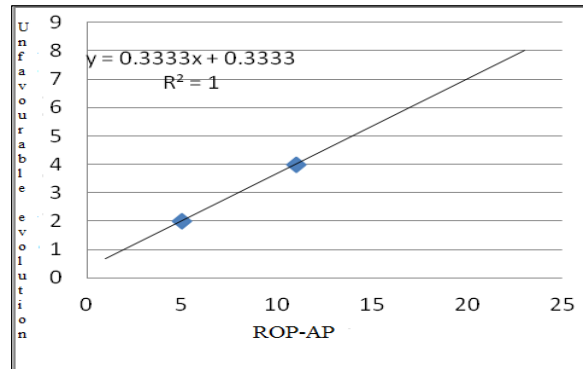


Figure 5. The correlation between the unfavourable post-surgery evolution and the posterior aggressive form of the disease when laser therapy was applied.

**Discussions:**

The results obtained are similar to the ones reported in the relevant scientific literature. In the United States, in a study performed in 2009, retinopathy of prematurity was diagnosed in 68% of the premature infants weighing less than 1250 grams at birth [4]. In the United Kingdom, the

reported incidence of retinopathy of prematurity varied between 66 and 68% in infants with less than 1251 grams at birth [5].

The inclusion criteria in our study were more permissive (with reference to the guidelines established in the program for national screening and laser therapy of

retinopathy of prematurity), the considered weight at birth being under 2000 grams.

The incidence of ROP will remain high, although the screening and therapy conditions have improved due to the increase in the number of premature births and the quality improvement in the medical care of premature infants, allowing more extremely premature infants to survive.

A correctly performed screening allows the timely identification of severe forms of the disease. This is very important, in order to have a favourable post-therapy evolution. Screening by indirect ophthalmoscopy is difficult, as it needs to be performed by an experienced physician. Such physicians can be found only in the large medical centres. Repeated examinations (as clinical signs vary from one examination to the other) are difficult to perform on infants being born in the areas surrounding big cities, as such examinations require the transportation of the fragile infant to the place where the screening takes place. In these conditions, a viable alternative to the screening by indirect ophthalmoscopy needs to be found:

- tele-medicine allows a non-specialist to take images of a child using the RetCam, regardless of the place the infant is located, and the transmission of such images in order to be interpreted by a specialist, who will then establish the monitoring and therapy strategy to be applied [6,7];

- the study of genetic polymorphisms;

It has been proved that there is a high genetic susceptibility in case of retinopathy of prematurity [8]. There are high levels of VEGF in the hypoxia affected retina. This has an important role in the development of retinopathy of prematurity. The gene for VEGF (VEGFA, ID: 7422) is localized on the chromosome 6q21.3 and it contains 8 exons and 7 introns. The genetic polymorphism of VEGF may influence the progress of retinopathy of prematurity. An option is to follow the correlation between the genetic polymorphisms of VEGF and the retinopathy of prematurity, by selecting the VEGF polymorphisms which, based on the studies performed, proved to have major influences in the development and severity of this disease [9, 10, 11].

Finding other screening alternatives which are much easier to perform (such as DNA-extraction from the oral mucosa by using a swab, or the DNA extraction from the umbilical chord blood), and the transportation of samples to the laboratory, without the need to move a fragile infant (often in poor health condition, under mechanical

ventilation or oxygen-dependent) to the centres for ophthalmologic screening, would allow a more efficient selection of infants with risk of a severe form of retinopathy of prematurity and the timely application of therapy (the retinopathy of prematurity in stage 3 requires emergency treatment to be applied within 48 to 72 hours from diagnosis). An important aspect to be mentioned is the fact that the results of genetic screening for VEGF polymorphisms can be obtained in the first day after birth allowing a rapid identification of the infants that will need laser therapy.

The visual stimulation program needs to start with the stimuli tolerated by the child and it needs to be enjoyable, so that the child is motivated to actively participate. The intensity and variety of the stimuli will be increased as soon as the child tolerates such changes, one step at a time. The child needs to be allowed to control the duration and intensity of the stimuli. Visual stimulation in a child with retinopathy of prematurity is precise work because, until the child learns to accept the sensory input, sensory over-stimulation is as harmful as lack of stimulation. Although it is important to systematically expose the child to various stimuli, one needs to use the activities motivating children to use their remaining sight and to integrate information into new contexts.

Early visual stimulation results in the development of visual perception in children with retinopathy of prematurity. More significant functional progresses were noted in the children who were included in the program from early age (first months of life).

### Conclusions

Screening of retinopathy of prematurity is justified by the large number of premature infants and by the presence of multiple associated risk factors. Retinopathy of prematurity remains an important cause of severe visual impairment and blindness in children.

Although the identification and study of genetic polymorphisms offers an alternative to the screening performed by the ophthalmologist, the studies up to now have been mostly done on Asian populations. Based on our knowledge, there has not been such a study performed on a risk group from Romania. Therefore, we believe it would be very useful to identify the specific polymorphisms and to use them in the screening stage of the retinopathy of prematurity.

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