

## CASE REPORT

# Fulminant evolution in one case of choroidal melanoma – predictive factors for general metastasis

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## Abstract

Comparing with other malignant tumors, uveal melanomas determine tardive general spreading. Even in the absence of local treatment, general metastasis is less than 20%, in the first five years, many factors being involved. This paper present a particular case of choroid melanoma with very unusual and unpredictable evolution of the disease; the high growth of tumor size (in only half year the tumor almost doubled up dimensions), was associated with hepatic metastasis developed in only several months. In our opinion, this case, presenting a very atypical evolution may contribute to increase our knowledge of the mechanisms underlying metastasis and the identification of reliable progression parameters as prognostic markers in primary uveal melanoma. Several histological characteristics and demographic factors have been associated with disease evolution: presence of retinal neovessels and neovascular glaucoma, rapid increasing tumor thickness, scleral invasion, epithelioid cell types with large nucleoli, lymphocytic infiltration, and vascular pattern with networks of loops. Sclera invasion is uncommon in choroid tumors, but it is associated with an unfavorable bad prognosis, giving a great propensity to metastasize and to affect the liver. The median survival following diagnosis of hepatic metastasis is only several months.

**Keywords:** choroid melanoma, scleral invasion, epithelioid cells, hepatic metastasis.

## Introduction

Choroidal melanoma is the most frequent primary intraocular malignant tumor in adults and old people, with an incidence varying on 5.3 to 8.7 per one million subjects, per year [1, 2].

Despite diagnostic advances and the introduction of new treatment modalities over the last several decades, the rate of metastatic disease from these tumors has not been substantially reduced [3]. Uveal melanomas metastasize relatively late: the 5-, 10- and 15-year survival rates based on tumor-related mortality are reported to be 72%, 59% and 53 %, compared to age-matched controls [4, 5]. Metastatic spread of uveal melanoma is associated with an extremely poor prognosis. The median survival following diagnosis of hepatic metastasis is only several months. For patients with uveal melanoma, there is no effective therapy if metastases have developed [5].

## Aim

The purpose of this study is to evaluate histological characteristics and demographic factors associated with disease evolution and to identify the progression parameters in a particular case of choroid melanoma with fulminant progression to hepatic metastasis.

## Case report

Patient, T.I., 61-year-old, female, has been diagnosed with malignant choroidal tumor in June 2012. At the first

visit in the Department of Ophthalmology, Emergency County Hospital of Craiova, Romania, the patient accused some floaters on her left eye, and a moderate decrease of visual acuity. In the ambulatory, the ophthalmologist suspected only a choroidal nevus but he decided the hospitalization to evaluate the case. Complete ocular examination, consisting in functional and imagistic evaluation has been performed:

- functional exams: corrected bilateral visual acuity, non-contact aplanotometry, biomicroscopy, direct and indirect ophthalmoscopy;
- imagistic exams: bilateral ocular echography, magnetic resonance imagery (MRI), optical coherence tomography (OCT), angiofluorography, hepatic echography, pulmonary radiography.

## First clinical examination

Visual acuity of right eye 0.9, visual acuity of left eye 0.7, ocular pressure of right eye 17 mmHg, ocular pressure of left eye 16 mmHg.

The biomicroscopy of both eyes was normal, only several small anterior and nuclear opacities have been noticed on the anterior ocular pole.

The fundus eye examination has been performed by direct and indirect ophthalmoscopy and Hruby lens biomicroscopy. On the left eye, we discovered mushroom-shaped pigmented choroidal mass, 7 mm superior from the optic disc, measuring 4.5 mm at its largest basal dimension, with associated subretinal fluid and retinal ischemia.

Fluorescein angiography revealed areas of hyperfluorescence and areas with blocked fluorescence. B-scan ultrasonography confirmed a 4.7 mm thick choroidal melanoma. There was no evidence of vitreous hemorrhage or extrascleral extension. Optical coherence tomography (OCT) showed full thickness replacement of the retina with tumor and abrupt posterior shadowing. Resonance imagery (MRI) certified the diagnosis of choroidal melanoma.

Enucleation *versus* plaque radiotherapy was discussed, but the patient did not agree to any of the two medical options. He decided to repeat all investigation in the University Clinical Hospital of Bucharest, Romania, in order to have a second opinion, therefore we did not start any treatment, even the diagnosis was certain.

Patient returned to our hospital in December 2012; in our clinic all functional and imagistic examination has been reevaluated, the results revealing the increased tumor size comparing with the last visit, with extremely decreasing of visual acuity of left eye, and very important ocular pain.

### Second clinical examination

Visual acuity of right eye 0.9, visual acuity of left eye – LP (light perception), ocular pressure of right eye 16 mmHg, ocular pressure of left eye 56 mmHg.

The biomicroscopy of the left eye presented perilimbic congestion, with dilated episcleral vessels, iridian neovessels, obstructed anterior chamber angle by neovessels and hemorrhages. The fundus eye examination was extremely difficult because the important corneal edema; it revealed the increasing of choroidal mass, that approximately measured 8–9 mm, very rich pigmented, with hemorrhages on its surface, and necrotic areas. B-scan ultrasonography confirmed the increasing of the tumor 8.4 mm on its long diameter, and some areas of very thin sclera.

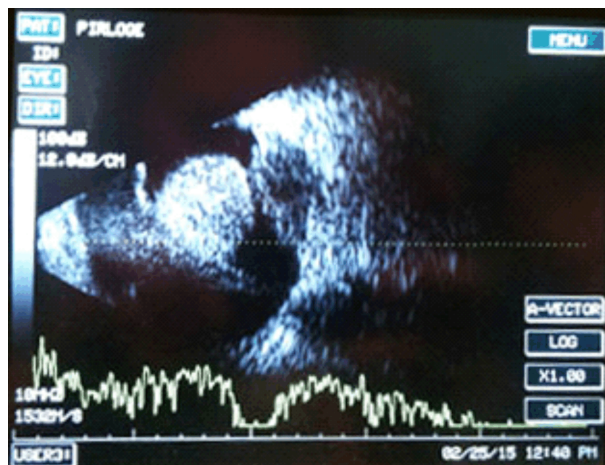


Figure 1 – Choroidal melanoma (B-scan ultrasonography).

The positive clinical diagnosis: choroid melanoma of left eye; secondary neovascular absolute glaucoma.

Because of this fulminant increasing of tumor, we decided and we performed immediately the enucleation of the right eye. Two days after surgery, we advised the patient to repeat the abdominal echography, and several opacities in the hepatic area were detectable. The suspicion

of hepatic dissemination was confirmed by blood biochemical results and by abdominal tomography. Patient was referred to oncologist to start the immunochemotherapy treatment.

### Anatomopathological exam

The optic globe section exposed a large tumor (8 mm on its large diameter and 3 mm in thickness), extremely dark, from the equator to 3 mm superior of optic nerve papilla; the superjacent retina was ischemic and infiltrated by the tumoral tissue with neovessels; macular and perimacular regions were involved.

To perform the histopathological exam, the eye was initially treated with the regular method of paraffin inclusion and the resulting sections were stained with Hematoxylin and Eosin (HE) and trichromic protocols: Goldner–Szekely (GS) and van Gieson.

The histopathological exam confirmed the diagnosis of choroidal melanoma with scleral and optical nerve invasion. The most prominent tumoral cells were represented by epithelioid cells. The tumor was characterized by the presence of vascular networks, defined as at least three back-to-back closed vascular loops, alternating with ischemic areas without vessels or with neovessels.

Comparing with other malignant tumors, uveal melanomas determine tardive general spreading. Usual, general metastasis in the first five years is less than 20%, even in the absence of local treatment, many factors being involved. Therefore, only approximately 15% of patients with choroid melanoma require primary enucleation.

Our patient with choroid melanoma presented a very unusual and unpredictable evolution of the disease; the uncommon high increasing in tumor size (in only six months the tumor almost doubled up dimensions), was associated with hepatic metastasis developed in only several months. In our opinion, this case, presenting a very atypical evolution may contribute to increase our knowledge of the mechanisms underlying metastasis and the identification of reliable progression parameters as prognostic markers in primary uveal melanoma.

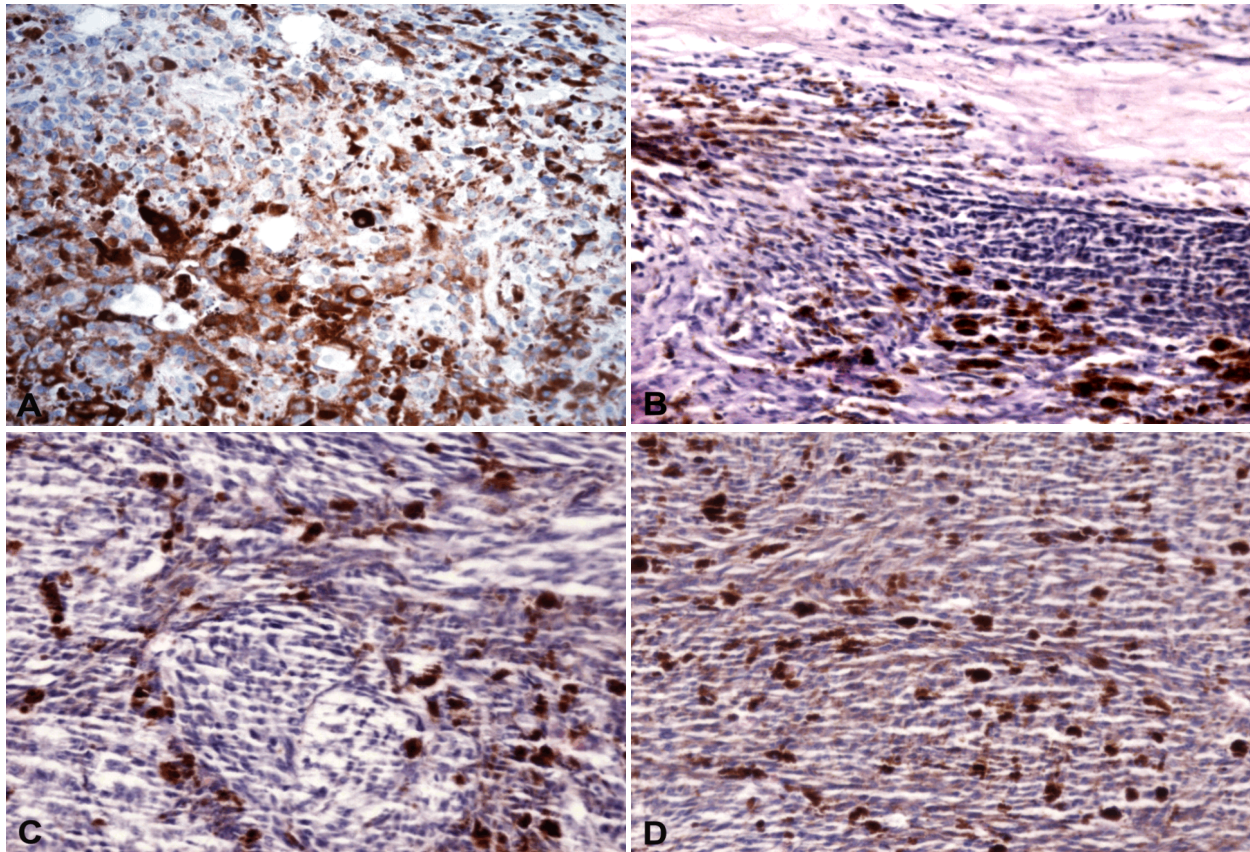
In our patient, the tumor growth rate was very rapid, in only several months the tumor doubled its dimensions and the eye lost all functional capacity. The ocular tension increased in only six months from 16 mmHg to 56 mmHg.

In this case, the ophthalmoscopy showed from the beginning a very vascularized and pigmented tumor.

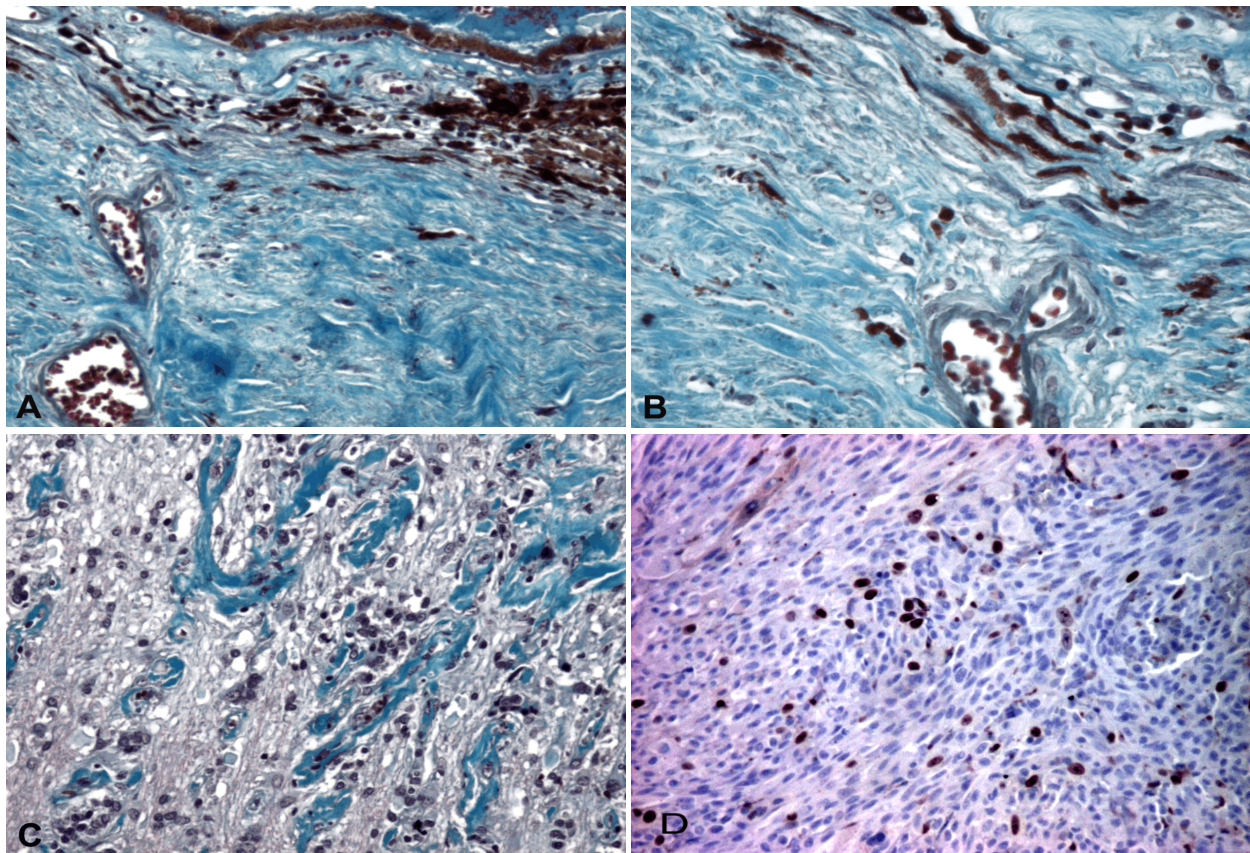
These modifications were certified by angiofluorography, where we have noticed extended hyperfluorescence areas with leakage from damaged intraretinal capillaries at the margin of the infiltrated retina, alternated with areas with blocked fluorescence, and double circulation pattern. We considered the obliteration of retinal arterioles and venules overlying choroidal melanoma as a reliable marker of retinal invasion.

Immunohistochemistry examination was performed using Ki-67 marker, frequently used for the differentiation between benignant and malignant melanocytes proliferation.

Ophthalmological examination corroborated with histopathological and immunohistochemistry result confirmed the diagnosis of choroidal melanoma of left eye.



**Figure 2 – Choroidal melanoma with epithelioid cells. Tumor cells are arranged in parallel cords (A and B) or crossing cords (C and D) that are separated by bands of connective tissue. HE staining: (A)  $\times 100$ ; (B–D)  $\times 200$ .**



**Figure 3 – Choroidal melanoma with sclera invasion. Pigmented tumor cells that disseminate throughout the sclera vessels (A and B). Optical nerve invasion by tumor cells (C). Melanocytes nuclei positive for Ki-67 (D). GS staining: (A and C)  $\times 100$ ; (B)  $\times 200$ . Ki-67 immunostaining: (D)  $\times 200$ .**

## Discussion

The most important issue related to general metastasis is represented by histopathological characteristic of malignant tumors. Uveal melanomas have a spectrum of cell types, ranging from thin and plump spindle cells to epithelioid cells. The following types of melanoma cells were recognized: spindle A-cells, spindle B-cells and epithelioid cells. Spindle cell tumors tend to grow in a compact cohesive fashion, with a dense framework of reticulin fibers. Epithelioid cells grow less cohesively than spindle cells and are not surrounded by a network of reticulin. Melanomas of the mixed cell type are composed of a mixture of epithelioid and spindle cells.

McLean *et al.* demonstrated that spindle cells include a spectrum of benign and malignant cells and they proposed a modification of the Callender's classification: spindle cell malignant melanomas, mixed cell melanomas and epithelioid cell melanomas [6]. They also demonstrated that all melanomas containing epithelioid cells had more than 50% chance of metastatic spread, larger size epithelioid cells was more predictable to develop metastasis [7].

In other studies, the presence or absence of any epithelioid cells (spindle cell melanoma *versus* a combination of mixed cell type and epithelioid cell tumors) has been preferred; less than two epithelioid cells per high-power field is associated with a favorable outcome [8].

Another recent study demonstrated that most of posterior uveal melanomas had mixed cellularity with spindle B-cells in a fascicular pattern of growth between epithelioid cells. The spindle B-cells had oval nuclei with distinct nucleoli and no evident cell borders. In the cytoplasm, we observed variable amount of melanin [9].

The presence of vascular networks, defined as at least three back-to-back closed vascular loops, is a feature strongly associated with death from metastatic melanoma. Other significant factors included (in descending order of importance), mitotic rate, the parallel with cross-linking vascular pattern, age, the presence of tumor-infiltrating lymphocytes. It was also demonstrated that the presence of vascular networks shortens survival and that the aggressive behavior of ciliary body melanomas appears to be related to the tendency for vascular networks to develop in this location [10].

The presence or absence of each of nine previously reported microcirculation patterns (normal blood vessels, silence area in the tumor, straight vessels, arcs, arcs with branching, parallel pattern, parallel with cross links, loops, and networks of loops) was recorded for each tumor from sections stained by Periodic Acid-Schiff without Hematoxylin counterstaining by viewing sections with a green filter as originally described by Folberg *et al.* [11]. He determined the prognostic value of tumor blood vessel morphology in primary uveal melanoma; they concluded that melanomas that have the same vascular profile as nevi (normal vessels, straight and parallel vessels) have a low risk to general spreading, whereas melanomas with vascular networks are strongly associated metastatic disease; 32% of patients whose melanomas had vascular

patterns other than those seen in nevi died of metastatic melanoma, comparing to 14% with a combined pattern between nevi and melanomas.

Foss *et al.* failed to credit any vascular pattern with independent prognostic value and argued that some of the vascular patterns, including networks, are generated by fibrovascular septa and not by the vessels themselves. However, the conflict regarding the histogenesis of these patterns does not explain the difference in results. The presence or absence of a single pattern is a dichotomy, and decision-making is likely to be subjective [12].

Several retrospective clinical studies, including more than eight thousands eyes with uveal melanoma identified the following factors predictive of metastasis: high patient age, ciliary body location, increasing tumor diameter/thickness, deeply pigmented tumor, presence of subretinal fluid or intraocular hemorrhage, extraocular extension. Retinal invasion has been shown to increase the risk for tumor recurrence but not metastasis or death [13, 14].

Visual acuity reduced to light perception (LP) or no light perception (NLP), intraocular pressure greater than 24 mmHg, juxtapapillary location, non-visible fundus were significantly associated with post-laminar optic nerve invasion [15].

High ocular pressure and chronic vascular occlusive disease may induce optic nerve ischemia and edema, also facilitating the growth of malignant tumor cells [16].

The tumor size is extremely important for evolution: recently, 300 mm<sup>3</sup> has been defined as the critical volume, which differentiates a large tumor from a small one. Survival rates in patients with tumors less than 300 mm<sup>3</sup> are significantly greater than those of the patients with larger lesions [17]. More, in several studies it has been emphasize the relationship between tumor growth rate and patient survival [18].

Typical angiofluorographic lesions in choroid melanoma are represented of broadened intercapillary spaces (54%), large zones of retinal capillary non-perfusion (35%) and tumor-retinal vascular anastomoses (15%) [19]; the presence of hyperfluorescence is less frequent, in relationship with neovessels appearance and it is associated with bad evolution.

In our patient, the most prominent cellular type was represented by epithelioid cells, with both scleral and retinal invasion. The microcirculation revealed areas with vascular networks of loops, alternating with large ischemic areas. These modifications together with the presence of neovessels, generating neovascular glaucoma, represented in our opinion the most significant factors for the unfavorable prognostic.

The most frequent metastasis in uveal melanoma affects liver, lung, bones, skin, and lymph nodes (Table 1) [20]. Metastases to the heart, kidney, and CNS were found only in patients with optic nerve invasion, perhaps reflecting a different metastatic pattern in uveal melanomas with optic nerve invasion than in uveal melanomas without this feature [21, 22].

**Table 1 – Studies of prognostic indicators in uveal melanoma (adapted from Mooy & De Jong, 1996 [20])**

Authors	Sample size	Melanoma deaths	Covariate	Scoring	Hazard ratio	P
Folberg <i>et al.</i> (1992) [8]	234	67 (28.6%)	Vascular networks	Present or absent	2.9	0.0006
			Largest tumor dimension	Continuous value	1.1	0.0054
			Mitotic count	Continuous value	1.2	0.0001
McLean <i>et al.</i> (1983) [6]	740	453 (61.2%)	Mean of the largest nucleoli	Continuous value	1.8	$<10^{-10\pm}$
			Largest tumor dimension	Continuous value	1.1	$<10^{-25\pm}$
Lattman <i>et al.</i> (1997) [23]	36	13 (33.3%)	Bromodeoxyuridine labeling	Log <sub>2</sub> transformed	2.3	0.01
			Mitotic index	Log <sub>2</sub> transformed	2.4	0.006
Seregard <i>et al.</i> (1998) [22]	212	71 (33.5%)	PC-10 count	Arranged in two groups <sup>‡</sup>	2.4 (5.8)	$<10^{-10}$ ( $<10^{-8}$ )
			Largest tumor dimension	Arranged in two groups <sup>‡</sup>	1.8 (2.8)	$<10^{-5}$ (0.00001)
Egan <i>et al.</i> (1988) [1]	267	114 (42.7%)	Number of epithelioid cells	Arranged in four groups	2.5	$<0.001$
			Largest tumor dimension	Arranged in three groups	2.0	$<0.01$
Foss <i>et al.</i> (1996) [12]	120	Unknown	Microvessel count	Square root transformed	1.4	$<0.00005$
			Largest tumor dimension	Continuous value	1.1	0.0003

<sup>‡</sup>Divided by median; <sup>±</sup>Estimated from *t*-value.

A possible route of dissemination in patients with post-laminar optic nerve invasion was by seeding through the cerebrospinal fluid because all these tumors had gained access to the subarachnoid space [24].

Local spread occurs through the overlying Bruch membrane, giving access to the subretinal space; the invasion of sclera is estimated in less than 5% of cases, and the optic nerve infiltration is estimated in less than 1% of patients [25].

The risk of metastasis depends on several factors: among them are the cell type, largest tumor diameter, mean of the larger nucleoli, lymphocytic infiltration, and vascular pattern, chromosomal aberration in the tumor, scleral invasion, age, gender, and location of the tumor.

Methods for assessing cellular proliferation include: mitotic count, DNA synthesis immunohistochemical methods, and assessment of nucleolar organizer regions.

The management of malignant melanoma has to prevent local and general spreading of tumor; it is not a consensus whether enucleation or conservative treatment offers the best prognosis. Although retrospective studies have suggested that the method of treatment makes no difference in the systemic prognosis, new studies in which the various therapeutic modalities are being compared are currently under way.

Some small choroidal melanomas can be treated with laser photocoagulation. Alternatively, radiotherapy (either episcleral application of a radioactive plaque or charged particle irradiation) can be used. Although the two methods of radiotherapy seem equal relative to the development of systemic metastatic lesions, plaque radiotherapy is associated with fewer and less severe local complications. Local resection has theoretical advantages, but the surgical procedure is associated with potentially greater immediate complications.

Enucleation is generally indicated for advanced melanomas that occupy most of the intraocular structures or have caused severe glaucoma. In addition, it is usually recommended for tumors that have invaded the optic

nerve. Although chemotherapy and immunotherapy have not been shown to provide a therapeutic cure for uveal melanomas, some studies revealed efficiency in advanced or in fulminant evolution, especially in administration of multi-drug treatment (Methotrexate, Cisplatin and Bleomycin) [26].

## Conclusions

Several histological characteristics have been associated with atypical fulminant evolution in this case of choroid melanoma: scleral invasion, presence of epithelioid cell types with large nucleoli, lymphocytic infiltration, and vascular pattern with networks of loops. Sclera invasion is rare in choroid tumors, this aspect being considered a particularity of this case. Another particularity was represented by retinal neovessels that spread rapidly the entire globe, generating increased ocular pressure and creating the context to early general metastasis.

## Conflict of interests

The authors declare that they have no conflict of interests.

## References

- [1] Egan KM, Seddon JM, Glynn RJ, Gragoudas ES, Albert DM. Epidemiologic aspects of uveal melanoma. *Surv Ophthalmol*, 1988, 32(4):239–251.
- [2] Reese AB. Pigmented tumors. In: Reese AB (ed). *Tumors of the eye*. 3<sup>rd</sup> edition, Harper & Row, Hagerstown, MD, 1976, 173–262.
- [3] Isager P, Østerlind A, Engholm G, Heegaard S, Lindegaard J, Overgaard J, Storm HH. Uveal and conjunctival malignant melanoma in Denmark, 1943–97: incidence and validation study. *Ophthalmic Epidemiol*, 2005, 12(4):223–232.
- [4] Lorigan JG, Wallace S, Mavligit GM. The prevalence and location of metastases from ocular melanoma: imaging study in 110 patients. *AJR Am J Roentgenol*, 1991, 157(6):1279–1281.
- [5] Scotto J, Fraumeni JF Jr, Lee JA. Melanomas of the eye and other noncutaneous sites: epidemiologic aspects. *J Natl Cancer Inst*, 1976, 56(3):489–491.
- [6] McLean IW, Foster WD, Zimmerman LE, Gamel JW. Modifications of Callender's classification of uveal melanoma at the Armed Forces Institute of Pathology. *Am J Ophthalmol*, 1983, 96(4):502–509.

- [7] McLean IW, Gamel JW. Prediction of metastasis of uveal melanoma: comparison of morphometric determination of nucleolar size and spectrophotometric determination of DNA. *Invest Ophthalmol Vis Sci*, 1988, 29(4):507–511.
- [8] Folberg R, Pe'er J, Gruman LM, Woolson RF, Jeng G, Montague PR, Moninger TO, Yi H, Moore KC. The morphologic characteristics of tumor blood vessels as a marker of tumor progression in primary human uveal melanoma: a matched case-control study. *Hum Pathol*, 1992, 23(11):1298–1305.
- [9] Baderca F, Solovan C, Boghian L. Epidemiological and morphological data of ocular melanocytic lesions. *Rom J Morphol Embryol*, 2013, 54(1):77–83.
- [10] Shields JA, Shields CL. Current management of posterior uveal melanoma. *Mayo Clin Proc*, 1993, 68(12):1196–1200.
- [11] Folberg R, Rummelt V, Parys-Van Ginderdeuren R, Hwang T, Woolson RF, Pe'er J, Gruman LM. The prognostic value of tumor blood vessel morphology in primary uveal melanoma. *Ophthalmology*, 1993, 100(9):1389–1398.
- [12] Foss AJ, Alexander RA, Jefferies LW, Hungerford JL, Harris AL, Lightman S. Microvessel count predicts survival in uveal melanoma. *Cancer Res*, 1996, 56(13):2900–2903.
- [13] Gündüz K, Shields CL, Shields JA, Cater J, Freire JE, Brady LW. Radiation complications and tumor control after plaque radiotherapy of choroidal melanoma with macular involvement. *Am J Ophthalmol*, 1999, 127(5):579–589.
- [14] Shields JA, Shields CL. Posterior uveal melanomas: clinical features. In: Shields JA, Shields CL. *Intraocular tumors: an atlas and textbook*. 2<sup>nd</sup> edition, Lippincott Williams & Wilkins, Philadelphia, PA, 2008, 86–92.
- [15] Giarelli L, Falconieri G, Cameron JD, Pheley AM. Schnabel cavernous degeneration: a vascular change of the aging eye. *Arch Pathol Lab Med*, 2003, 127(10):1314–1319.
- [16] Diener-West M, Earle JD, Fine SL, Hawkins BS, Moy CS, Reynolds SM, Schachat AP, Straatsma BR; Collaborative Ocular Melanoma Study Group. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma, III: initial mortality findings. COMS Report No. 18. *Arch Ophthalmol*, 2001, 119(7):969–982.
- [17] Kersten RC, Tse DT, Anderson R. Iris melanoma. Nevus or malignancy? *Surv Ophthalmol*, 1985, 29(6):423–433.
- [18] Davidorf FH. The melanoma controversy. A comparison of choroidal, cutaneous, and iris melanomas. *Surv Ophthalmol*, 1981, 25(6):373–377.
- [19] Augsburger JJ, Golden MI, Shields JA. Fluorescein angiography of choroidal malignant melanomas with retinal invasion. *Retina*, 1984, 4(4):232–241.
- [20] Mooy CM, De Jong PTVM. Prognostic parameters in uveal melanoma: a review. *Surv Ophthalmol*, 1996, 41(3):215–228.
- [21] Herlyn M, Thurin J, Balaban G, Bennicelli JL, Herlyn D, Elder DE, Bondi E, Guerry D, Nowell P, Clark WH, Koprowski H. Characteristics of cultured human melanocytes isolated from different stages of tumor progression. *Cancer Res*, 1985, 45(11 Pt 2):5670–5676.
- [22] Seregard S, Spångberg B, Juul C, Oskarsson M. Prognostic accuracy of the mean of the largest nucleoli, vascular patterns, and PC-10 in posterior uveal melanoma. *Ophthalmology*, 1998, 105(3):485–491.
- [23] Lattman J. Anophthalmic socket reconstruction techniques highlighted. *Ophthalmology Times*, 1997, 22(2):14–17.
- [24] Madajewicz S, Karakousis C, West CR, Caracandas J, Avellanosa AM. Malignant melanoma brain metastases. Review of Roswell Park Memorial Institute experience. *Cancer*, 1984, 53(11):2550–2552.
- [25] Spencer WH. Optic nerve extension of intraocular neoplasms. *Am J Ophthalmol*, 1975, 80(3 Pt 1):465–471.
- [26] Maurya RP, Bhushan P, Singh VP, Singh MK, Pandey M, Shahi UP, Kumar M. Clinico-pathological response of neo-adjuvant chemotherapy in advance ocular carcinoma. *World J Surg Med Radiat Oncol*, 2012, 1:6.

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*Received: March 12, 2014*

*Accepted: July 8, 2015*