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Research Article

Fractal and lacunarity analysis of human retinal vessel arborisation in normal and amblyopic eyes

[1]**Ştefan Ţălu**, [2]**Cristina Vlăduţiu**, [3]**Livia A. Popescu**, [4]**Carmen A. Lupaşcu**, [5]**Ştefan C. Vesa**, [2]**Simona D. Ţălu**

[1] The Technical University of Cluj-Napoca, Faculty of Mechanical Engineering, Department of AET, Discipline of Descriptive Geometry and Engineering Graphics, Cluj-Napoca, Romania; [2] "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Medicine, Department of Surgical Specialties and Medical Imaging, Discipline of Ophthalmology, Cluj-Napoca, Romania; [3] County Emergency Hospital Cluj-Napoca, Ophthalmology Department, Cluj-Napoca, Romania; [4] Università degli Studi di Palermo, Dipartimento di Matematica e Informatica, Palermo, Italy; [5] "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Medicine, Department of Pharmacology, Cluj-Napoca, Romania.

Abstract. Objective: The purpose of this paper is to determine a global assessment of the human retinal vascular network for patients with amblyopia. Fractal geometry and lacunarity parameters are used in this study. Materials and methods: A set of 12 segmented and skeletonized human retinal images, corresponding to both normal (6 images) and amblyopia states of the retina (6 images), was analyzed using the Image J software with box-counting method. Statistical analyses were performed for these groups using Microsoft Office Excel 2003 and GraphPad InStat software. Results: The human retinal vascular network architecture can be estimated using the fractal geometry. The average of fractal dimensions D for the amblyopia images (segmented and skeletonized versions) is slightly higher than the corresponding values for normal images (segmented and skeletonized versions). However, the average of lacunarity parameter Λ for the amblyopia images (segmented and skeletonized versions) is slightly lower than the corresponding values for normal images (segmented and skeletonized versions). Conclusions: The fractal and lacunarity analysis may be used for an early diagnosis of patients with amblyopia.

Key Words: retina, retinal vessels, amblyopia, strabismus, fractal dimension

Corresponding Author: S. Ţălu, stefan_ta@yahoo.com

Introduction

Amblyopia occurs in approximately 2 % of the general population and is the most common cause of decreased vision in childhood. Amblyopia is caused by abnormal visual stimulation during visual development, resulting in abnormalities in the visual centers of the brain. Children are susceptible to amblyopia between birth and 7 years old (Wright et al 2006).

Investigating the human retinal vascular network, as a part of an in-vivo analysis, is helpful in detection and diagnose of retinal disorders (Ţălu 2005a; Ţălu 2005b; Ryan et al 2006; ; Holz & Spaide 2010; Ţălu et al 2009; Ţălu 2011a; Ţălu et al 2011; Ţălu & Ţălu 2012; Ţălu 2013a).

The human retinal vascular network has a branching pattern and it is considered to be a fractal structure at low resolution, in a "scaling window" which normally ranges in two to three orders of magnitude (Kyriacos et al 1997; Losa et al 2005). It can be observed in its natural living state using a retinal camera (Masters 2004).

The fractal and multifractal analysis of human retinal vascular network depends on the experimental and methodological parameters involved as: diversity of subjects, image acquisition, type of image, image processing, fractal methods, including the algorithm and specific calculation used etc. (Kyriacos et al 1997; Mendonça et al 2007; Ţălu 2011b; Ţălu & Giovanzana 2011; Fraz et al 2012; Ţălu & Giovanzana 2012; Ţălu 2012a; Ţălu 2012b; Ţălu et al 2012; Ţălu 2012c; Ţălu 2012d; Ţălu et al 2013; Ţălu 2013b; Ţălu & Ţălu 2013).

Material and methods

Fractal dimension

The fractal analysis of fundus photographs may allow a quantitative measure of complexity of the retinal vessel branching. In fractal analysis, box-counting is one of the most widely used and practical methods to determine the fractal dimension of a figure (Falconer 2003; Lopes & Betrouni 2009; Napolitano et al 2012). Let's consider a fractal object recorded into a digital image. Let A be any nonempty bounded subset of R^n (n-dimensional Euclidian space R^n). The fractal dimension gives the scaling between the smallest number of n-dimensional ε boxes needed to cover the set *A* completely, and the boxes' size *ε*. The box-counting dimension of *A* is expressed by (Falconer 2003);

$$D_B(A) = \lim_{\varepsilon \to 0} \frac{\log N_\varepsilon(A)}{\log(1/\varepsilon)} \quad (1)$$

In equation (1) the zero limit cannot be applied to biological images and $D_B(A)$ can be estimated by means of next equation (Grizzi et al 2005):

$$D_B(A) = D \quad (2)$$

where *D* is the slope of the regression line for the log-log plot of the scanning box size and the count from a box counting scan. The "count" usually refers to the number of grid boxes that contained pixels in a box counting scan. The slope of the linear region of the plot is (-*D*), where *D* is the box-counting dimension that corresponds to the fractal dimension.
The fractal dimension *D* of retinal vascular network quantifies the global measure of complexity of the vascular branching pattern (Ţălu 2011; Ţălu & Giovanzana 2011; Ţălu & Giovanzana 2012; Ţălu 2012a; Ţălu 2012b; Ţălu et al 2012).
(Kyriacos et al 1997; Masters 2004; Ţălu 2011b; Ţălu & Giovanzana 2012). Generally, arterioles have a lower fractal dimension than venules (Patton et al 2006).
Some investigators (Azemin et al 2012) observed a significant decrease in the fractal dimensions of human retinal vascular network with aging, consistent with observations from other human organ systems.
Investigators have also found different values in the fractal dimensions associated with the retinal pathological status (Avakian et al 2002; Lakshminanarayanan et al 2003; Kunicki et al 2009; Liberek et al 2010; Olujić et al 2011; Ţălu et al 2012).

Lacunarity analysis
Lacunarity analysis is a multiscale method for describing patterns of spatial dispersion. It can be considered a scale-dependent measure of heterogeneity or texture of an object, whether or not it is a fractal. Lacunarity measures the deviation of a geometric structure from translational invariance, or gappiness of geometric structure. It can be used with both binary and quantitative data in one, two, and three dimensions (Plotnick et al 1996; Ţălu & Giovanzana 2011; Ţălu & Giovanzana 2012; Ţălu 2012a; Ţălu 2012b).
Lacunarity can be evaluated both for fractal and non-fractal sets. Lacunarity may characterize different texture appearances, which may have the same fractal dimension value. Lacunarity is complementary to the fractal dimension, as shapes with the same fractal dimension may have different lacunarity values. If the fractal object is dense the lacunarity is small, the lacunarity increases with coarseness (Ţălu & Giovanzana 2011; Ţălu & Giovanzana 2012; Ţălu 2012a; Ţălu 2012b).
Various algorithms have been proposed to calculate and quantify lacunarity of an image. Among them, the gliding-box algorithm developed by Allain and Cloitre (1991) is one of the most widely used.
The lacunarity λ was evaluated using FracLac. The mean lacunarity (or *Λ*) was expressed as (Image J software; FracLac V 2.0f for Image J software):

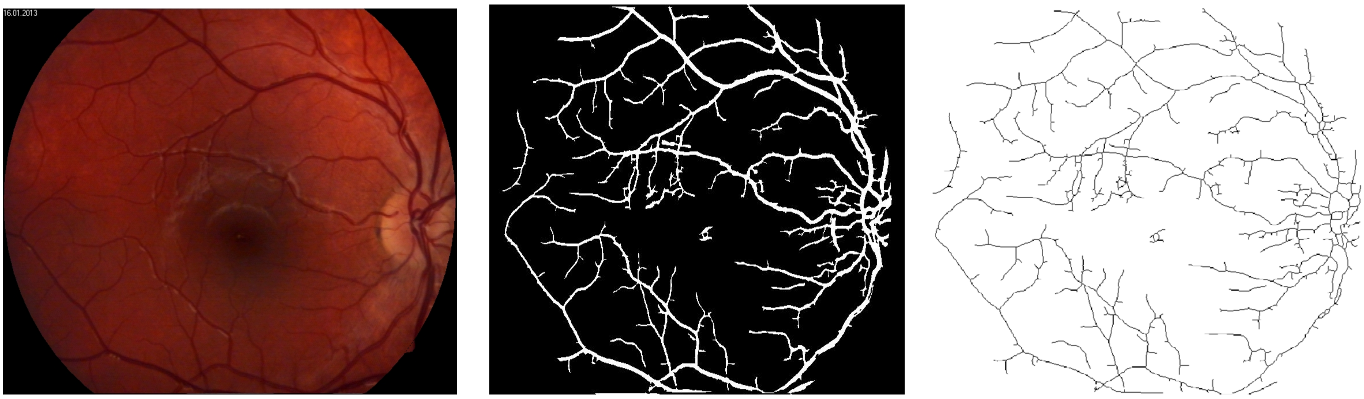
$$\Lambda = \left(\sum \left[1 + (\sigma/\mu)^2\right]\right)/n \quad (3)$$

where σ is the standard deviation; μ is the mean for pixels per box at this size, ε, in a box count at this orientation, g; and n is the number of box sizes.

Segmentation method
An automatic unsupervised method for the segmentation of retinal vessels was applied before fractal analysis. Finally the segmented images were post-processed, by eliminating small connected components in order to remove noisy pixels and to improve in this way the accuracy of the segmentation (Lupaşcu et al 2009; Lupaşcu et al 2010; Lupaşcu & Tegolo 2011).

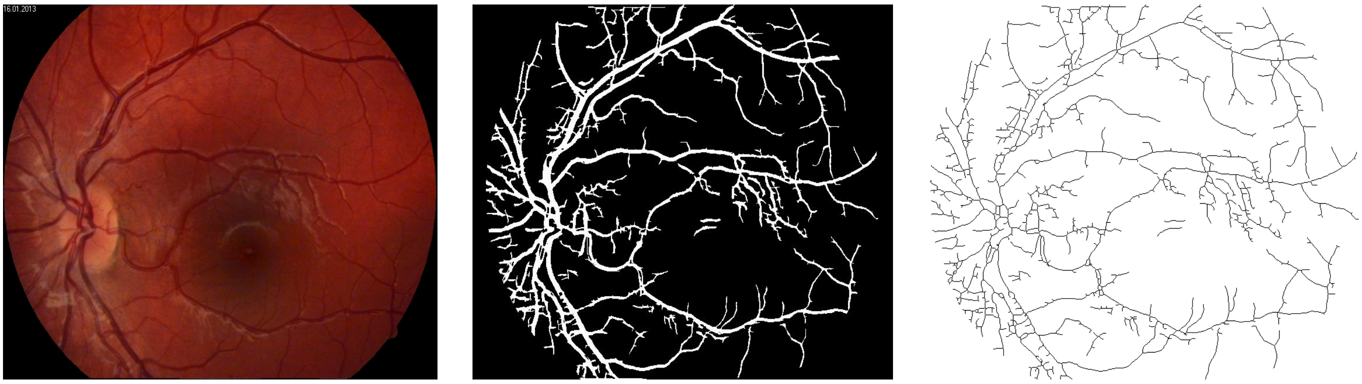
Fractal and lacunarity analysis
This study was conducted according to the recommendations of the Declaration of Helsinki for research in human subjects. The protocol was approved by the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca Romania. The child's parents gave informed written consent to participate.
Let us consider a set of 12 segmented and skeletonized human retinal images, corresponding to both normal (6 images) and amblyopia states of the retina (6 images), randomly selected from amblyopic children treated in the Ophthalmological Clinic in Cluj-Napoca, Romania. Six patients (50 %) were male and six (50 %) female. The slides were captured by a fundus camera at 45° field of view (VISUCAM^{Lite} Zeiss; Carl Zeiss Meditec AG 07740 Jena 2008, Germany).
After adjustments of every digital image, the segmented version of retinal vessel structure contains the vessel silhouettes extracted from the fundus photographs.
The binary skeletal structure (8-bit) of retinal vessel structure was obtained by mathematical morphologic processing from the original micrograph images with one single pixel in width, but without any change in relative location and configuration of each element. This procedure was applied for all analyzed structures. (Deserno 2011).
Figures 1 & 2 show the images of a normal and pathological state retinal vessel structure.
Fractal, and lacunarity analysis was performed in two cases: a) on the segmented images (seg.) and (b) on the skeletonized (skl.) version.
Fractal analyses were computed applying the standard box-counting algorithm to the digitized data, using the Image J software package (Wayne Rasband, National Institutes of Health, in Bethesda, Maryland, USA) (Image J software) together with the FracLac plug-in (A. Karperien - Charles Sturt University, Australia) (FracLac V 2.0f for Image J software). The algorithm for fractal analysis was applied with the following options: a) Grid positions - 10; b) Calculating of grid calibers - use default box sizes. The range of box sizes used for the fractal dimension calculation was: 2 pixels (the minimum box size) and 45 % from region of interest (the maximum box size).

Statistical analysis
Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) statistical functions were used to determine



a) b) c)

Fig. 1. Image of a normal state retinal vessel network, right eye (file 1.tif): (a) the color image version, (b) the segmented version and (c) the skeletonized version.



a) b) c)

Fig. 2. Image of an amblyopia state retinal vessel network, left eye (file 2.tif): (a) the color image version, (b) the segmented version and (c) the skeletonized version.

(average ± standard deviation) of the fractal dimensions *D* and lacunarity parameters *Λ* obtained with Image J software.
The statistical processing of the results obtained with Image J software (Table 1) was done using GraphPad InStat software program, version 3.20 (GraphPad, San Diego, CA, USA).
Normal distribution of variables was previously assessed by means of the Kolmogorov-Smirnov test. It was found that fractal dimensions and lacunarity parameters *Λ* of the vascular trees followed a normal distribution. An analysis of variance (ANOVA) was performed to see whether significant differences were found between different diseased states. Differences with a *P* value of 0.05 or less were considered statistically significant. The average results were expressed as mean value and standard deviation.

Results

Results of fractal and lacunarity analysis

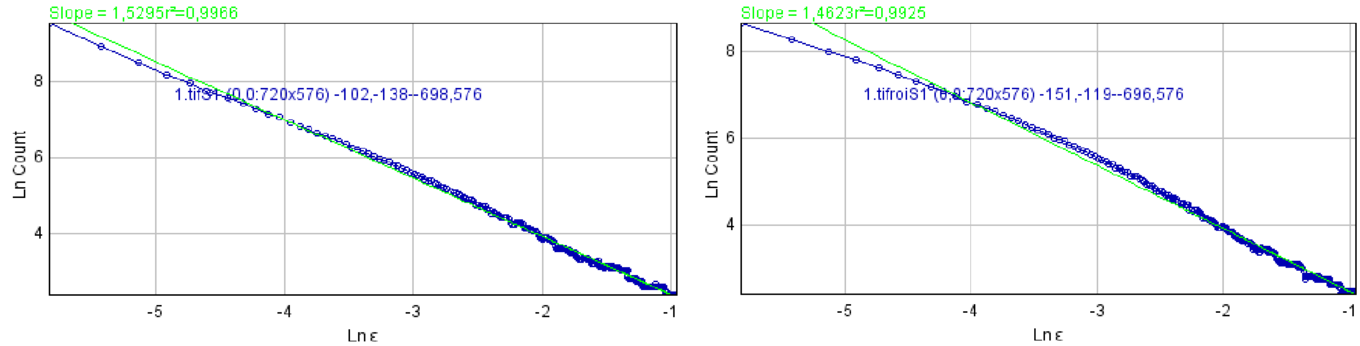
For all analyzed cases (Table 1), the coefficients of correlation (R^2) associated with fractal dimensions *D* were greater than 0.9925 representing a good linear correlation. An (R^2) of 1.0 indicates that the regression line perfectly fits the data.
In Figs. 3-4 are shown the results of fractal analyses for retinal digital image (file 1.tif – normal status) analyzed with the Image J software, in segmented and skeletonised variants.
In Figs. 5-6 are shown the results of fractal analyses for retinal digital image (file 2.tif – pathological status) analyzed with the Image J software, in segmented and skeletonised variants.

Table 1. The fractal dimensions and lacunarity parameter *Λ* with average ± standard deviation, of 12 analyzed images, for normal (N) and pathological (P) status, in segmented and skeletonized variants.

Status	Type	Value of fractal dimension (D)	Lacunarity parameter Λ
N	Seg.	1.5295 ± 0.0240	0.6766 ± 0.1136
N	Skl.	1.4544 ± 0.0407	0.5820 ± 0.0928
P	Seg.	1.6132 ± 0.0539	0.5069 ± 0.0659
P	Skl.	1.5380 ± 0.0671	0.4348 ± 0.0319

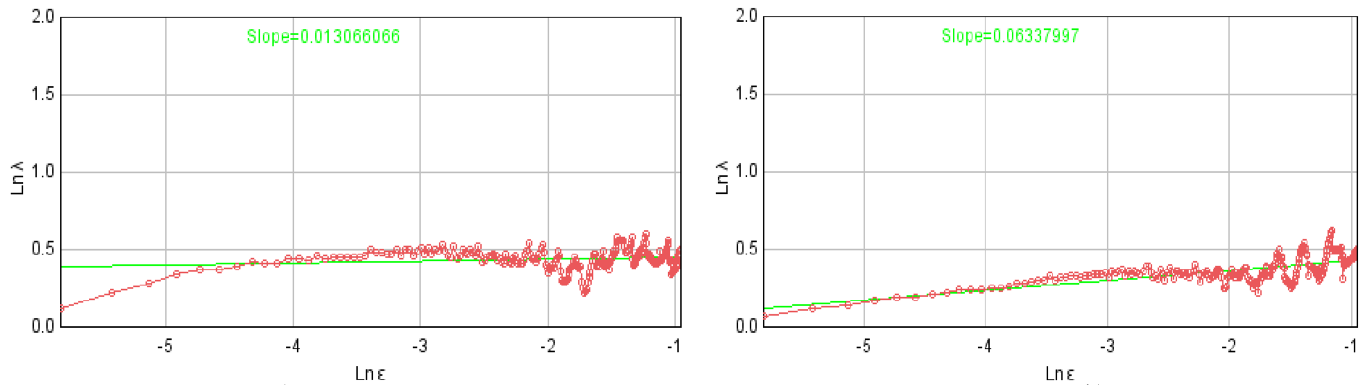
Discussion

The retinal diseases modify the human retinal vessel arborisation. The complex-dynamical fractal structure development of human retinal vascular network architecture can be estimated using the fractal geometry. The fractal dimension (as a quantitative estimator of the spatial complexity) and lacunarity parameter may be markers of subtle changes in retinal vascular architecture. The average of fractal dimensions *D* for the amblyopia



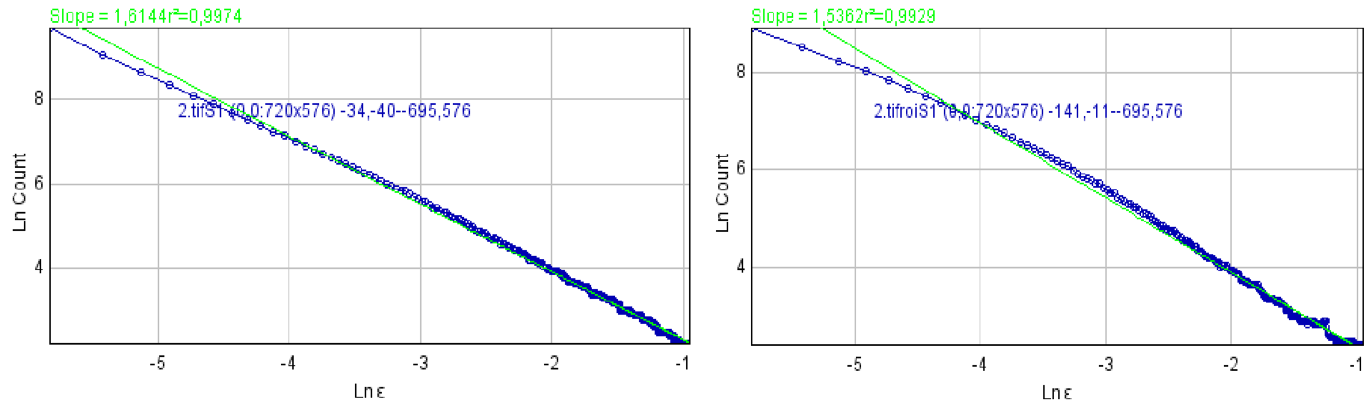
a) b)

Fig. 3. The Ln(count) versus Ln(ε) (file 1.tif): (a) the segmented version and (b) the skeletonised version.



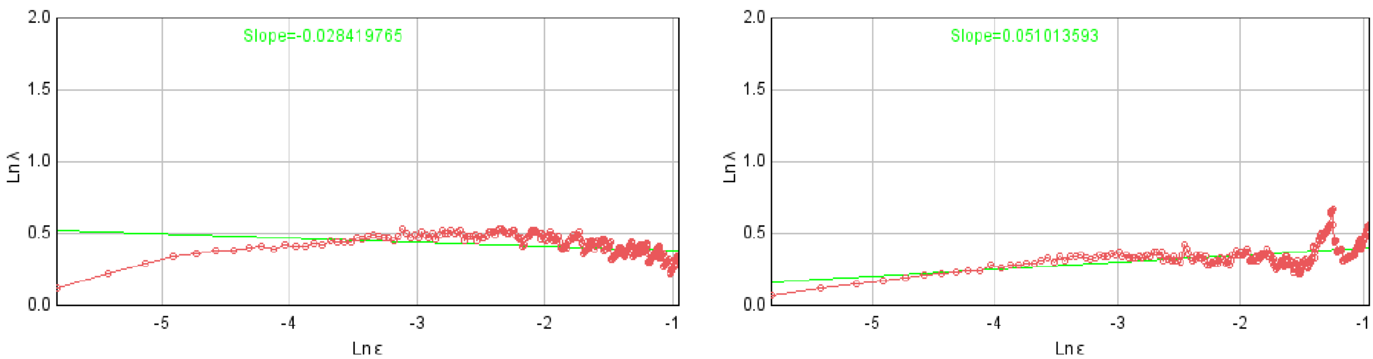
a) b)

Fig. 4. The Ln(λ) versus Ln(ε) (file 1.tif): (a) the segmented version and (b) the skeletonised version.



a) b)

Fig. 5. The Ln(count) versus Ln(ε) (file 2.tif): (a) the segmented version and (b) the skeletonised version.



a) b)

Fig. 6. The Ln(λ) versus Ln(ε) (file 2.tif): (a) the segmented version and (b) the skeletonised version.

images (segmented and skeletonized versions) is slightly higher than the corresponding values for normal images (segmented and skeletonized versions). However, the average of lacunarity parameter Λ for the amblyopia images (segmented and skeletonized versions) is slightly lower than the corresponding values for normal images (segmented and skeletonized versions). This statistical significance suggests a different complex morphology of the human retinal vascular network architecture in two evaluated groups.
A good correlation between the appearance of the retinal vessel structures and the interpretations of fractal and lacunarity descriptors were found.

Conclusions

The potential for using non-invasive clinical assessment of the human retinal microvasculature offers clear advantages. The fractal and lacunarity analysis may be used for detecting and quantifying of patients with amblyopia, for comparing its multifarious retinal architecture under physiological and diseased conditions. These results confirm the usefulness of the described methods for retinal image analysis and processing in medical practice.
This study confirms the results of fractal analysis determined by Liberek et. al, where the fractal dimensions of fundus eye images corresponding to the strabismus states of the retina have higher average values in comparison with the corresponding values of the normal cases.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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Authors

•Ştefan Ţălu, Technical University of Cluj-Napoca, Faculty of Mechanical Engineering, Department of AET, Discipline of Descriptive Geometry and Engineering Graphics, 103-105th B-dul Muncii St., 400641, Cluj-Napoca, Cluj, Romania, e-mail: stefan_ta@yahoo.com

•Cristina Vlăduţiu, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Medicine, Department of Surgical Specialties and Medical Imaging, Discipline of Ophthalmology, 8th Victor Babeş St., 400012, Cluj-Napoca, Cluj, Romania, e-mail: cristivladutiu@yahoo.com

•Livia Alina Popescu, County Emergency Hospital Cluj-Napoca, Ophthalmology Department, 3-5 Clinicilor St., Cluj-Napoca 400006, Romania.

•Carmen Alina Lupaşcu, Università degli Studi di Palermo, Dipartimento di Matematica e Informatica, Via Archirafi, 34, Palermo 90123, Italy, e-mail: lupascu@math.unipa.it

•Ştefan Cristian Vesa, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Medicine, Department of Pharmacology, 6 Pasteur St., Cluj-Napoca 400349, Cluj, Romania, e-mail: stefanvesa@gmail.com

•Simona-Delia Ţălu, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Faculty of Medicine, Department of Surgical Specialties and Medical Imaging, Discipline of Ophthalmology, 8th Victor Babeş St., 400012, Cluj-Napoca, Cluj, Romania, e-mail: talusimona@gmail.com

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