Uveal melanoma – testing of abnormalities of chromosome 3 and 8 in the Czech Republic

Veronika Matuskova[a], Pavla Hornackova[a], Radoslava Uhmannova[a], Lenka Zlamalikova[b], Kvetoslava Liskova[b], Michal Uher[c], Eva Vlkova[a]

Aim. The aim of this cytogenetic study is to confirm the significance of chromosome 3 loss (monosomy 3) and of the gain of chromosome 8 as prognostic markers in histopathological samples of enucleated eyes with uveal melanoma in the Czech population.

Methods. This is a retrospective study of 52 enucleated eyes. Chromosome 3 and 8 status were tested by CISH, and in a few samples FISH was used. The correlation between monosomy 3 and gain of chromosome 8 and clinical features (histopathological type, size of the tumour) were evaluated. A follow up for the detection of metastases was conducted in all patients. The statistical significance of chromosomal abnormalities as a prognostic factor for the development of metastases was calculated.

Results. There were 52 patients, 27 men (51.9%) and 25 women (48.1%) enrolled in our study group. The mean age was 63 ± 14 years. Loss of the one copy of chromosome 3 (monosomy 3) was detected in 26 (50.0%) patients, monosomy 8 was present in 34.6% of patients with monosomy 3. After 5 years there were no metastases in 82% of patients without monosomy 3 as opposed to 40% of patients with monosomy 3. We confirmed a statistically significant association between progression free survival (PFS) and the presence of monosomy 3 (*P*=0.017). The association between PFS and gain of chromosome 8 was significant as well (0.010).

Conclusions. Our data showed the association of progression-free survival with the presence of monosomy 3 in uveal melanomas. We provided a good prognostic value of monosomy 3 in uveal melanoma.

Key words: uveal melanoma, chromosome 3, chromosome 8, CISH, FISH, progression-free survival

Received: April 24, 2019; Revised: June 19, 2019; Accepted: July 22, 2019; Available online: September 13, 2019
https://doi.org/10.5507/bp.2019.037

[a]Department of Ophthalmology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic
[b]Department of Pathology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic
[c]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic
Corresponding author: Veronika Matuskova, e-mail: v.matuskova@email.cz

INTRODUCTION

Although uveal melanoma (UM) is a rare cancer, it is, however, the most frequent primary intraocular malignant tumour in adults[1]. The incidence of uveal melanoma in Europe was analysed in a European Cancer Registry-based study (EUROCARE) where it was estimated that there were 1.3-8.6 cases per million per year in Europe[2]. These data are almost equal to a study by the United States National Cancer Institute (SEER) where the overall incidence of uveal melanoma was 5.1 per million per year in the United States[3]. The incidence of uveal melanoma has remained stable since the 1970s with approximately 7000 new cases of uveal melanoma worldwide[4].

The mean age at presentation is 60 years. There is a progressive increase with age, peaking at 70-75 years and then reaching a plateau. There is a slight predominance in males[5]. Uveal melanoma typically affects Caucasians in 98% of cases. The risk factors are light skin, inability to tan and light iris colour[6]. This is the reason we can see a north-to-south decreasing gradient of melanoma incidence in Europe[2]. The role of exposure to sunlight has been broadly discussed, but has not been proven[7]. Other predisposing conditions include choroidal nevus and ocular melanocytosis[8].

Ocular melanoma arises from melanocytes that are differentiated from pluripotent neural crest cells[1]. Uveal melanoma can be located in the iris (4% of patients), in the ciliary body (6% of patients) and in the choroid (90% of patients) (ref.[9]).

Despite a high success rate of therapy for the primary tumour, 50% of patients die from metastases[10,11]. It is presumed that this is due to the presence of micrometastases already at the time of the primary diagnosis[12]. For a long time, effective metastases treatment had been lacking. In recent years, immunotherapy has begun to be used. There are currently several studies in this area (https://app.emergingmed.com/mrf/trials/). Some immunostimulants have been approved by the FDA: Imlygic (T-VEC), Yervoy + Opdivo, Opdivo (nivolumab), Keytruda (pembrolizumab), Yervoy (ipilimumab), Interleukin-2 (IL-2) and Interferon alpha 2-b.

Of great importance is to identify high risk patients who may develop metastases. Clinical features predisposing a higher risk for metastasis are large tumour size, ciliary body involvement and extrascleral extension of the tumour. Shields evaluated the risk of tumour size for metastasis where every millimetre increases the risk of metastasis by 5% (ref.[13]). The above factors are the basis of the Tumour, Node, Metastasis (TNM) description[14]. The risk of metastasis is also dependent on the histopathological characteristics of the tumour. The first Callander classification was modified by McLean who divided melanoma cell type into spindle, epithelioid and mixed[15]. Risk factors for metastatic spread are epithelioid cell type, high mitotic activity, increased tumour-infiltrating lymphocytes, the presence of networks of closed microvascular loops, and vascular invasion[16].

The presence of chromosomal aberrations provides the possibility of a more accurate assessment of the risk of metastasis. Frequently observed non-random gross chromosomal changes are specific for uveal melanoma. They are of prognostic significance for the progression of the tumour. Prescher described the loss of one copy of chromosome 3 (monosomy) in tumours with a high tendency for metastatic spread[17]. This strong correlation was shown in several clinical studies[18-21]. The other chromosomal mutation associated with reduced life survival is a gain of 8q. Other unfavourable chromosomal alternations are a loss of 6q or 1p; contrariwise, a gain of 6p is a sign of a better prognosis[22].

Prognostic biopsy evaluating metastatic risk is standard in some oncology centres in the management of patients with uveal melanoma in the United States[23] and in Europe[18,21,24-27]. Presently, chromosomal analysis is not used in the Czech Republic.

The aim of this cytogenetic study is to confirm the significance of chromosome 3 loss (monosomy 3) and of the gain of chromosome 8 as a prognostic marker in histopathological samples of enucleated eyes with uveal melanoma. We want to map the genomic profile in the Czech Republic and we want to report the first results from the Czech population. Finally, we want to discuss the clinical implications of our cytogenetic findings. The aim of the study is to show the effectiveness of introducing a cytogenetic examination into clinical practice in patients with uveal melanoma in the Czech Republic.

MATERIAL AND METHODS

A retrospective non-randomised study of eyes of 52 patients who underwent enucleation due to the diagnosis of choroidal melanoma or ciliary body melanoma. This cytogenetic study was conducted between January 2008 and December 2017.

The patients were treated in the Department of Ophthalmology of the University Hospital at Masaryk University in Brno.

The clinical data included age and gender of the patients, tumour location (ciliary body, posterior pole, nasal part, and temporal part) and histological type. The measurement of thickness and diameter (mm) of the tumour were assessed using ultrasound, A and B scans (Compact touch, Quantel Medical, France). For statistical evaluation we used the mathematical approximation of the total size of the tumour. Other clinical features included the presence of rupture of Bruch's membrane, secondary retinal detachment and orbital extension. The staging of the disease was accessed at the time of the diagnosis. All of the patients were followed up in our Department, the staging of the disease was repeated every 3 months during the first 2 years of the following period and then every 6 months.

Other treatments, such as brachytherapy or Cyberknife, were not carried out because of the size of the tumour or because of the tumour being located too close to the optic nerve.

The follow-up data, including the time and cause of death, were obtained from clinical records and by contacting the Institute of Health Information and Statistics of the Czech Republic. Based on these data we distinguished the metastatic death (disease specific survival rate) and death due to other causes. Informed consent was obtained from all the participants. We followed Good Clinical Practice (GCP) guidelines according to the Declaration of Helsinki.

Archival formalin-fixed, paraffin-embedded tissues were used in this study. The samples were stored in the archives of the Department of Pathology of the University Hospital at Masaryk University in Brno. A histopathologic examination was conducted according to standardised protocols. Haematoxylin and eosin stained sections were reviewed by a pathologist to determine the histological type and to choose the sample for the cytogenetic study. We tested samples for monosomy 3, and in patients with monosomy 3 we also tested the gain of chromosome 8 by chromogenic in situ hybridisation (CISH) and by fluorescence in situ hybridisation (FISH).

Chromogenic in situ hybridisation (CISH)

The CISH method was performed on tissue sections in VENTANA BenchMark Ultra (*Ventana* Medical Systems, USA) using the VENTANA ultraView RED ISH DIG Detection Kit (*Ventana* Medical Systems, USA). Tissue sections were deparaffinised and pretreated with Cell Conditioning 2 (CC2) at pH 6 at 82°C. Enzymatic digestion of proteins was performed with ISH Protease 3 for 32 min. The double-stranded DNA was denatured for 12 min at 80 °C, the hybridisation of the probe proceeded at 44 °C for eight hours. Chromosome 3 DIG Probe (*Ventana* Medical Systems, USA) hybridising to the centromere of the chromosome was used. A stringency wash was performed at 72 °C to wash off unbound or weakly bound probes. The ultra*View* Red ISH DIG detection kit was used for the detection of the CEN3 signal. Tissue sections were then counterstained in hematoxylin II and bluing reagent to enhance the contrast. Images were scanned by a DM 5500 B microscope equipped with a Leica DFC 290 HD camera. Red signals were analysed using Leica

Table 1. Baseline characteristics of patients by sex.

		Total (n = 52)	Females (n = 25)	Males (n = 27)	*P*
Sex – female		25 (48.1%)	25 (100.0%)	0 (0.0%)	–
Age at the time of diagnosis (years)		63 ± 14	61 ± 16	65 ± 12	0.409
		67 (56; 71)	65 (51; 71)	68 (57; 71)	
Location	Posterior pole	8 (15.4%)	3 (12.0%)	5 (18.5%)	0.774
	Nasal part	13 (25.0%)	6 (24.0%)	7 (25.9%)	
	Temporal part	19 (36.5%)	11 (44.0%)	8 (29.6%)	
	Ciliary body	12 (23.1%)	5 (20.0%)	7 (25.9%)	
Histological cell type*	Epithelioid	7 (14.0%)	2 (8.3%)	5 (19.2%)	0.797
	Spindle. type A	14 (28.0%)	7 (29.2%)	7 (26.9%)	
	Spindle. type B	10 (20.0%)	5 (20.8%)	5 (19.2%)	
	Mixed	19 (38.0%)	10 (41.7%)	9 (34.6%)	
Thickness of melanoma (mm)		8.3 ± 3.7	8.3 ± 3.7	8.4 ± 3.8	0.653
		8.5 (5.5; 12.0)	9.0 (5.5; 10.5)	8.0 (7.0; 12.0)	
Size in diameter (mm)		14.6 ± 4.0	15.6 ± 3.5	13.7 ± 4.3	0.114
		14.0 (12.0; 17.0)	16.0 (13.0; 19.0)	14.0 (11.0; 16.0)	
Total size of melanoma (cm^3)		1.29 ± 1.08	1.33 ± 1.08	1.26 ± 1.10	0.700
		0.99 (0.48; 1.88)	1.01 (0.66; 1.80)	0.96 (0.45; 1.98)	
Rupture of Bruch's membrane		23 (44.2%)	14 (56.0%)	9 (33.3%)	0.162
Secondary retinal detachment		39 (75.0%)	20 (80.0%)	19 (70.4%)	0.528
Extraocular extension		2 (3.8%)	2 (8.0%)	0 (0.0%)	0.226

*Histological cell type is unknown in 1 man and 1 woman.
Continuous variables are described by mean ± SD, median (25th percentile; 75th percentile) and significance of differences are assessed by Mann-Whitney test.
Categorical variables are described by absolute (relative) frequencies and significance of association are assessed by Fisher's exact test.

LAS AF software (Leica Microsystems GmbH, Wetzlar, Germany). To define the monosomy of chromosome 3 we used a cut-off value of 20%.

Fluorescence in situ hybridisation (FISH)

For the detection of chromosome 3 monosomy, the ZytoLight® CEN3 Probe was used (ZytoVision GmbH, Bremerhaven, Germany). Hybridisation was performed according to the manufacturer's instructions. Images were scanned by a DM 5500 B microscope equipped with a Leica DFC 290 HD camera. Fluorescence signals were analysed using Leica LAS AF software (Leica Microsystems GmbH, Wetzlar, Germany). 100 cells per case were analysed. To define the monosomy of chromosome 3 we used a cut-off value of 20%.

For the analysis of chromosome 8q status, ZytoLight ® SPEC FGFR1/CEN 8 Dual Color Probe and ZytoLight ® SPEC MYC/CEN 8 Dual Color Probe were used. Gain of the long arm of chromosome 8 was defined by amplification of the MYC gene (located in the chromosomal region 8q24.21) and copy loss of the FGFR1 gene (located in the chromosomal region 8p11.23-p11.22). To avoid uncertainty, another probe was used located in the long arm of chromosome 8 - RUNX1T1 located in the region 8q21.3-q22.1.

Statistical analysis

Continuous variables were described using mean (standard deviation) and median (25th and 75th percentile), and the statistical significance of differences was tested by the Mann-Whitney test. Categorical variables were described by absolute and relative frequencies. The statistical significance of association between categorical variables was assessed with Fisher's exact test. Disease specific survival and progression free survival were estimated using the Kaplan-Meier method with the log-rank test as a measure of statistical significance of different survival rates between groups. The Cox PH regression model was applied to estimate hazard ratios (HR) between groups. The level of statistical significance was set to 0.05 in all hypothesis testing.

RESULTS

There were 52 patients, 27 men (51.9%) and 25 women (48.1%) enrolled in our study group. The mean age of the patients was 63 ± 14 (median 67, 56–71 years). Table 1 shows the patients' principal clinical characteristics. Posterior uveal melanoma was present in 40 patients (76.9%) and ciliary body melanoma in 12 (23.1%) patients. The average thickness of the melanoma was 8.3 ± 3.7 mm and the average size 14.6 ± 4.0 in diameter. We observed the rupture of Bruch's membrane in 23 (44.2%) and secondary retinal detachment in 39 (75.0%) cases. Extraocular extension was diagnosed in 2 (3.8%) patients. The most common histological cell type was mixed, in 19 (38.0%) patients; epithelioid cell type was present in 7 patients (14.0%); and spindle cell type in 24 (48.0%)

Table 2. Baseline characteristics of patients by abnormalities of chromosome 3.

		Total (n = 52)	Monosomy 3 (n = 26)	Without monosomy 3 (n = 26)	*P*
Sex – female		25 (48.1%)	12 (46.2%)	13 (50.0%)	1.000
Age at the time of diagnosis (years)		63 ± 14	65 ± 12	62 ± 16	0.819
		67 (56; 71)	67 (59; 71)	67 (51; 71)	
Location	Posterior pole	8 (15.4%)	6 (23.1%)	2 (7.7%)	0.235
	Nasal part	13 (25.0%)	8 (30.8%)	5 (19.2%)	
	Temporal part	19 (36.5%)	7 (26.9%)	12 (46.2%)	
	Ciliary body	12 (23.1%)	5 (19.2%)	7 (26.9%)	
Histological cell type*	Epithelioid	7 (14.0%)	3 (12.5%)	4 (15.4%)	0.937
	Spindle, type A	14 (28.0%)	7 (29.2%)	7 (26.9%)	
	Spindle, type B	10 (20.0%)	4 (16.7%)	6 (23.1%)	
	Mixed	19 (38.0%)	10 (41.7%)	9 (34.6%)	
Thickness of melanoma (mm)		8.3 ± 3.7	9.1 ± 3.5	7.6 ± 3.9	0.193
		8.5 (5.5; 12.0)	10.0 (6.4; 12.0)	7.0 (4.0; 12.0)	
Size in diameter (mm)		14.6 ± 4.0	15.1 ± 3.9	14.1 ± 4.2	0.368
		14.0 (12.0; 17.0)	15.0 (12.0; 18.0)	14.0 (11.0; 17.0)	
Total size of melanoma (cm^3)		1.29 ± 1.08	1.47 ± 1.13	1.11 ± 1.01	0.098
		0.99 (0.48; 1.88)	1.18 (0.79; 1.53)	0.72 (0.35; 1.98)	
Rupture of Bruch's membrane		23 (44.2%)	14 (53.8%)	9 (34.6%)	0.264
Secondary retinal detachment		39 (75.0%)	19 (73.1%)	20 (76.9%)	1.000
Extraocular extension		2 (3.8%)	1 (3.8%)	1 (3.8%)	1.000

*Histological cell type is unknown in 2 patients with monosomy 3.
Continuous variables are described by mean ± SD, median (25th percentile; 75th percentile) and significance of differences are assessed by Mann-Whitney test.
Categorical variables are described by absolute (relative) frequencies and significance of association are assessed by Fisher's exact test.

Table 3. Survival by abnormalities of chromosome 3.

	Disease-specific survival		Progression free survival	
	5-year survival (95% CI)	HR (95% CI)	5-year survival (95% CI)	HR (95% CI)
Total (n = 52)	77.8 (62.8; 92.8)	–	62.3 (47.2; 77.3)	–
Without monosomy 3 (n = 26)	92.9 (79.4; 100.0)	Reference category	81.6 (64.8; 98.5)	Reference category
Monosomy 3 (n = 26)	60.9 (35.0; 86.7)	4.14 (0.84; 20.25)	40.4 (18.2; 62.6)	3.21 (1.13; 9.09)*

*Significant (P<0.05) change in HR (hazard ratio) between categories.

patients. The median follow-up was 4.3 ± 3.2 years. Metastases after 5 years were present in 27.4%, and after 5 years 40.7% of patients had died. In all the cases distant metastases were present, in one patient local recurrence was also observed. The first metastases were diagnosed in the liver in 17.3% of patients.

Disease specific survival was not statistically significant when associated with the size of the tumour (P=0.064), the thickness of the tumour (P=0.053) and the size in diameter (P=0.134). Progression free survival was statistically significant when associated with the diameter size above 14 mm (P=0.008) (Fig. 1); this was not statistically significant regarding the thickness of the melanoma (P=0.303) and with the size of the melanoma (P=0.250).

Loss of one copy of chromosome 3 (monosomy 3) was detected in 26 (50.0%) patients. A more detailed description of the subgroup with monosomy 3 is shown in Table 2. Patients with monosomy 3 were a little older and they had a larger tumour with a greater thickness as well as diameter, and rupture of Bruch's membrane was more frequent; but the results are not statistically significant. Disease specific survival and progression free survival were longer in patients without monosomy 3. Disease specific survival did not quite reach a statistically significant level (P=0.053), but the difference of progression free survival between both groups was statistically significant (P=0.017), Fig. 2. When we evaluate five-year survival and monosomy 3, then patients with monosomy 3 lived longer than 5 years (61% of patients) and without monosomy 3 (93% of patients). The strongest association is between monosomy 3 and progression free survival, HR 3.21 (Table 3). After 5 years there are no metastases in 82% of patients without monosomy 3 as opposed to 40% of patients with monosomy 3. The risk of progression

Table 4. Baseline characteristics of patients by abnormalities of chromosome 3 and 8.

		Total (n = 52)	Monosomy 3 with chr. 8 abnorm. (n = 9)	Without monosomy 3 or without chr. 8 abnorm. (n = 43)	*P*
Sex – female		25 (48.1%)	3 (33.3%)	22 (51.2%)	0.469
Age at the time of diagnosis (years)		63 ± 14 67 (56; 71)	62 ± 6 61 (57; 67)	63 ± 14 67 (56; 71)	0.327
Location	Posterior pole	8 (15.4%)	4 (44.4%)	4 (9.3%)	0.098
	Nasal part	13 (25.0%)	2 (22.2%)	11 (25.6%)	
	Temporal part	19 (36.5%)	2 (22.2%)	17 (39.5%)	
	Ciliary body	12 (23.1%)	1 (11.1%)	11 (25.6%)	
Histological cell type*	Epithelioid	7 (14.0%)	3 (33.3%)	4 (9.8%)	0.098
	Spindle, type A	14 (28.0%)	1 (11.1%)	13 (31.7%)	
	Spindle, type B	10 (20.0%)	0 (0.0%)	10 (24.4%)	
	Mixed	19 (38.0%)	5 (55.6%)	14 (34.1%)	
Thickness of melanoma (mm)		8.3 ± 3.7 8.5 (5.5; 12.0)	9.8 ± 3.5 11.0 (7.2; 12.0)	8.0 ± 3.7 7.2 (5.0; 12.0)	0.240
Size in diameter (mm)		14.6 ± 4.0 14.0 (12.0; 17.0)	15.7 ± 3.8 16.0 (12.0; 19.0)	14.4 ± 4.1 14.0 (12.0; 17.0)	0.437
Total size of melanoma (cm^3)		1.29 ± 1.08 0.99 (0.48; 1.88)	1.67 ± 1.28 1.32 (0.96; 1.38)	1.21 ± 1.03 0.79 (0.40; 1.97)	0.147
Rupture of Bruch's membrane		23 (44.2%)	5 (55.6%)	18 (41.9%)	0.486
Secondary retinal detachment		39 (75.0%)	6 (66.7%)	33 (76.7%)	0.674
Extraocular extension		2 (3.8%)	0 (0.0%)	2 (4.7%)	1.000

*Histological cell type is unknown in 2 patients with monosomy 3 without chromosome 8 abnormalities.
Continuous variables are described by mean ± SD, median (25th percentile; 75th percentile) and significance of differences are assessed by Mann-Whitney test.
Categorical variables are described by absolute (relative) frequencies and significance of association are assessed by Fisher's exact test.

to metastatic disease is 3.2 fold higher in patients with monosomy 3 (Table 3).

The gain of chromosome 8 was present in 9 patients with monosomy 3 (34.6%). The subgroup of patients with monosomy 3 and gain of chromosome 8 is described in Table 4. We did not find a statistically significant association of this subgroup with disease specific survival (*P*=0.317) (Fig. 3). But progression free survival was statistically significant (*P*=0.010) (Fig. 4).

DISCUSSION

One of the typical markers of the cancer is the presence of genetic mutations. Uveal melanoma has only a few specific mutations we can identify. A specific non-random occurrence of chromosome 3 abnormalities in melanoma was published in the early 1990s (ref.[28]). A few patients showed chromosomal abnormalities of chromosome 3, 6 and 8. Complete loss of chromosome 3 (monosomy 3) was present in 43% of patients and was often connected with chromosome 8 abnormalities. These changes are probably secondary in uveal melanoma in contrast to cutaneous melanoma and they are probably the reason for the genetic difference between cutaneous and uveal melanoma[17]. These observations of monosomy 3 were confirmed by other authors[29,30]. The first observation of the loss of one copy of chromosome 3 and its association with metastatic spread of UM was published by Prescher in 1996 (ref.[17]). In 54 patients they found monosomy 3 in 56% of enucleated eyes; this abnormality was the most significant risk factor for metastatic disease after enucleation[17]. During the following years, several clinical trials of enucleated eyes were conducted to confirm the association between certain genetic mutations and the risk of developing metastases[18,24,27,31,32]. Some clinical work carried out has dealt with the association of genetic abnormalities and tumour characteristics. Sisley showed a significant association of cytogenetic characteristics with ciliary body involvement[18]. However, this observation was probably influenced by the composition of the study group in which 57% of the patients had ciliary body melanoma. Kilic and Damato emphasised the connection with tumour size[20,33].

Cytogenetic testing of chromosomal abnormalities in fine-needle aspiration biopsy (FNAB) samples from enucleated eyes was presented by Sisley[34]. In 2002 Naus reported that genetic testing of samples obtained by fine-needle aspiration biopsy could be as reliable as testing by open biopsy after enucleation in melanomas[35]. They performed fine-needle aspiration of tumours in 40 enucleated eyes; for determining genetic abnormalities the FISH method was used. The first published study of FNAB in vivo before brachytherapy of uveal melanoma included eight patients[36]. The most extensive work presenting results with genetic testing of FNAB samples in patients with uveal melanoma is the study of 500 cases published by Shields et al. They described complete monosomy 3 in 25% of patients and partial monosomy 3 in 27% of patients. Shields tried to calculate the cumulative probability according to the tumour thickness and cytogenetic

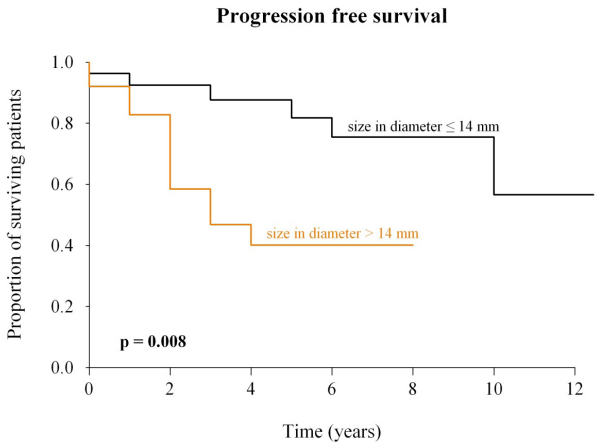

Fig. 1. Progression free survival (until presence of metastasis or death due to observed diagnosis of melanoma) by size of melanoma in diameter (P value of log-rank test is presented).

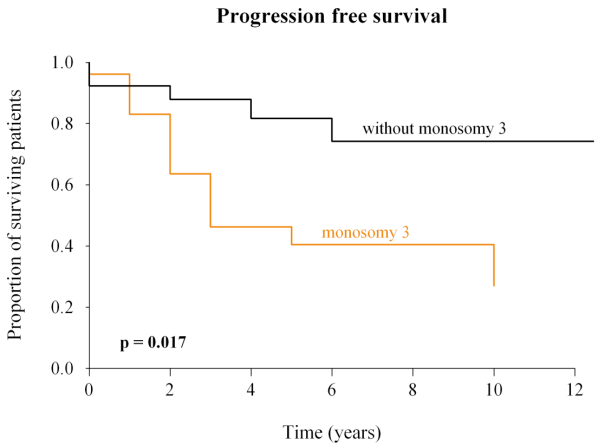

Fig. 2. Progression free survival (until presence of metastasis or death due to observed diagnosis of melanoma) by abnormalities of chromosome 3 (P value of log-rank test is presented).

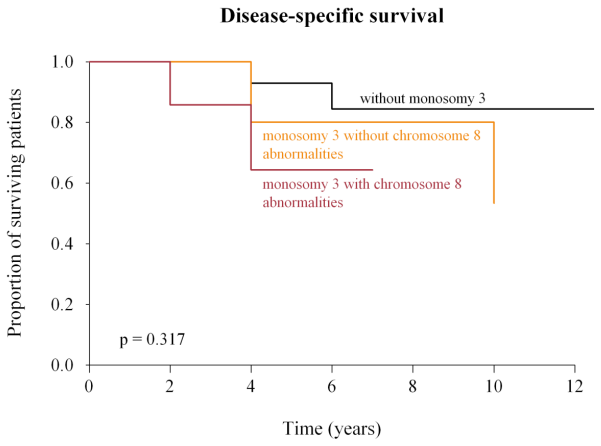

Fig. 3. Disease-specific survival (until death due to observed diagnosis of melanoma) by abnormalities of chromosome 3 and 8 (P value of log-rank test is presented).

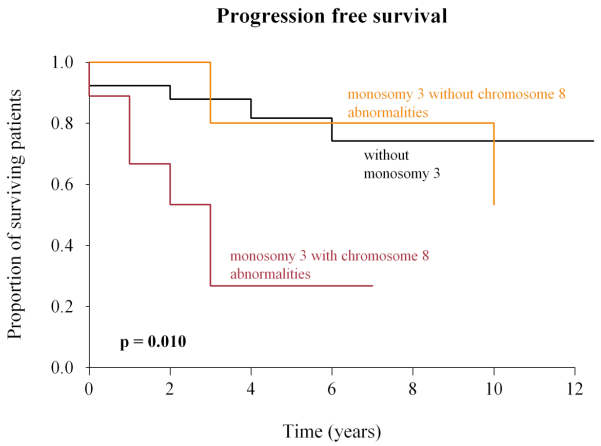

Fig. 4. Progression free survival (until presence of metastasis or death due to observed diagnosis of melanoma) by abnormalities of chromosome 3 and 8 (P value of log-rank test is presented).

characteristics. At 3 years the cumulative probability of metastases for tumours with monosomy 3 was 0% for small melanomas, almost 25% for medium and 57.5% for large melanomas. In contrast the cumulative probability of tumours with disomy was 0% for small, 1.4% for medium and 23.1% for large tumours. Shields suggested that the higher rate of incidence of metastases in disomy 3 tumours could be caused by a sampling error of FNAB in larger tumours[23].

The most important predictive factors of monosomy 3 included increasing tumour thickness and increasing distance to the optic disc. According to the observation of some authors, monosomy 3 is related to another prognostic factor, such as diameter size and ciliary body involvement[28,33].

The basic characteristic of our study group corresponds to other study groups. The study group contains fewer patients, because this genetic test is not routinely used in the Czech Republic. Our Department of Ophthalmology is the oncology centre for the South Moravian region, which has 1 200 000 inhabitants. The frequency of monosomy 3 in our study group is comparable with other studies. When comparing the incidence of monosomy with these studies, we should take into account that we have examined enucleated eyes and this implies that the tumours are larger. Le Guin studied monosomy 3 in the subgroup treated with brachytherapy and by enucleation. The proportion of monosomy 3 is higher in tumours treated by enucleation (71% vs. 32%) (ref.[12]).

In our study group tumours with monosomy 3 were a little higher, more often localised on the posterior pole and more often associated with the rupture of Bruch's membrane, but this was not statistically significant. Alterations of chromosome 3 and 8 were found occurring together in 17.3% of patients. These melanomas, with both chromosomal abnormalities, were less frequent in women, and these tumours were more frequently present

in the posterior pole; they were also higher and larger. These associations were not statistically significant. At the edge of statistical significance, despite the smaller study group size, was the connection between epithelioid histological type and presence of both chromosomal abnormalities. We observed a statistically significant association between monosomy 3 and progression free survival. There are no metastases in 82% of patients without monosomy 3 as opposed to 40% of patients with monosomy at a 5-year follow-up. We can compare our study group with the subgroup of large tumours in the study of Shields. The cumulative probability of metastases in patients with disomy 3 after 3 years[23] was 23%, and in patients with monosomy 58%. The risk of progression to metastatic disease is 3.2 fold higher in patients with monosomy 3 in our study group where larger tumours are involved. Prescher already reported a 50% metastases rate in patients with monosomy 3 after 3 years[17]. Le Guin showed a significant association between monosomy 3 and tumour-related survival not only in patients after enucleation but also in the subgroup after brachytherapy[12].

Damato published that 5% to 20% of disomy 3 uveal melanoma patients unexpectedly develop metastases[33]. There were 3 patients without monosomy 3 with the presence of metastasis at the initial examination in our study group. They were women, the diameter of the basis of the tumours were 15-20 mm. In one patient we observed extrascleral spread of melanoma. It has been suggested that translocations or partial deletions of chromosome 3 are the cause of this[37,38]. Attempts have been made to identify metastasis-suppressor genes, but without success[31].

A limiting factor of this cytogenetic examination is a group of tumours with the loss of only a part of the chromosome, referred to as partial monosomy 3. It is probably due to a mis-sampling of tumour samples with monosomy 3 or the incorrect interpretation of a sample with partial monosomy 3 (ref.[25]). Lake described Multiplex Ligation-Dependent Probe Amplification (MLPA) that can detect monosomy 3 in these "unfavourable" uveal melanomas, whereas FISH could not, especially in small tumours[39]. This is probably due to a higher sensitivity of the MLPA method and by cellular heterogeneity in the sample. The frequency of this abnormality differs greatly, from 0%, over 0.6% (ref.[32]) to 4% (ref.[25]) or 5.3% (ref.[23]). The mortality rate in these patients is comparable with monosomy 3 patients according to Damato[33], in contrast to the observation of Shields and Thomas[23,25].

Vaarwater demonstrated that specificity was equal for FISH and MLPA (ref.[40]). We did a retrospective study, thus only formalin-fixed paraffin-embedded (FFPE) tissue sections were available. Therefore, in situ hybridisation was a better choice than MLPA, which requires preferably fresh tissue. Monosomy 3 was tested by CISH by allowing the examination of CISH signal and tissue morphology simultaneously. Nevertheless, we *did not detect* any *signals* in the few samples, thus FISH was used in those cases.

The gain of chromosome 8 influences the metastatic progression of tumours with monosomy 3, but not of tumours with disomy 3. This coincidence of chromosome 3 and 8 abnormalities was referred by Patel[41]. The gain of chromosome 8 is known as late change during melanoma development and it is related to large tumour size[20]. The higher risk of metastatic death was present in patients with monosomy 3 with chromosome 8 abnormalities[18,25,42]. The combination of the anatomic and genetic characteristic as a prognostic factor has been shown by Damato[33], Dogrusöz[27] and van den Bosch[26]. Even a slightly stronger high correlation between tumour diameter and gain of chromosome 8 than with monosomy 3 was presented by van den Bosch[26]. In all the patients with low percentage monosomy 3 who died of metastasis during a follow-up period, they detected a high percentage gain of chromosome 8q. This is probably due to the great importance of chromosome 8 abnormalities – in low percentage monosomy 3 tumours, the high percentage gain of chromosome 8 is an indicator of worsening patient survival. Patel and Bronkhorst presume the cut-off limit to be 30%, because of the sensitivity of the probe[41,43]. On the other hand, van den Bosch did not consider the threshold 30% as the limit that can be used to exclude patients from the high risk group; he showed that a significant number of patients with 30% of monosomy 3 died of metastasis in his study group[26].

It is suggested that monosomy 3 tumour cells acquire their metastatic potential at an early stage in contrast to tumours with disomy 3, which acquire metastatic potential later[25]. Damato supported the idea of the transformation model. It was proposed that a natural cause is responsible for the subsequent loss of disomy 3 during the progression of the tumour[33]. However, later detailed genetic expression profiling (GEP) of monosomy 3 and disomy 3 tumours revealed that the development of monosomy 3 melanoma from disomy 3 melanoma is not probable[44].

In summary our data showed an association of progression free survival with presence of monosomy 3 and gain of chromosome 8 in uveal melanomas. We provided a good prognostic value of monosomy 3 in uveal melanoma. Only enucleated tumours were included, which implied that we studied larger tumours only. A limiting factor of our study group was the small number of patients, however, this was the first ever larger cytogenetic testing carried out in the Czech Republic. A cytogenetic study of a smaller study group of 11 patients was published by Sobotova[45].

CONCLUSION

Genetic testing in many countries is a standard diagnostic procedure in patients with uveal melanoma. According to the established risk of metastasis, we can adjust the interval of regular examinations. Another reason is that patients want to know their risk of developing metastases[46]. The importance of genetic testing will probably continue to increase due to the progressive development of immunotherapy, which would influence the development of metastases in patients with uveal melanoma.

Acknowledgements: Supported by Ministry of Health, Czech Republic – conceptual development of research organisation (FNBr, 65269705)

Author contributions: VM, EV: manuscript writing, VM: literature search, coordination of research, EV: coordination of financial support, PH, RU: patient follow up, clinical examination, PH: data collection, LZ, KL: genetic analysis, MU: statistical analysis, table and figures.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer 1998; 83(8):1664-78.
2. Virgili G, Gatta G, Ciccolallo L, Capocaccia R, Biggeri A, Crocetti E, Lutz JM, Paci E; EUROCARE Working Group. Incidence of uveal melanoma in Europe. Ophthalmology 2007;114(12):2309-15.
3. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. Ophthalmology 2011;118(9):1881-5.
4. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973–1997. Ophthalmology 2003;110(5):956-61.
5. Singh AD, De Potter P, Fijal BA, Shields CL, Shields JA, Elston RC. Lifetime prevalence of uveal melanoma in white patients with oculo(dermal) melanocytosis. Ophthalmology 1998; 105:195-8.
6. Weis E, Shah CP, Lajous M, Shields JA, Shields CL. The association between host susceptibility factors and uveal melanoma: a meta-analysis. Arch Ophthalmol 2006; 124(1):54-60.
7. Shah CP, Weis E, Lajous M, Shields JA, Shields CL. Intermittent and chronic ultraviolet light exposure and uveal melanoma: a meta-analysis. Ophthalmology 2005;112(9):1599-607.
8. Shields CL, Kaliki S, Furuta M, Mashayekhi A, Shields JA. Clinical spectrum and life prognosis of patients with uveal melanoma based on age at presentation in 8033 cases. Retina 2012;32:1363-72.
9. Shields CL, Furuta M, Berman EL, Zahler JD, Hoberman DM, Dinh DH et al. Choroidal nevus transformation into melanoma: analysis of 2514 consecutive cases. Arch Ophthalmol 2009; 127(8):981-7.
10. Augsburger JJ, Corrêa ZM, Shaikh AH. Effectiveness of treatments for metastatic uveal melanoma. Am J Ophthalmol 2009;148(1):119-27.
11. Kujala E, Mäkitie T, Kivelä T. Very long-term prognosis of patients with malignant uveal melanoma. Invest Ophthalmol Vis Sci 2003;44(11):4651-9.
12. Le Guin CHD, Metz KA, Lehmann N, Kreis SH, Bornfeld N, Rudolf Lohmann D, Zeschnigk M. Chromosome 3 is a valid marker for prognostic testing of biopsy material from uveal melanoma later treated by brachytherapy. Biomarkers 2018;28:1-7.
13. Shields CL, Furuta M, Thangappan A, Nagori S, Mashayekhi A, Lally DR et al. Metastasis of uveal melanoma millimeter-by-millimeter in 8033 consecutive eyes. Arch Ophthalmol 2009; 127(8):989-98.
14. Edge SB,Compton CC. The American Joint Committee on Cancer: the 7th editionof the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471-4.
15. 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):502-9.
16. The Collaborative Ocular Melanoma Study (COMS) randomized trial of pre-enucleation radiation of large choroidal melanoma II: initial mortality findings. COMS report no. 10. Am J Ophthalmol 1998;125(6):779-96.
17. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jöckel KH, Becher R. Prognostic implications of monosomy 3 in uveal melanoma. Lancet 1996;347(9010):1222-5.
18. Sisley K, Rennie IG, Parsons MA, Jacques R, Hammond DW, Bell SM, Potter AM, Rees RC. Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. Genes Chromosomes Cancer 1997;19(1):22-8.
19. Singh AD, Tubbs R, Biscotti C, Schoenfield L, Trizzoi P. Chromosomal 3 and 8 status within hepatic metastasis of uveal melanoma. Arch Pathol Lab Med 2009;133(8):1223-7.
20. Kilic E, van Gils W, Lodder E, Beverloo HB, van Til ME, Mooy CM, Paridaens D, de Klein A, Luyten GP. Clinical and cytogenetic analyses in uveal melanoma. Invest Ophthalmol Vis Sci 2006;47(9):3703-7.
21. Damato B, Duke C, Coupland SE, Hiscott P, Smith PA, Campbell I, Douglas A, Howard P. Cytogenetics of uveal melanoma: a 7-year clinical experience. Ophthalmology 2007;114(10):1925-31.
22. Kaliki S, Shields CL, Shields JA. Uveal melanoma: estimating prognosis. Indian J Ophthalmol 2015;63(2):93-102.
23. Shields CL, Ganguly A, Bianciotto CG, Turaka K, Tavallali A, Shields JA. Prognosis of uveal melanoma in 500 cases using genetic testing of fine-needle aspiration biopsy specimens. Ophthalmology 2011;118(2):396-401.
24. Scholes AG, Damato BE, Nunn J, Hiscott P, Grierson I, Field JK. Monosomy 3 in uveal melanoma: correlation with clinical and histologic predictors of survival. Invest Ophthalmol Vis Sci 2003;44(3):1008-11.
25. Thomas S, Pütter C, Weber S, Bornfeld N, Lohmann DR, Zeschnigk M. Prognostic significance of chromosome 3 alterations determined by microsatellite analysis in uveal melanoma: a long-term follow-up study. Br J Cancer 2012;106(6):1171-6.
26. van den Bosch T, van Beek JG, Vaarwater J, Verdijk RM, Naus NC, Paridaens D, de Klein A, Kiliç E. Higher percentage of FISH-determined monosomy 3 and 8q amplification in uveal melanoma cells relate to poor patient prognosis. Invest Ophthalmol Vis Sci 2012;53(6):2668-74.
27. Dogrusöz M, Bagger M, van Duinen SG, Kroes WG, Ruivenkamp CA, Böhringer S, Andersen KK, Luyten GP, Kiilgaard JF, Jager MJ. The Prognostic Value of AJCC Staging in Uveal Melanoma Is Enhanced by Adding Chromosome 3 and 8q Status. Invest Ophthalmol Vis Sci 2017;58(2):833-42.
28. Prescher G, Bornfeld N, Becher R. Nonrandom chromosomal abnormalities in primary uveal melanoma. J Natl Cancer Inst 1990;82(22):1765-9.
29. Sisley K, Cottam DW, Rennie IG, Parsons MA, Potter AM, Potter CW, Rees RC. Non-random abnormalities of chromosomes 3, 6, and 8 associated with posterior uveal melanoma. Genes Chromosomes Cancer 1992;5(3):197-200.
30. Wiltshire RN, Elner VM, Dennis T, Vine AK, Trent JM. Cytogenetic analysis of posterior uveal melanoma. Cancer Genet Cytogenet 1993;66(1):47-53.
31. Tschentscher F, Prescher G, Horsman DE, White VA, Rieder H, Anastassiou G, Schilling H, Bornfeld N, Bartz-Schmidt KU, Horsthemke B, Lohmann DR, Zeschnigk M. Partial deletions of the long and short arm of chromosome 3 point to two tumor suppressor genes in uveal melanoma. Cancer Res 2001;61(8):3439-42.
32. Ewens KG, Kanetsky PA, Richards-Yutz J, Al-Dahmash S, De Luca MC, Bianciotto CG, Shields CL, Ganguly A; Genomic Profile of 320 Uveal Melanoma Cases: Chromosome 8p-Loss and Metastatic Outcome. Invest Ophthalmol Vis Sci 2013;54(8):5721-9.
33. Damato B Coupland SE. Translating uveal melanoma cytogenetics into clinical care. Arch Ophthalmol 2009;127:423-9.
34. Sisley K, Nichols C, Parsons MA, Farr R, Rees RC, Rennie IG. Clinical applications of chromosome analysis, from fine needle aspiration biopsies, of posterior uveal melanomas. Eye (Lond) 1998;12:203-7.
35. Naus NC, Verhoeven AC, van Drunen E, Slater R, Mooy CM, Paridaens DA, Luyten GP, de Klein A. Detection of genetic prognostic markers in uveal melanoma biopsies using fluorescence in situ hybridization. Clin Cancer Res 2002;8(2):534-9.
36. Midena E, Bonaldi L, Parrozzani R, Tebaldi E, Boccassini B, Vujosevic S. In vivo detection of monosomy 3 in eyes with medium-sized uveal melanoma using transscleral fine needle aspiration biopsy. Eur J Ophthalmol 2006;16(3):422-5.
37. Blasi MA, Roccella F, Balestrazzi E, Del Porto G, De Felice N, Roccella M, Rota R, Grammatico P. 3p13 region: a possible location of a tumor suppressor gene involved in uveal melanoma. Cancer Genet Cytogenet 1999;108:81-3.
38. Parrella P, Fazio VM, Gallo AP, Sidransky D, Merbs SL. Fine Mapping of Chromosome 3 in Uveal Melanoma. Cancer Res 2003; 63:8507-10.

39. Lake SL, Coupland SE, Taktak AF, Damato BE. Whole-genome microarray detects deletions and loss of heterozygosity of chromosome 3 occurring exclusively in metastasizing uveal melanoma. Invest Ophthalmol Vis Sci 2010;51(10):4884-91.
40. Vaarwater J, van den Bosch T, Mensink HW, van Kempen C, Verdijk RM, Naus NC, Paridaens D, Brüggenwirth HT, Kiliç E, de Klein A. Multiplex ligation-dependent probe amplification equals fluorescence in-situ hybridization for the identification of patients at risk for metastatic disease in uveal melanoma. Melanoma Res 2012;22(1):30-7.
41. Patel KA, Edmondson ND, Talbot F, Parsons MA, Rennie IG, Sisley K. Prediction of prognosis in patients with uveal melanoma using fluorescence in situ hybridisation. Br J Ophthalmol 2001;85(12):1440-4.
42. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma.Cancer 1998:;83(2):354-9.
43. Bronkhorst IH, Maat W, Jordanova ES, Kroes WG, Schalij-Delfos NE, Luyten GP, Jager MJ. Effect of heterogeneous distribution of monosomy 3 on prognosis in uveal melanoma. Arch Pathol Lab Med 2011;135(8):1042-7.
44. Martin M, Maßhöfer L, Temming P, Rahmann S, Metz C, Bornfeld N, van de Nes J, Klein-Hitpass L, Hinnebusch AG, Horsthemke B, Lohmann DR, Zeschnigk M. Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. Nat Genet 2013;45(8):933-6.
45. Sobotova M, Hecova L, Vaněček T, Rusňák Š. Genetické vyšetření u maligního melanomu cévnatky. In: Sborník abstrakt XX. Výročního sjezdu České oftalmologické společnosti; 2012 20-22 Sep; Plzeň, CZ. Ústí nad Labem: FKK Company;2012. p. 82. (in Czech)
46. Beran TM, McCannel TA, Stanton AL, Straatsma BR, Burgess BL. Reactions to and desire for prognostic testing in choroidal melanoma patients. J Genet Couns 2009;18(3):265-74.