Diploma Thesis

Epidemiological and clinical characteristics of patients with multiple primary melanomas

A retrospective data analysis and review of the literature

submitted by

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Statutory Declaration

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Graz, 29.01.2018 *Max Rogatsch eh*

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Abbreviations

AJCC	–	American Joint Committee on Cancer
ALM	–	acral lentiginous melanoma
ASR	–	age-standardized rate
BCC	–	basal-cell carcinoma
CI	–	confidence interval
CM	–	cutaneous melanoma
DFS	–	disease free survival
DN	–	dysplastic nevi
DNA	–	deoxyribonucleic acid
et al.	–	et alii/aliae/alia
i.e.	–	id est
IQR	–	interquartile range
LMM	–	lentigo maligna melanoma
MC1R	–	melanocortin-1 receptor
MIS	–	melanoma in situ
MM	–	malignant melanoma
mm	–	millimeter
MPM	–	multiple primary melanoma
NM	–	nodular melanoma
RR	–	risk ratio
SD	–	standard deviation
SLN	–	sentinel lymph node
SSC	–	squamous-cell carcinoma
SSM	–	superficial spreading melanoma
UICC	–	International Union Against Cancer
UV	–	ultraviolet
vs.	–	versus
WHO	–	World Health Organization
χ^2 test	–	Pearson´s chi-squared test

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Abstract

Background. With a rising incidence of malignant melanoma (MM), the rate of patients developing more than one melanoma during their life course is increasing. Over the past years, new insight on the risk factors and the development of multiple primary melanomas (MPM) was gained. This study addresses the issue of sex-specific differences in the clinical presentation of MPM.

Methods. Data of patients presenting with subsequent primary melanomas between the years 2010 – 2016 at the Dermatology department of the Medical University of Graz were obtained. Following an initial evaluation, a retrospective analysis of the clinical data with a focus on sex-specific differences was conducted.

Results. In our study collective of 87 patients, a total of 263 melanomas were diagnosed between the years 1973 and 2016. The mean age was 68 years (range: 34 to 92 years). Forty-six percent of the patients were women. The most common anatomic location in both sexes was the trunk (47.2%) followed by the upper extremity (21.2%). Women were younger at the time of the first diagnosed MM with a mean age of 54 years compared to 61 years in men. The difference between the medians was 12.5 years (p = .031). Furthermore, in women the presence of dysplastic nevi was associated with younger age (p < .001). In men, a higher rate of consecutive MM was reported after the fifth year of the previous diagnose (28.9% vs. 16.3% in women). Breslow thickness was lower in the subsequent melanomas of male patients compared to the first diagnosed melanoma (p < .05).

Conclusion. Our study suggests some novel gender-related differences in patients affected by multiple primary MMs.

Zusammenfassung

Hintergrund. Mit einer steigenden Inzidenz von malignen Melanomen (MM) nimmt auch die Anzahl der Patientinnen und Patienten zu, welche im Laufe ihres Lebens mehr als ein Melanom entwickeln. In den letzten Jahren konnten neue Erkenntnisse über die Risikofaktoren und die Entstehung von multiplen primären Melanomen (MPM) gewonnen werden. Diese Studie befasst sich mit der Thematik von geschlechtsspezifischen Unterschieden in der klinischen Präsentation von MPM.

Methodik. Es wurden die Daten von Patientinnen und Patienten gesammelt, welche zwischen 2010 – 2016 mit einem weiteren primären Melanom auf dem Institut für Dermatologie der Medizinischen Universität Graz vorstellig wurden. Nach einer initialen Evaluierung folgte eine retrospektive Analyse der klinischen Daten mit dem Fokus auf mögliche Unterschiede zwischen den Geschlechtern.

Ergebnisse. Zwischen den Jahren 1973 und 2016 wurden in unserem Studienkollektiv von 87 Personen eine Summe von 263 Melanomen diagnostiziert. Das Durchschnittsalter betrug 68 Jahre (Bandbreite: 34 bis 92 Jahren). Dreiundvierzig Prozent der Patienten waren weiblich. Die häufigste anatomische Lokalisation in beiden Geschlechtern war der Stamm (47.2%) gefolgt von den oberen Extremitäten (21.2%). Frauen waren bei der Diagnose des ersten Melanoms jünger mit einem Durchschnittsalter von 54 Jahren im Vergleich zu 61 Jahren bei Männern. Die Differenz zwischen den Medianen betrug 12.5 Jahre (p = .031). Des Weiteren war bei Frauen die Anwesenheit von dysplastischen Naevi mit einem jüngeren Alter assoziiert (p < .001). Bei Männern wurde eine höhere Rate von MM nach dem fünften Jahr der vorangegangenen Diagnose verzeichnet (28.9% vs. 16.3%). Die Breslow Dicke verringerte sich bei männlichen Patienten im Vergleich zur initialen Läsion (p < .05).

Schlussfolgerung. Unsere Studie weißt auf neue geschlechtsspezifische Unterschiede in Patientinnen und Patienten mit multiplen primären MM hin.

1 Introduction

1.1 Cutaneous melanoma

1.1.1 Definition

Derived from the Greek *μέλας, melas* meaning “dark” or ”black” [1], the term *melanoma* describes a malignant neoplasm, most commonly presenting itself as dark macule or nodule on the surface of the skin. Evolving from genetically altered melanocytes in the hair follicle, epidermis and dermis, the malignant cell population infiltrates the surrounding tissue and spreads through lymphatic or hematogenous dissemination [2,3]. Based on the origin of the melanocytes undergoing the malignant transformation, three subtypes can be distinguished: Mucosal melanoma, uveal melanoma and cutaneous melanoma, representing the most common subtype [4]. While they only make up a single-digit percentage of all skin cancer cases, cutaneous melanoma (CM) are reported to cause 80% of all deaths in this group [3,5]. While representing a considerable health issue in Europe, Northern America and Oceania, the numbers of cases on the Asian and African continent are low [6].

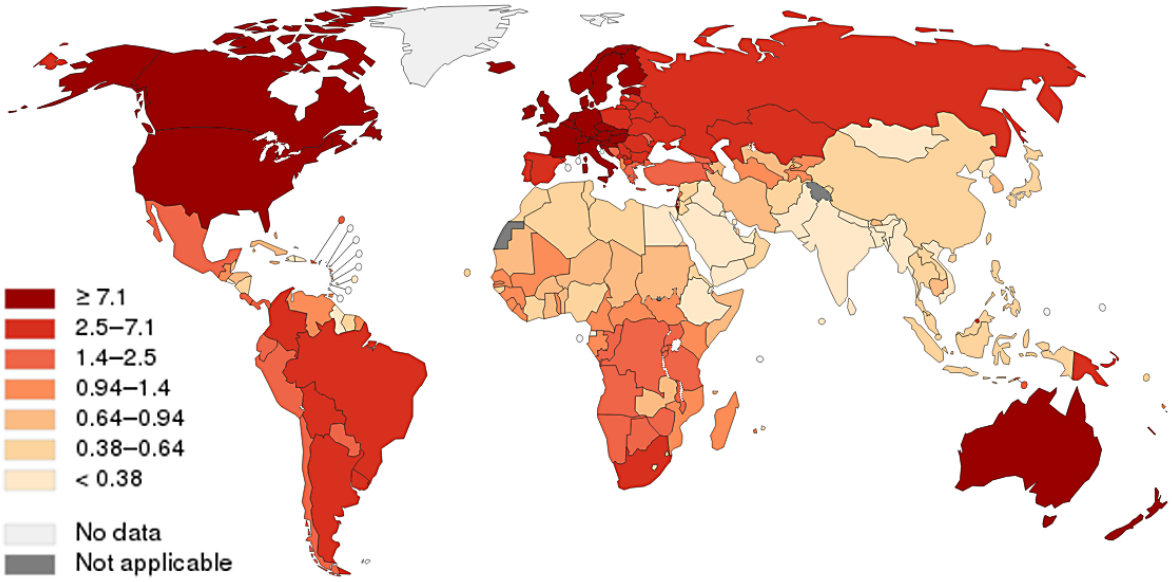

Figure 1: Estimated age-standardized rates (World) of incident cases, both sexes, melanoma of the skin, worldwide in 2012 (Source: WHO IARC)

1.1.2 Epidemiology

Incidence. According to data acquired in the GLOBOCAN project [7], the worldwide age-standardized rate per 100 000 residents (ASR) for melanoma of the skin is estimated to be around 3.0, with a slightly higher probability in men. Since mainly fair-skinned populations are affected, the incidence rates are widely variable around the globe (Figure 1). They range from an ASR below 0.5 in South-Eastern and South-Central Asia regardless of gender to rates of 30.5 in female and 40.3 in male inhabitants of Australia and New Zealand. The mean ASRs in Northern America (14.1) and Europe (8.8) lie in between those two extremes. Even within the European continent, the different populations show highly variable rates of incidence. From the ASRs as high as 19.2 in Switzerland and above 16 in the Scandinavian countries to lower numbers under 3 per 100 000 in Eastern Europe and the Mediterranean regions, the incidence rates show a descending north-south and west-east gradient throughout the continent. Primarily the variation of skin phenotypes and variable environmental factors in the different latitudes play an important part in this observation. Furthermore, this disparity can partly be explained by inconsistent attitudes towards recreational outdoor activities, traveling to countries with a higher ultraviolet (UV) index and the resulting increased exposure to UV radiation [6,8,9].

Increase of incidence. While being a relatively rare cancer entity in the early and mid-20th century, the incidence rates of CM have been steadily rising in the countries with a major fair-skinned population. Over an investigated time of nearly 30 years, various countries of the northern hemisphere have reported a threefold increase of incidence rates [6]. This makes melanoma the cancer with the fastest growth of incidence in white populations [4,10]. Epidemiological data from the last two decades suggested a future stabilization and slowing down of this trend, with the focus on young-aged individuals [11,12]. Nevertheless, a rise in incidence is still observed in elderly men. Besides an augmented exposure to potential risk factors, more frequent visits for dermatological checkup and an increased rate of skin biopsy account for peaks of increased incidence in the past [10].

Mortality. In comparison to steady increasing annual cases of CM, the total numbers of deaths showed a different development in the last three centuries. In regions with higher incidence, namely Australia/New Zealand, Northern America and Europe, the mortality rate did not follow the expected trend that higher incidence rates would imply. While showing a concurrent rise in the beginning, the mortality rates stabilized in many developed countries by the end of the 20th century [11,13]. In middle-aged adults the death rate plateaued and a decline has been observed in younger age groups since the 1990s. This development could be the result of improved comprehension and education on the dangers of extensive UV radiation and a broader health care utilization of the general public [11,14]. Early detection of suspected lesions and preventive management is a further key factor for the reduction of mortality [14,15].

Analogous to the incidence, the ASRs of mortality show a global variance with an average value of 0.7 in 100 000. The lowest rates are reported in the region of South-Central Asia with 0.1 annual deaths per 100 000 of the female residents. In the high incidence countries Australia and New Zealand the age-standardized mortality rates range from 2.4 in women up to 5.9 in men [7]. By studying the worldwide data on incidence and mortality of CM, a higher burden in more developed countries can be observed. The inequality and the wide gap between the figures can be explained with the variance in geography and demography, with focus on ethnic and racial differences. On the other hand, the different lifestyles in countries with high numbers of CM cases could partly contribute to this trend [16].

Age and Sex. While non-melanoma skin cancers, such as squamous-cell (SSC) and basal-cell carcinoma (BCC), commonly present in older age groups, the majority of CM diagnoses are made in earlier life years. From adolescence up to the age of approximately 50 years, the incidence increases linearly before slowing down. The median age at diagnose in the studied cohorts [6,10] has been described to lie between 55 and 57 years. Considering the reported age distribution, the data reveals that half of the cases of CM occur during the time of the third to the sixth decennium [10].

Comparing the epidemiological data of female and male individuals, various trends can be observed around the globe. While in high-incidence countries the ratio tends

to shift to a higher number of cases in male, in regions with low incidence more women get diagnosed with CM. European countries with previously higher incidence rates in women show an almost equal sex ratio in the present [6]. In relation to age, there appears to be a female preponderance until the age of 44 years [17]. On the contrary, most cases of CM in men arise later on, with the highest rates of incidence at ages beyond 60 [9]. Also, the reported mortality rates differ between female and male. Pooled worldwide data from cancer registries showed 1.3 times higher mortality in men [7]. Consistently, death rates in male patients rose to a higher extent in the past [18].

Host and risk factors. In the past years of comprehensive research, various theories for the emergence of CM have been developed. The studied causes range from environmental agents to acquired and hereditary genetic alteration. Today the etiology of CM is considered to be multifactorial, resulting from constitutional and environmental factors.

Phenotype. As the global incidence rates indicate, CM is almost exclusively a disease of light-skinned populations. A diminished amount of skin pigmentation, but also blue eyes, fair or red hair and presence of freckles are indicators for an increased risk of this cancer. This can partly be explained by a higher sensitivity to sunlight and easy sunburn in comparison to individuals with darker color of the hair, skin and iris [19]. These phenotypic features are partly defined by the specific allele configuration of the gene encoding for the melanocortin-1 receptor (MC1R). A reduced activity of the protein causes changes within the intracellular signal transduction, with effects on a wide range of genetic and biochemical processes. One influenced cascade is the synthesis of the dark-colored pigment eumelanin, which by default is produced by melanocytes in an alternative pathway to pheomelanin, the lighter red-colored pigment. Through the impaired function higher amounts of pheomelanin are present, resulting in the phenotypic traits mentioned above [3].
In addition, an involvement of MC1R variants in complex interactions with other melanoma susceptibility genes and further biochemical changes have been described [20].

Family history. According to current knowledge, one of the most important host factors and an indicator for heightened risk of CM and multiple primary melanomas (MPM) in particular, is a past history of melanoma in first-degree relatives. Research on the genetic pathways involved in the familial melanoma syndrome has identified CDKN2A and CDK4 as the most common mutation loci. The affected patients often present with dysplastic nevi (DN) and early forms of melanocytic cancer, even at a younger age [10].

Multiple nevi. While only about 30% of CM transform directly from melanocytic nevi, their number and the histologic subtype are important criteria for risk evaluation [10]. A large amount of congenital and acquired nevi express a considerable risk for CM, with the highest risk ratio (RR) in persons with multiple dysplastic nevi [21,22]. DN are still considered by some to have higher malignant potential than common ones and it has been speculated that an atypical nevus pattern reflects genetic alterations or environmental exposures in early life, contributing to accelerated melanomagenesis [15]. Sun exposure is an important driver for the evolution of melanocytic nevi, nevertheless, studies provided data suggesting them not to be viewed as consequential and consolidated, but rather as individual factors with additive effects in the development of CM [23].

Ultraviolet radiation. Similar to BCC and SCC, the by far most extensively studied environmental factor is sun exposure [24,25]. While UV radiation is a well-recognized risk factor in SCC, the exact role in CM is not as clear [26]. Through genetic investigation, it was possible to identify a series of pathways that lead to the malignant transformation of melanocytes [27,28]. Exogenous carcinogens like UV-A and UV-B radiation can damage the deoxyribonucleic acid (DNA) of melanocytes, which could trigger further steps in the oncogenesis [29]. In combination with alterations in certain gene loci, this development becomes more likely.
Polymorphisms in DNA-repair genes, which render this mechanism less efficiently, have been identified as low-penetrance cancer susceptibility genes [30,31]. While examining patients with CM, a reduced DNA repair capacity and a higher tendency to sunburn, represented by a low minimum erythema dose, have been observed

[26]. Numerous epidemiological studies investigated the association between the risk for CM and sun exposure, with findings showing strong evidence for an increased RR following a history of sunburns during childhood as a surrogate for intermittent, intense exposure to UV radiation [25,32].

Other factors. Immunosuppression seems to be a driving force in the development of CM, as higher numbers of melanoma have been found in patients with acquired or iatrogenic immunodeficiency. Ionizing radiation, vinyl chloride, polychlorinated biphenyls and petrochemicals have been discussed as further risk factors, however, the degree of significance is speculated to be low [10]. Following observations of an increased risk in women with breast cancer or during pregnancy, past studies investigated potential hormonal influences. By the current state of knowledge, no indications exist for a negative impact on the survival [33].

1.1.3 Clinical presentation

Diagnose. The importance of prevention, efficient screening and an early diagnose of CM is nowadays a well-established fact and has been postulated by numerous authors in the past [15,34]. Regarded as the effective and most widely used approach for an early detection is the self-examination performed by the patient and a regular inspection through a healthcare professional. It is only feasible if both, patients and treating physicians, are involved in this process. While utilizing acquired knowledge about the disease and following the guidance of the dermatologist, the person at risk for CM can ultimately help to detect melanomas in an earlier, possibly still in an in situ form form. The trained clinician got several diagnostic options at hand. From physical examination and evaluation of suspected lesions with the naked eye to more in-depth study using dermatoscopy, full-body photography or confocal microscopy, the aim is to spot suspicious pigmented lesions, differentiate between benign and malignant and thereby increase early detection and reduce the rate of unnecessary excisions. The standard for clinically equivocal lesions remains excision and subsequent histological examination. With a steady increase in incidence, these tactics are the key factors to ensure the best possible outcome of this, in many ways preventable disease [35].

Histopathologic subtypes. Melanoma shows two phases of growth, a horizontal spreading at the dermal-epidermal junction followed by a vertical infiltration to deeper layers of the dermis. Based on the clinics and the tumor growth pattern, CM is classified into four major subtypes (Table 1) [36].

Subtype	Growth pattern	Typical clinical features	% of CM*
Superficial spreading melanoma (SSM)	Mainly horizontally with latent infiltration	Associated with intermittent sun exposure, presents most frequently on women's legs and men's trunk	54,6%
Nodular melanoma (NM)	Rapid vertical growth often with ulceration and early metastasis	Shows no distinct predilection site and is generally found in older individuals.	24%
Acral lentiginous melanoma (ALM)	Biphasic, initial radial growth followed by rapid vertical spread	Occurs on soles, palms and subungual sites with the highest incidence in people of African or Asian descent.	8,2%
Lentigo maligna melanoma (LMM)	Latent proliferation from precursor lesion	Commonly found in severely sun damaged skin of the head and neck area of elderly patients	4,5%
*Data used from Elsaeßer et al.[37]: n=1819, Melanoma not other specified n=158 (8,7%)			

Table 1: Classification of histopathologic subtypes of CM and clinical presentation

Tumor thickness. The depth of invasion, also known as Breslow thickness, is a valuable indicator for the advance and prognosis of the CM [38]. Assessed from histological slides by a trained pathologist, the thickness is measured in millimeters (mm) from the top of the stratum granulosum to the deepest point of infiltration of the malignant melanocytes. Measurements are rounded to the nearest 0.01 mm. While thin melanomas tend to have a lower tendency to metastasize, deep invasion (2.0 mm or more) is a negative marker for disease-free survival (DFS) [3].
In comparison to the assessment of the deepest infiltrated microscopic layer of the skin (i.e. level of invasion) postulated by Clark et al. [39], the prognostic value of tumor thickness has been shown to be greater and is implemented in the current American Joint Committee on Cancer (AJCC) staging system [40]. While being considered the most important prognostic factor in early stages, more aspects have to be taken into account to estimate DFS in progressive disease [41].

Ulceration. Formerly described as an interruption of continuity in the epidermal layer overlying the primary melanoma, microscopic ulceration is another important feature in the staging of CM [42]. A more comprehensive approach widely used by pathologists defines melanoma ulceration *as the combination of a full-thickness epidermal defect, evidence of reactive changes and thinning, effacement, or reactive hyperplasia of the surrounding epidermis* [43]. The presence of ulcerations result in a more negative outcome with reduced DFS in comparison to non-ulcerated melanoma with the same Breslow thickness, and is therefore used as a parameter in the AJCC staging system [40]. Furthermore, patients with ulcerated melanomas in advanced stage show an increased response to adjuvant pegylated-interferon resulting in improved long-term survival rates [44].

Mitotic rate. As proposed by Schmoekel and Braun-Falco in 1978 [45], the sum of mitoses is counted in the histological slides under 400-fold magnification (i.e. high-power field) and converted to number per square millimeter. As an independent negative predictor of survival, a mitotic count of 6/mm^2 or greater goes along with a high probability of metastasis [46]. By studying a cohort of patients with thin melanoma (defined as having a Breslow thickness ≤1.0mm), a significant association between increased mitotic activity in the tumor nests and sentinel lymph node (SLN) positivity was found [47]. In the 2009 7th edition of the AJCC cancer staging manual, early-stage melanomas with an increased mitotic rate of ≥1/mm^2 get uprated to the next T-category and share a similar prognosis with thick melanomas lacking mitotic figures [40]. In the newly revised 8th edition, mitotic rate is recommended to be assessed and reported but is dropped as a staging criterion [48].

Staging. The TNM classification, built on the three categories tumor-node-metastasis, is the most widely used system to classify malignant tumors, representing the basis for the current versions of staging guides. Developed by Pierre Denoix [49], it has been pursued and continuously updated by the International Union Against Cancer (UICC) [50] and adapted by the AJCC [40]. The purpose of the TNM system is to document the anatomical spread of cancer in a standardized manner, through clinical examination and histological evaluation. In comparison to the UICC classification with a sole focus on the extent of the disease, the AJCC staging manual includes various prognostic factors, depending on the specific type of tumor. In the case of CM, the lesion gets categorized in the T section by thickness, mitotic rate and presence of ulceration. The number of metastatic lymph nodes defines the N- and the spread to distant sides the M-subgroup. With this set of variables, the patients can be assigned to stage groups, reflecting the estimated survival rate. The current AJCC staging system is listed below (Table 2).

TNM - Classification			Clinical Staging			
T	Thickness (mm)	Ulceration Status/Mitoses	Stage	T	N	M
Tis	-	-	0	Tis	N0	M0
T1	≤ 1.00	a: Without ulceration/mitosis <1/mm^2 b: With ulceration/mitosis ≥1/mm^2	IA	T1a	N0	M0
T2	1.01 – 2.00		IB	T1b	N0	M0
T3	2.01 – 4.00	a: Without ulceration		T2a	N0	M0
T4	> 4.00	b: With ulceration	IIA	T2b	N0	M0
N	No. of metastatic nodes	Nodal Metastatic Burden		T3a	N0	M0
N0	0	-	IIB	T3b	N0	M0
N1	1	a: Micrometastasis		T4a	N0	M0
N2	2 – 3	b: Macrometastasis	IIC	T4b	N0	M0
N3	4 + metastatic nodes	c: in transit metastases/satellites without metastatic nodes	III	Any T	N > N0	M0
M	Site	Serum LDH	IV	Any T	Any N	M1
M0	No distant metastases	-				
M1a	Distal skin, subcutaneous, or nodal metastases	Normal				
M1b	Lung metastases	Normal				
M1c	All other visceral	Normal				
	Any distant metastasis	Elevated				

Table 2: AJCC 2009 staging system for melanoma of the skin

1.2 Multiple primary melanomas

Definition. MPM are defined as either more than 1 melanoma or subsequent melanomas, distinguished from potential metastasis, in patients with a prior history of CM. The reported incidences of a second primary melanoma are not uniform, ranging from 1% to more than 10%. Differences in study design and composition of the study population most likely account for this variation. In at-risk patients, the rate for a second primary melanoma is 2% in the first year after the diagnosis of the first melanoma and continuously 1% in the following years. [51]

Differences. There are possible epidemiological differences in patients with MPM regarding the age of first diagnose of the index primary melanoma. A higher incidence of MPM in elderly patients with a late onset of the first melanoma has been described. According to the authors of the study, alteration in immunocompetence as a result of the aging process could be the driving factor in this observation. The research group found a high percentage of the consecutive primary melanomas presenting within 6 months from the initially diagnosed melanoma in patients aged 65 or more. Furthermore, they reported an accumulation in occurrences of sequential melanomas after a median of 5 years. Therefore, regular surveillance of these patients is recommended [52]. Past clinical studies have described a trend for thinner consecutive melanoma when compared to the initial primary melanoma [51,53,54]. The identified exogenous risk factors for MPM are basically the same as for single CM. Multiple DN and a family history of melanoma have been identified as risk factors in patients developing MPM [55,56].

1.3 Aim of this study

With a still rising incidence in populations of Europe, Australia or the USA, progress in the detection and management of CM is crucial. While a vast amount of cofactors and associations have been investigated in persons presenting with MPM, no distinct scientific approach has been made in the field of possible clinical differences in this disease related to gender. With this study, we would like to contribute a part and hopefully encourage further research on this topic.

2 Material and Methods

2.1 Data

2.1.1 Patients

Inclusion & exclusion criteria. The study was approved by the local ethics committee (EK-Nr: 1519/2017) We searched the database of the Dermatology Department of the University Clinic of Graz for patients with MPM between 2010 and 2016. No restrictions regarding age or sex were set. We considered data entry acceptable when the last appointment at the clinic was not earlier than 2010 to ensure up-to-date medical history. Previously known mutations in CDKN2A or CDK4 and diagnosed Xeroderma pigmentosum were collected in order to assign the patients to risk groups. Also, data regarding history of leukemia, lymphoma, chemo-/radiotherapy or long-term immunosuppression were included.

Data. The collected patients demographics included gender, age by the end of the study collection (2016) and at time of the first melanoma, total numbers of melanomas including melanoma in situ (MIS), the highest AJCC staging, and whether or not multiple patients had a high nevus count including DN. Based on previous studies suggesting a higher risk after the age of 65 years, patients were assigned to two age groups (< 65 years and ≥ 65 years) [57].

2.1.2 Melanoma

Reevaluation. After the identification of the patients meeting the inclusion criteria, the past medical histories were collected from the patients' hospital documentation sheets. To rule out recurrent melanoma and metastatic melanoma, we reevaluated the clinical and histopathologic reports following the suggestion of Kornberg et al. [58]. Patients with indication of metastatic or recurrent disease, who did not reach a count of 2 or more primary melanomas, were excluded from the study collective.

Data. From the medical reports, we extracted the relevant clinical data of the individual lesions. This includes Breslow thickness measured in mm, date of first diagnose and body site. The locations were defined as follows: head and neck,

trunk, upper extremity, lower extremity, and special anatomic body sites, summarizing melanomas presenting at acral surfaces, the ear, scalp, flexural areas and surfaces along the embryonic milk line (i.e. breast, axillae, umbilicus, genitalia).

From the collected data, new variables were generated. T-classification was defined by Breslow thickness, presence of ulceration and reported mitotic rate following the AJCC melanoma staging manuals of 2009 and 2017, respectively. The time between diagnoses of the first and subsequent melanomas was calculated in months using the date on the pathology report. Melanomas occurring within 1 month of the last diagnose were specified as synchronous lesions, all others as metachronous.

2.2 Statistics

The obtained data was organized with the spreadsheet software Microsoft Excel 2011. The statistical analysis and creation of graphical depiction were performed in IBM SPSS 23. The data was specified as follows:

Patient		
Variable	**Value**	**Scale**
Sex	female/male	nominal
Age at 1st melanoma	in years	metric
Age group	< 65 years, ≥ 65 years	ordinal
Dysplastic nevi	yes/no	nominal
High-risk group	yes/no	nominal
Highest AJCC-Stage	0, IA, IB, IIA, IIB, IIC, IIIA, IIIB, IIIC, IV	ordinal
Total amount of melanomas	number	metric
Portion of melanomas in situ	number	metric
Observation time	in months	metric

Table 3: Structure of the patient data

Melanoma		
Variable	**Value**	**Scale**
Melanoma number	figure (01, 02, 03 …)	nominal
Breslow's depth	in millimeters	metric
Mitotic rate ≥1/mm^2	yes/no	nominal
Ulceration	yes/no	nominal
Location	head/neck, trunk, upper extremity, lower extremity, special	nominal
T stage AJCC 2009	Tis, T1a/b, T2a/b, T3a/b, T4a/b	ordinal
T stage AJCC 2017	Tis, T1a/b, T2a/b, T3a/b, T4a/b	ordinal
Time to next melanoma	in months	metric
Synchronicity	yes/no	Nominal

Table 4: Structure of the melanoma-related data

Descriptive statistics & exploratory data analysis. For quantitative variables, the arithmetic mean with 95% confidence interval (CI) and median were reported as measures of central tendency. To characterize dispersion, the quartile and standard deviation (SD) were calculated. Histograms and Q-Q Blots were generated for graphical depiction of the data. Following initial review, the Kolmogorov-Smirnov test for normality was executed. For continuous variables with a normal distribution of values, the skewness was specified additionally. To visualize assumed differences between two groups, box blots were created, composed of median, quartile, whiskers from minimum to maximum values and as appropriate the outliers (marked with °) or extreme values (marked with *). Qualitative variables were interpreted through frequency tables and illustrated with bar graphs or circular charts. Median and the interquartile range (IQR) were used as measures of dispersion. When comparing groups with different case numbers, relative frequencies were stated.

Inferential statistical analysis. The assumed differences in distribution were then tested for statistical significance. The null hypothesis H_0 was rejected when the probability for a random output was smaller than 5% (i.e. *p*-value < .05). Non-parametrical tests were used for interval and metrical scaled data without normal distribution. For independent variables, the Mann-Whitney U test was performed. In order to verify differences in frequencies between defined groups, nominal and ordinal data was sorted in cross-tabulations and tested with Pearson's chi-squared (χ^2 test) or Fisher's Exact Test.

3 Results

3.1 Patients

We identified 135 patients presenting with consecutive melanoma between the beginning of the year 2010 and the end of 2016. From this collective of eligible subjects, a total of 48 were excluded after revision of the clinical data because of missing complete data. Following reevaluation, in 32 cases the consecutive lesions were specified as secondary melanoma resulting in a total count of fewer than two primary melanomas. Thereof, 21 were classified as recurrences and 11 lesions were metastases. After failing to appear for scheduled examinations, 5 patients with their last appointment before 2010 were excluded. 11 patients were excluded due to missing clinical data. The study cohort is illustrated in Figure 2.

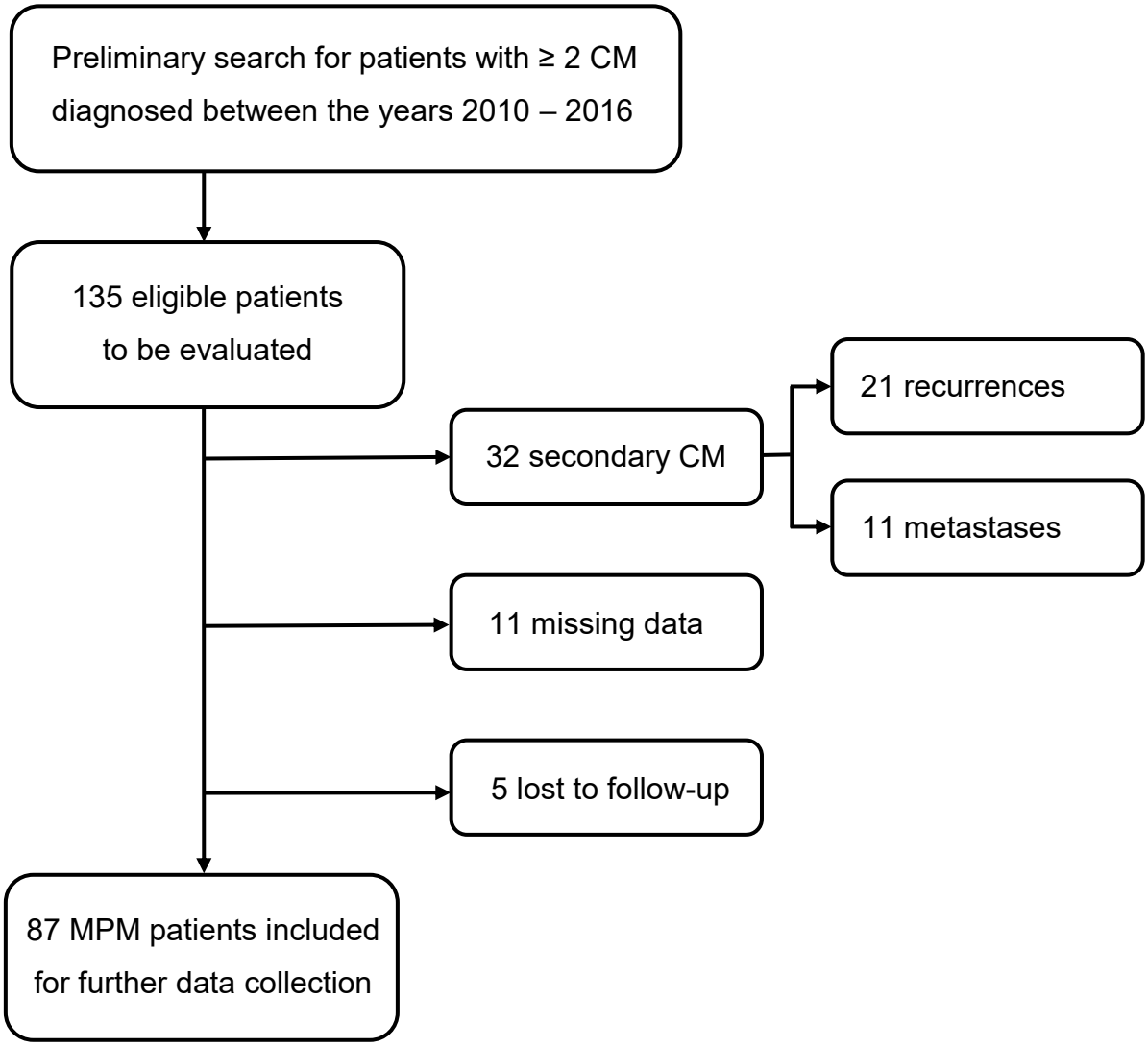

Figure 2: Study cohort of 87 MPM patients suitable for statistical analysis

The definite study cohort consisted of 87 patients including 40 women (46%) and 47 men (54%). The mean age of the entire cohort was 68 (SD 14.34, median 70) years with a range from 34 to 92 years. The observation time ranged from 0 to 521 (mean 95.6, SD 97.9, median 69, IQR 96) months from the data of the first melanoma diagnosis. Overall 51 of the patients (58.6%) were younger than 65 years at time of their first melanoma diagnosis, while 36 (41.4%) developed melanomas after 65 years of age. The mean age at first melanoma diagnosis was 57.6 (SD 15.6, median 61, IQR 26) years. The youngest and oldest patients diagnosed with first melanoma were aged 20 and 90 years, respectively. The overall distribution of age at initial diagnose of melanoma is depicted below in Figure 3.

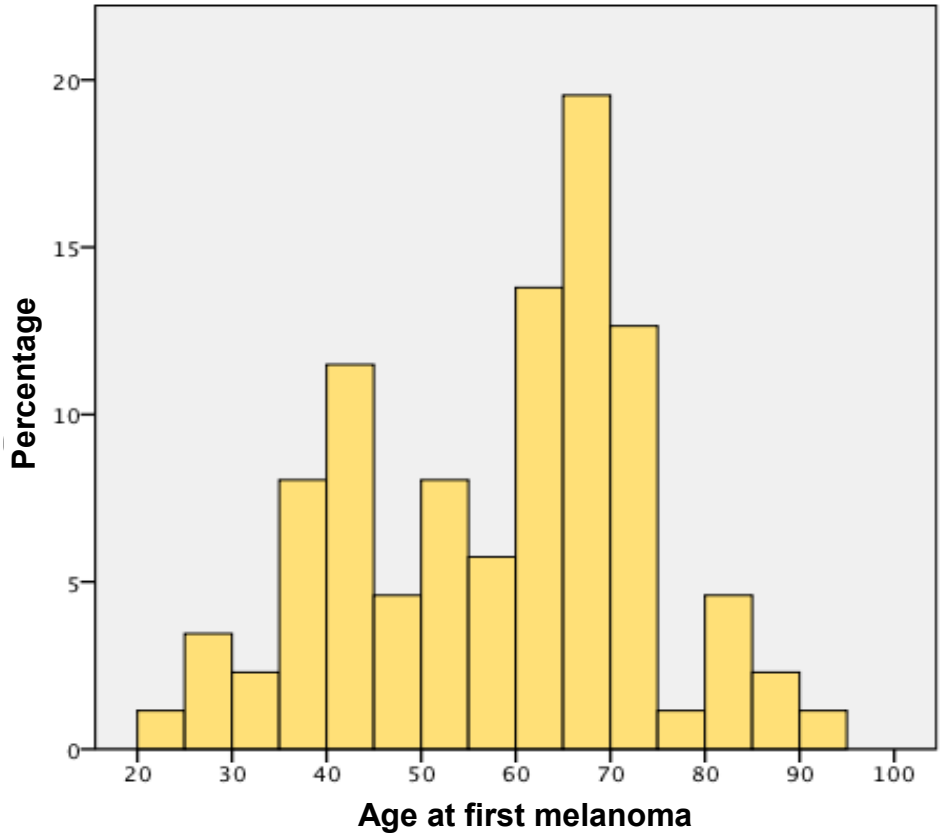

Figure 3: Distribution of age at first diagnosis in our study collective

When dividing the collective into two groups regarding the presence or absence of multiple DN, 36 patients (41.4%) had multiple DN compared to 51 patients (58.6%) without them.

Moreover, 9 patients (10.3%) had additional high risk factors for melanoma. Five patients had an impaired immune system including 3 patients with a history of lymphoma, 1 with a kidney transplant and 1 had a history of chemotherapy because of an anal carcinoma. Moreover, 3 patients had a dysplastic nevus syndrome with confirmed CDKN2A mutation in one case and 1 patient had Xeroderma pigmentosum.

In our cohort of 87 patients, 44 (49.4%) patients had 2 primary melanomas, 24 developed (27.6%) 3 melanomas, and 11 (23%) were diagnosed with 4 or more melanomas. The mean number of melanomas per person was 3.0 (SD 1.45, median 3, IQR 1). Non-invasive forms of melanoma (i.e. MIS) represented 35.6% (n=31) of all examined melanomas. Metastases were reported in 5 (5.7%), spreading to lymph nodes in 15 patients (17.2%). The break down of the study collectives considering the highest diagnosed AJCC-Stage can be observed in the following Table 5.

		Total		Sex			
				female		male	
		Count	%	Count	%	Count	%
AJCC-Stage	0	2	2.3%	2	5.0%	0	0.0%
	IA	43	49.4%	22	55.0%	21	44.7%
	IB	13	14.9%	5	12.5%	8	17.0%
	IIA	7	8.0%	3	7.5%	4	8.5%
	IIB	4	4.6%	3	7.5%	1	2.1%
	IIC	3	3.4%	0	0.0%	3	6.4%
	IIIA	3	3.4%	1	2.5%	2	4.3%
	IIIB	3	3.4%	1	2.5%	2	4.3%
	IIIC	4	4.6%	2	5.0%	2	4.3%
	IV	5	5.7%	1	2.5%	4	8.5%

Table 5: Total count and percentages of patients divided per highest AJCC-Stage

3.2 Melanoma

The 87 patients in our cohort developed overall 263 melanomas (mean thickness = 0.79 mm) between January 1973 and July 2016. The range of melanomas per

person varied from 2 up to 9 melanomas. Data regarding the Breslow thickness were available in 254 cases, with 49 (18.6%) of the lesions classified as in situ. The mean tumor thickness for invasive melanomas was 0.98 (SD 1.36, median 0.6, IQR 0.5) mm. The mean tumor thickness of all first melanomas was 1.29 (range 0.00 to 15) mm compared to 0.55 (range 0.00 to 3.80) mm for all subsequent cases. With 78.1%, the majority of the lesions were classified as T1. When comparing the former and present edition of the AJCC manual, according to the 2009 version 157 lesions were specified as T1a and 3 as T1b, while 137 T1a and 23 T1b were diagnosed following the version of 2017, respectively. No changes were made for the other categories and the rest of the melanomas were staged as follows: 26 T2 (12.7%), 14 T3 (6.8%), and 5 T4 (2.4%).

Mitotic rate and ulceration. Histopathology charts reported on ulceration in 8 cases and an increased mitotic rate in 2 cases.

Synchronicity and time to next melanoma. Of the 176 subsequent melanomas, 45 (25.6%) presented synchronously (i.e., diagnosis within 1 month of the initial diagnose). In metachronous melanomas (n = 131 cases), the time to the subsequent melanoma was at average 35 (IQR 54, mean 47.63, SD 47.63) months.

Lesion site. In 259 cases, data on the location of the melanoma was available. The overall case numbers and the breakdown of patients regarding gender can be found in Table 6. The by far most common site of emergence was the trunk (47.2%) followed by upper extremity (21.2%), lower extremity (15.8%), head/neck (10.8%), and special areas (5.0%).

			Count	%
Location	head/neck	total	28	10.8%
		male	11	8.2%
		female	17	13.6%
	trunk	total	122	47.2%
		male	86	64.2%
		female	36	28.8%
	upper extremity	total	55	21.2%
		male	22	16.4%
		female	33	26.4%
	lower extremity	total	41	15.8%
		male	10	7.5%
		female	31	24.8%
	special	total	13	5.0%
		male	5	3.7%
		female	8	6.4%

Table 6: Observed numbers and percentages of CM in the 5 defined body sites

3.3 Group differences

3.3.1 Gender-related differences

Overall 47.9% (n = 126) of CM occurred in women and the remaining 137 CM were found in men.

Age at first melanoma. In women, the mean age at the first melanoma was 53.98 (SD 16.55) years. In comparison, diagnosis of melanoma in men occurred nearly 7 years later (mean age 60.66 years, SD 14.23). The data of the female patients showed a normal distribution, while the male data did not. By looking at the median of 52.5 years (IQR 26) in female versus 65 years (IQR 15) in men, respectively, the difference in dispersion is recognizable and was tested to be statistically significant (p = .031). A graphical depiction of the distribution can be seen in Figure 4.

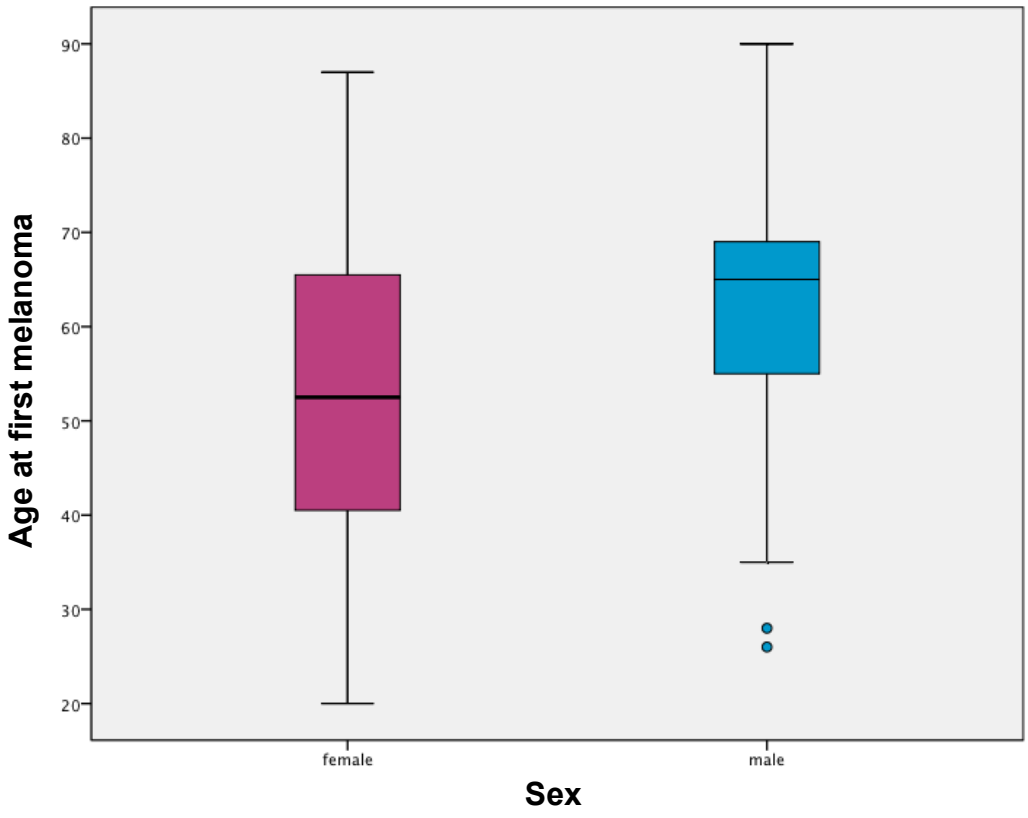

Figure 4: Distribution of age at first melanoma in female and male

Dysplastic Nevi. The presence of DN was marginally higher in women (42.5% vs. 40.4%) without statistical significance ($p > .05$). When comparing the age groups with respect to gender, we found a female predominance in the younger age group (57.2% vs. 52.2%) and a higher percentage of men in the age group ≥ 65 years (29.2% vs. 8.3%). However, χ^2 test showed no statistical significance.

High-risk group. There were no differences in the frequency of high risk factors between women and men (10.0% of female vs. 10.6% of male).

Highest AJCC-Stage. The majority of patients (67.8%) were diagnosed with stages below IIA. The percentages for the highest AJCC-Stage by gender can be found in Table 6 (page 29). Comparing the gender groups, the median stage was stage IA for women and IIA for men, respectively ($p = .095$). A total of 27.5% women had a stage above IIA compared to over a third (38.3%) of men. In Figure 5, an increment of men in higher stages is noticeable.

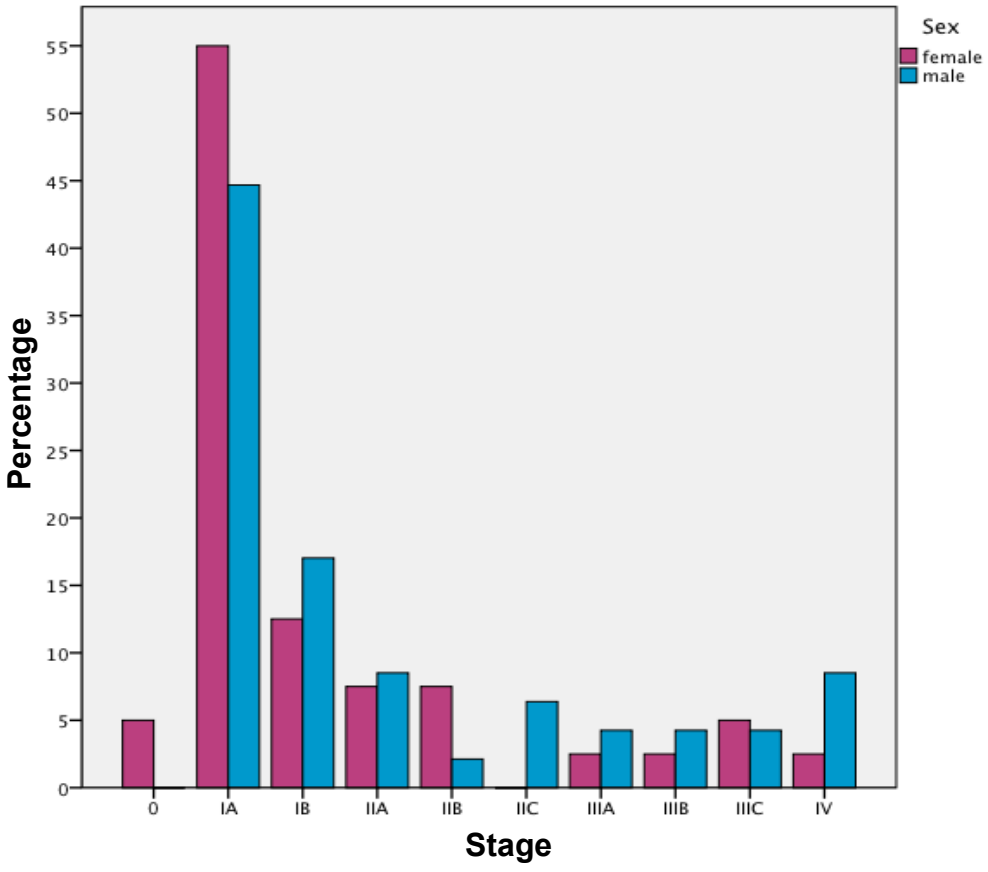

Figure 5: Ratios of highest diagnosed AJCC-Stage in the study population

Amount of melanomas. The count of melanomas per patient was widely variable (Figure 6). The median number of melanomas per female patient was 3 melanomas (IQR 2, mean 3.13, SD 1.68) and 2 women had overall 9 melanomas. Instead median number of melanomas in men was 2 (IQR 1, mean 2.89, SD 1.26) and 1 man had 7 melanomas. The mean rate of MIS showed slight variation with an average of 0.78 (SD 1.09) lesions in female and 0.43 (SD 0.85) lesions in male, respectively. Neither in the total amount of melanomas nor in the fraction of MIS, differences of the distribution were shown to have statistical significance.

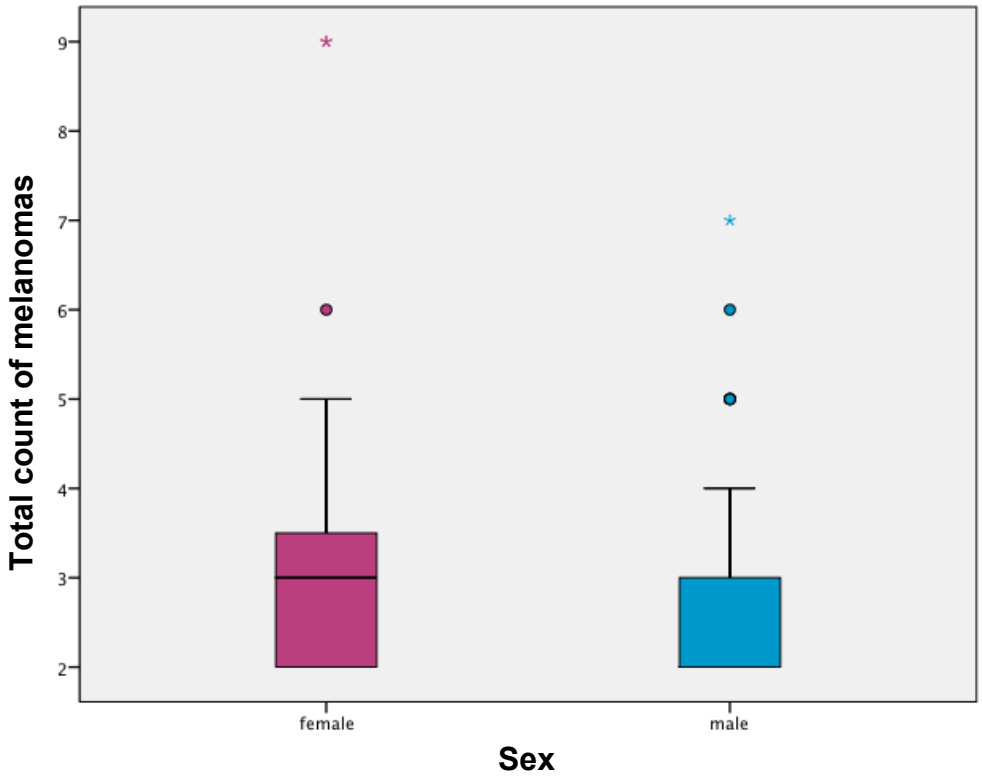

Figure 6: Distribution of melanoma count per person between sexes

Synchronicity and time to next melanoma. The 45 synchronous CM were evenly distributed among women and men (46.7% and 53.3%). Excluding synchronous melanomas from the analysis of time until the diagnosis of the next melanoma, a total of 131 lesions (65 in female, 66 in male) remained. In women, subsequent CM appeared at average 15.82 months earlier than in men. The median time to the subsequent melanomas was 29 months (IQR 46) in women and 40 months (IQR 62) in men, respectively. However, this difference did not reach statistical significance (p = .081).

Lesion site. The majority of melanomas in both genders were located on the trunk (64.2% and 28.8%, respectively), although there was a male predominance for this site. For the remaining body sites, the frequency of melanomas in women was higher (Figure 7). In descending order, the frequency of melanoma in women in comparison to men were as follows: upper extremity (26.4% vs. 16.4%), lower extremity (24.8% vs. 7.5%), head/neck (13.6% vs. 8.2%) and special sites (6.4% vs. 3.7%). The null hypothesis, stating an equal site distribution of lesions between sexes, was rejected with a *p*-value < .001.

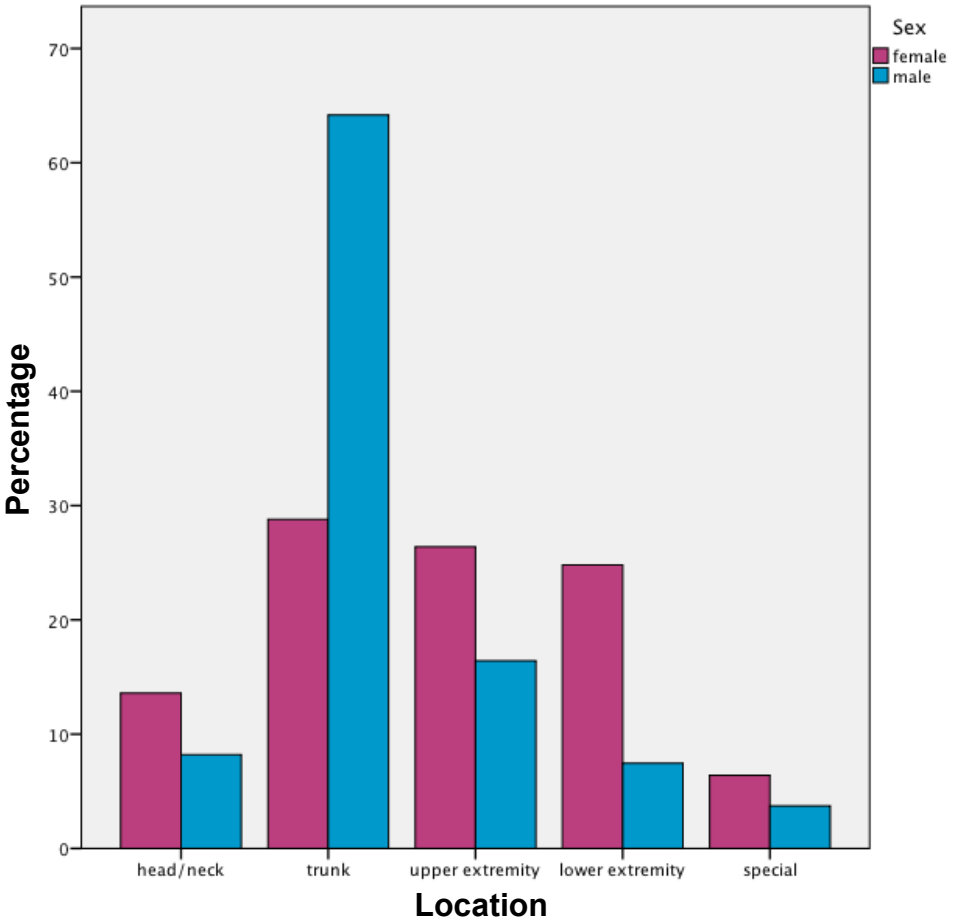

Figure 7: Anatomic distribution of lesions with regard to sex

Breslow thickness. In both genders, the median for depth of infiltration was 0.5 mm (IQR 0.57 female and IQR 0.59 male). The mean thickness was 0.65 mm (SD 0.75) in women and 0.92 mm (SD 1.61) in men, respectively. The variations were not statistically significant (*p* = .52). While 30 (24.8%) of melanomas in women were in situ, only 19 (14.3%) of in situ melanomas were encountered in the male group (*p* = .032).

3.3.2 Dysplastic nevi

Age at first melanoma. Patients without DN had a melanoma diagnosis at average 14 years later than individuals with DN (mean age 64.73 vs. 50.25 years, respectively). The median age in the group with DN was 52 years (IQR 24) compared to 65 years (IQR 16) in the group without DN (Figure 8). These findings were highly significant ($p < .001$). When the study collective was subdivided by sex, no statistic significance ($p = .113$) was found in men (54.89 years with and 63.21 years without DN). Whereas the age differences in women (42.75 years vs. 62.22 years) showed high statistic significance ($p < .001$).

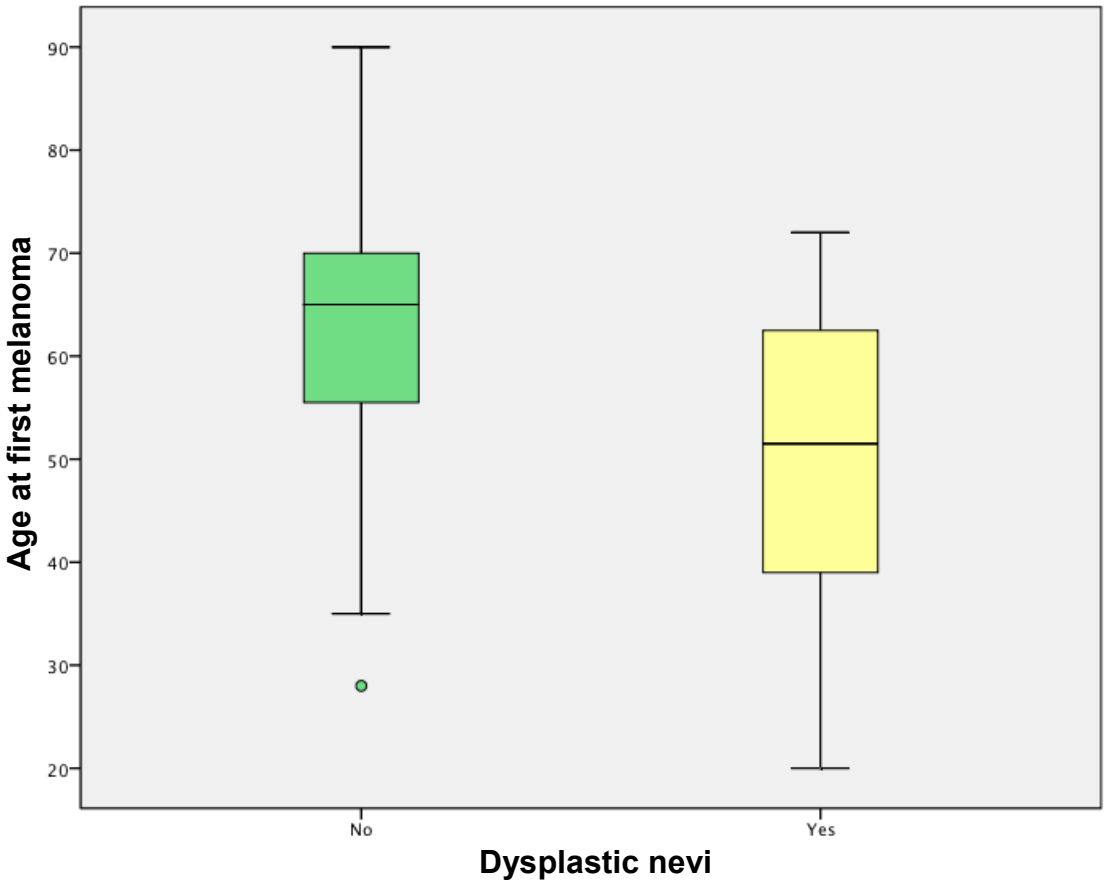

Figure 8: Distribution of age at the diagnose of the first melanoma in patients with and without DN

Observation time. The mean follow up time between patients with and without DN was 109.83 vs. 85.55 months, respectively ($p = .009$).

3.3.3 Melanoma count

Age at 1st melanoma. In the group with a total count of 3 melanomas or less, the mean age was 58.04 years (SD 15.66, median 61.5, IQR 25). On average, patients with a sum of 4 melanomas or more were aged 54.45 years (SD 15.64, median 54, IQR 30). No statistical significance was reached (p = .641).

3.3.4 Consecutive Melanoma

Melanoma in situ. The percentage of invasive melanomas reduced notably from the first to the following ones. While only 8.4% of all first diagnosed CM were in situ, the percentage steadily rose to 19.0% in the second, 20.5% in the third and 23.8% in the forth melanoma. In melanoma number 5 to 9, more than half of the lesions (54.5%) were non-invasive. The findings were statistically significant (p <.05).

Breslow thickness. Furthermore, the overall depth of infiltration decreased in subsequent melanomas in comparison to the initial lesion. To enable an execution of the Mann-Whitney U test, we assigned the lesions into 5 groups according to the number of melanoma. As seen in Table 7, in men the reduction of Breslow thickness from the first melanoma was statistically significant in all following melanomas. In contrast, melanomas in women did not show a constant decreasing depth of infiltration.

			Breslow thickness				
			Mean	SD	Median	IQR	*p*-value*
Melanoma number	1st	total	1.29	1.97	0.75	1.00	
		female	0.94	0.98	0.70	0.73	-
		male	1.60	2.52	0.75	1.10	
	2nd	total	0.64	0.70	0.50	0.41	**.002**
		female	0.54	0.48	0.50	0.76	.072
		male	0.73	0.83	0.50	0.45	**.007**
	3rd	total	0.49	0.53	0.45	0.30	**< .001**
		female	0.47	0.65	0.20	0.50	**.007**
		male	0.51	0.39	0.50	0.45	**.002**
	4th	total	0.50	0.42	0.50	0.69	**.016**
		female	0.51	0.46	0.50	0.90	.188
		male	0.49	0.40	0.50	0.60	**.036**
	≥5th	total	0.32	0.66	0.00	0.43	**< .001**
		female	0.49	0.84	0.2	0.69	.017
		male	0.12	0.24	0.00	0.21	**< .001**

Table 7: Infiltration depth from first to last melanoma in millimeter,
*Mann-Whitney U test executed for differences from initial lesion

Time to next melanoma. The count and frequency of lesions subdivided into time periods from last diagnosed melanoma can be found in Table 8. A total of 44.3% of all subsequent CM were diagnosed within the 1st following year. The percentage of synchronicity increased until the 4th lesion. Melanoma numbers 5 to 9 were less likely to be diagnosed simultaneously or within 1 month from the prior lesion. Nearly half of them (47.8%) were detected within the first 2 years. A considerable number (22.7%) of MPM arose after 5 years from the previous diagnosed CM.

Time group		Number of melanoma				Total
		2nd	3rd	4th	≥ 5th	
synchronous	Count	11	8	8	2	29
	%	12.6%	17.8%	38.1%	8.7%	16.5%
within the 1st year	Count	25	14	4	6	49
	%	28.7%	31.1%	19.0%	26.1%	27.8%
within the 2nd year	Count	7	4	2	5	18
	%	8.0%	8.9%	9.5%	21.7%	10.2%
within the 3rd year	Count	15	2	1	1	19
	%	17.2%	4.4%	4.8%	4.3%	10.8%
within the 4th year	Count	6	5	0	3	14
	%	6.9%	11.1%	0.0%	13.0%	8.0%
within the 5th year	Count	1	2	3	1	7
	%	1.1%	4.4%	14.3%	4.3%	4.0%
after the 5th year	Count	22	10	3	5	40
	%	25.3%	22.2%	14.3%	21.7%	22.7%
Total	Count	87	45	21	23	176

Table 8: Cross tabulation of sequential melanoma subdivided by years from respective prior lesion

The ratios for the time periods of occurrence are illustrated in Figure 9. As stated above, two peaks of high frequency can be observed. In addition, there was a variation of rate for lesions after the 5th year between sexes (16.3% of female vs. 28.9% of male, respectively). Fischer's exact test showed no statistical significance (p = .481).

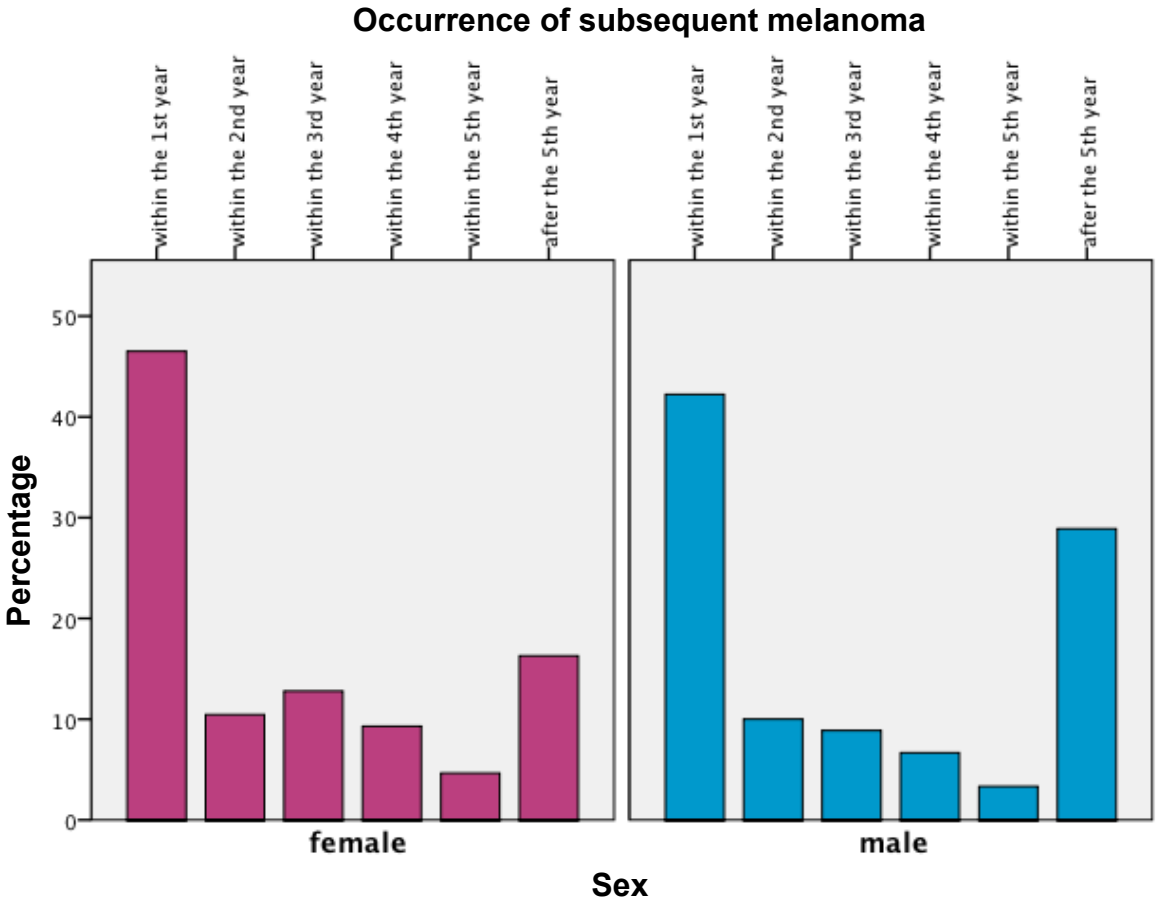

Figure 9: Frequency of lesions in women and men subdivided into time periods

By analyzing the years between diagnoses of CM, we found a descending median from prior lesion until the 4th lesion (Figure 10). The differences were not statistically significant (Table 9). Various outliers characterized the findings.

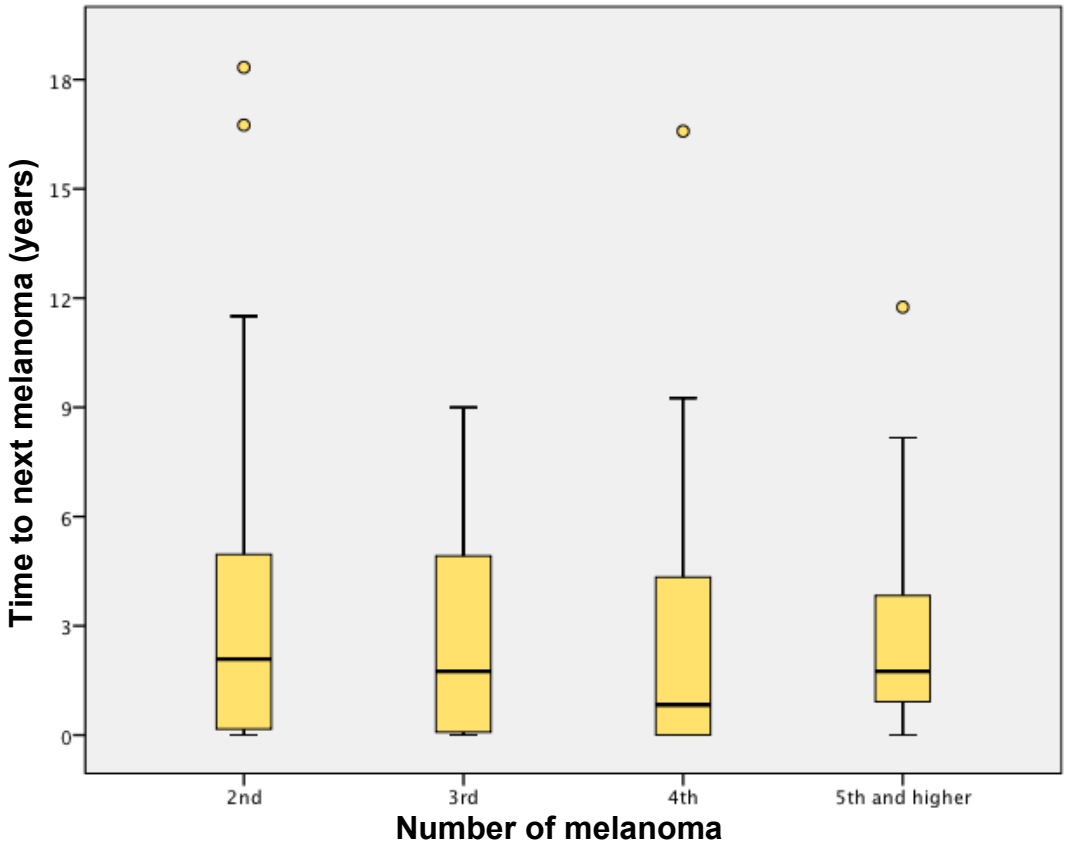

Figure 10: Average time in years between the diagnoses with marked outliers

		Time to next melanoma in years				
		Mean	SD	Median	IQR	*p*-value*
Melanoma number	2nd	3.32	4.44	2	5	-
	3rd	2.45	2.59	1.75	5	.557
	4th	2.66	5	.83	4	.325
	5th and higher	2.89	3	1.75	3	.153

Table 9: Years to the subsequent melanoma, *Mann-Whitney U test executed for differences from prior lesion

4 Discussion

The steady rise of incidence in fair-skinned populations resulted in extensive epidemiological research on CM in the last decades. Grand scale multicenter observations and genetical studies were conducted to identify risk factors and gain insight into the pathogenesis of this malignant skin tumor. This led to the development of improved therapeutic options, namely immunotherapies and molecular targeted therapies [59,60]. Besides the promising effects of the new therapy regimens, the screening for melanoma and progress in the field of early detection helped to substantially increase the probability of survival in the past [61]. Nevertheless, the entirety of influences and risk factors for the development of MPM has yet not been uncovered.

By analyzing the clinical data of patients with MPM and subsequently the available variables of the tumors, we were able to show gender-specific differences and clinical trends in this specific patient collective.

Incidence. The currently available epidemiological data (2014) [62] on the incidence of melanoma in Austria shows an ASR (European standard population) of 21.1 per 100.000 persons (Figure 11). Consistently to other European countries, a steady increase of incidence from the 1970s up to the present has been reported and is projected to continue. In the course of analyzing the epidemiological data of the Austrian cancer registries, *Duschek et al.* [63] have found gender-related differences in this development. The steepest rise of incidence rate was observed in middle-aged men, with a flattening in both sexes in ages over 65. From an equal risk for melanoma in the beginning of the 1980s, nowadays the overall incidence in men is significantly higher.

Surprisingly, in outpatient clinics around the globe, a greater percentage of women are reported to undergo skin examination. While this problem of sex-specific attitudes towards CM screening and dermatological checkup was described in the past [64,65], this bias is expected to only have a marginal impact on our study collective. The majority of the patients have been under medical guidance and surveillance for long-time periods and one can therefore assume that female and male patients in our study share an equal sensitivity for consecutive lesions of the skin.

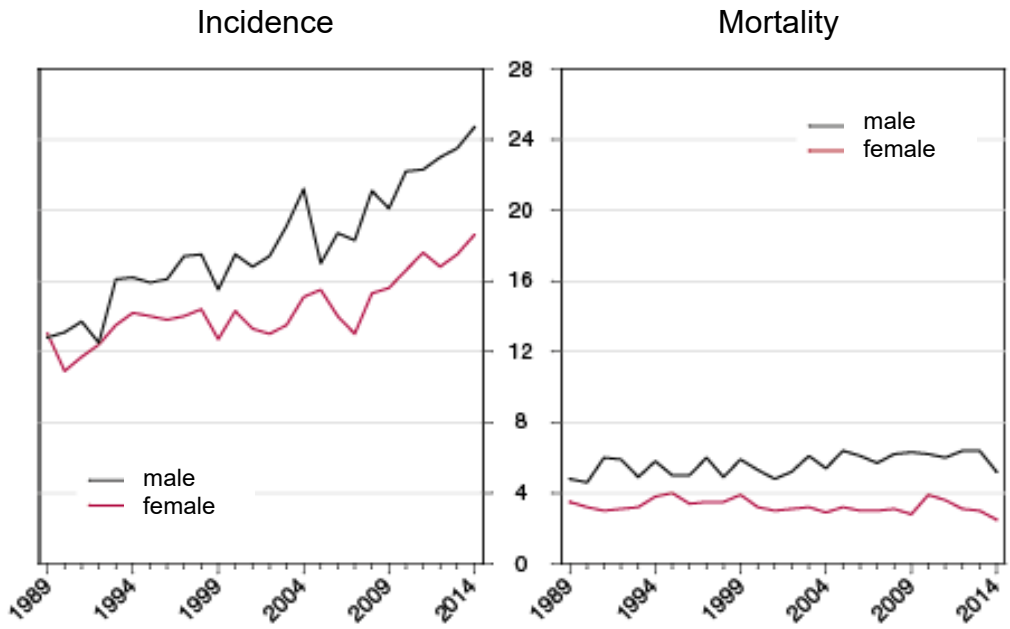

Figure 11: Cutaneous melanoma incidence and mortality ASR per 100.000 Austrian citizens from 1989 to 2014

The sex ratio in our study collective was virtually equal, with a slight preponderance in men. These findings are coherent with incidence data from the Austrian cancer registries [12], revealing a 1.1 ratio of men over women in the last decades. Furthermore, a comparable study conducted by Savoia *et al.* [66] in Italy showed a similar distribution of MPM between the sexes. In contrast to these observations, northern European countries reported a higher incidence of CM in women in the past [67]. Likewise, Menzies et al. [68] described a higher number of women in their Irish MPM patients. Considerations on the cause of this discrepancy could be the higher latitude with different attitudes towards sun exposure or more frequent dermatological visits of women resulting in possible overdiagnosis.

Comparison of studies. Our data and the findings of the most recent studies on MPM collectives are listed in the following Table 10. The mean age at first diagnosis and the total number of women and men are stated as demographic characteristics. If available, data on the ratio of in situ to invasive disease and the mean Breslow thickness is specified for the first 3 lesions. Furthermore, the timing of subsequent melanoma is listed.

	Rogatsch 2017 Austria n = 87	Menzies et al. 2017 [68] Ireland n = 46	Moore et al. 2015 [51] USA n = 1122	Rowe et al. 2015 [69] Australia n = 190	Savoia et al. 2012 [66] Italy n = 270	Hwa et al. 2012 [70] USA n = 61
Patients						
Age, y						
Mean ± SD	57.6 ± 15.6	66 ± 14	64.4 ± 13.7	48.4 ± 15.9	55 (21–85) *	63.7 ± 16.0
Sex, n (%)						
Female	40 (46)	25 (54)	384 (34.2)	84 (44.2)	127 (47)	22 (36)
Male	47 (54)	21 (46)	738 (65.8)	106 (55.8)	143 (52)	39 (64)
Melanoma						
Invasiveness, n (%)						
1st	87	46	1122			
In situ	7 (8.4)	11 (23.9)	476 (42.4)			
Invasive	80 (91.6)	35 (76.1)	646 (57.6)			
2nd	87	46	1122			
In situ	16 (18.4)	24 (52.2)	599 (53.4)			
Invasive	71 (81.6)	22 (47.8)	523 (46.6)			
3rd	45	11	172			
In situ	9 (20.0)	7 (63.6)	92 (53.5)			
Invasive	36 (80.0)	4 (36.4)	80 (46.5)			
Breslow thickness						
Mean mm ± SD						0.96 (0.2–12) º
1st	1.29 ± 1.97		1.05 ± 1.34	0.85 ± 0.88	1.48 ± 1.44	
2nd	0.64 ± 0.70		0.83 ± 1.21	-	0.60 ± 1.44	
3rd	0.49 ± 0.53		0.85 ± 1.32	-	0.81 ± 0.24	
Timing of MPM, %						
Synchronous	16.5		2nd MM Δ		25.9	
≤ 1 year	27.8		28.5		16.7	
1 < year ≤ 5	33		40.5		28.1	
> 5 year	22.7		31		29.3	

Table 10: Comparison of MPM studies regarding study collective and clinical data
* only median age (range) provided, º only median thickness (range) provided, Δ only data on 2nd melanoma available

Age. By studying the distribution of age at the first diagnosed melanoma, we found different patterns between men and women of our study collective. In general, women were younger at time of the initial diagnosis, with 70.0% of them aged less than 65 years compared to 48.9% of the men. According to a study by *Markovic et al.* [10], approximately half of the melanoma incidence is observed in middle-aged individuals. This seems to be the case in the female part of our collective, while the incidence in men shifts towards older ages.

The difference of 12.5 years (p = .031) between the medians suggests clinical importance and could be taken into consideration in future prevention and screening programs. In comparison to our data from MPM-patients, *Joosse et al.* [71] reported a 2.6 years lower median age in women with single CM. To verify our findings, more extensive data evaluation with a control group of singular CM should be performed.

Dysplastic nevi. Past studies emphasized the influence of multiple DN on the risk for CM [22] and especially MPM [55,56,72]. The overall likelihood for the presence of DN in our study was nearly the same in women and men. When taking the age at first diagnosed melanoma into consideration, a much higher percentage of men showed DN in advanced age. Noteworthy, the overall mean age at first diagnosis of individuals with DN was 14 years lower compared to the rest of the patients. Similar findings with an average age difference of 10 years in a collective of MPM patients were published by *Kang et al.* [56].

Higher numbers of men with a late diagnose stand in contrast to the observed younger age at diagnose of female patients. This would suggest that the presence of DN is an indicator of higher susceptibility to develop CM in younger ages, with a stronger effect in female than in men. A search for confounding factors and multivariate analysis is necessary to evaluate the validity of this assumption.

Breslow thickness. The percentage of MIS was significantly lower in men, depicting a shift to higher tumor stages. This is coherent with previous reports on the incidence in the Austrian population [63]. In our study collective, invasive melanomas in women were on an average 30% thinner (0.65 mm in women vs. 0.92mm in men, respectively), although this was not statistically significant. This is most likely due to a limited sample size, resulting in weak statistical power. A significantly lower mean Breslow thickness (approximately 25% thinner) in women was described by *Moreno-Ramírez et al.* [73] in patients with early-stage singular melanoma. The data gathered by *Haenssle et al.* [74] showed significantly lower tumor thickness in young women. Nevertheless, it is not possible to draw reliable inferences on our findings because of differences in the selection of patients, making the study collectives only comparable up to a certain point.

One theory for higher Breslow thickness in men relies on the assumption that women have a higher awareness for CM and therefore are diagnosed earlier [75]. As stated before, in our view this assumption is only valid for the initial tumor and not for subsequent CM in our study. Further studies are necessary to gain insight into the probable differences between patients with singular versus multiple CM.

AJCC-Stage. Both, the number of spreading to lymph nodes and the rate of metastasizing disease, was higher in men, thus the AJCC-Stage varied between the sexes. Nearly three-fourths of the female patients were diagnosed with stages lower than IIA. Men showed higher numbers of advanced disease. While no statistical significance was found in our analysis, various sources, most recently a German study [33], described an improved clinical outcome in women over men.

Survival benefit. The increased hazard ratio in men, described by Mervic *et al.*, was only apparent in patients up to an age of 60 years. This observation could be the result of decreasing levels of sexual hormones in the menopause and andropause, which had been described to influence the response of the immune system in advanced age [76]. The overall process of decreasing immunity in the elderly, also known as immunosenescence, has a major impact on how the human organism deals with malignant cell lines [77]. To what extent the sex plays a role in this development has yet not been described in detail.

The authors came to the conclusion that the observed survival benefit of female patients in younger aged groups resulted from a combination of biological factors and varying attitudes towards screening and prevention. In an extensive analysis of prognostic factors conducted by the AJCC, a reduced hazard ratio of 0.836 was reported for female patients with localized disease in comparison to men [78]. Another survival analysis conducted by *Enninga et al.* [79], utilizing information from the US population-based SEER dataset, showed a female survival advantage in all age groups, without the confinements described in the German study collective. Conversely, an Australian study group described the female survival benefit only to be significant in ages over 60 years [80]. This highlights the heterogeneity of the studied patient collectives and the need for standardization and analysis of global data.

In principle, all three studies came to the mutual conclusion that sex has an impact on survival outcome. Therefore, further research on the underlying mechanisms in the evolution of melanoma should be conducted. One approach is to evaluate differences in the exposure and reaction to solar irradiation and environmental factors in women and men. Following minimal erythema dose testing of healthy patients, *Broekmans et al.* [81] stated a higher skin sensitivity in men. By analyzing the relationship between the daily UV index and the incidence rates of CM in regions of Europe, Australia and the United States, *Liu-Smith et al.* [82] were able to show an association between the regional UV index and the male ASRs. While UV radiation is a major risk factor for the development of CM, it cannot thoroughly explain the differences in survival. Other potential endogenous influences are the varying hormonal milieu, changes in the immune system and responses to oxidative stress [83].

To summarize, the course of disease relies on a complex interaction between exogenous, endogenous and behavioral factors, with age dependency and variations between the sexes.

Staging and survival. The AJCC revised the staging guidelines for melanoma of the skin in their 8th edition cancer-staging manual, which will be implemented for clinical use in January 2018. As in the preceding editions, the survival rate of the individual is calculated with consideration of the highest diagnosed stage. The numbers of previous CM or MIS are not taken into account. Various studies [84,85] elaborated the impact of consecutive melanoma on the long-term survival, with contradicting results. These disparities are in large part due to differences in the study concept and the chosen date for measuring the time of survival. While the calculation from the first melanoma until the endpoint introduced a positive effect on the overall survival, a measurement from the last diagnosed lesion caused the opposite. Youlden *et al.* addressed this problem in a recent publication [86]. In their analysis, they adjusted for this survival bias and found evidence for a higher risk of death in patients with MPM in comparison to single melanoma.

Lesion site. The location of CM is viable data holding clinical information and helps to draw conclusions on sun exposure pattern. Obviously, a lesion arising on the face will be detected earlier by the patient than lesions on less examinable body sites like the back or scalp. Melanomas found on the trunk have been described to show a higher rate of metastasis and thus a less favorable outcome [87]. Differences in the anatomical distribution of single CM between sexes have been extensively studied in the past [57,87–89]. Previous research seems to validate the view that CM arises most commonly on the trunk of men and the lower extremities of women. In contrast, women in our study showed a different distribution of lesions. Concordant with men (64.2%), the most common body site for CM was the trunk (28.8%). Upper extremity followed only with a marginally lower percentage (26.4%). Similarly, *Warren et al.* [89] reported the highest percentages of MPM in both sexes on the trunk (46% of the male and 29% of the female lesions, respectively). As seen in our patient collective, upper extremity ranged second in women (28%). In comparison to these findings in MPM patient cohorts, the study of *Lasithiotakis et al.* [57] showed the highest rates of singular primary melanoma in women to be on the lower extremities (42.7%). Consistently, the most common body site in men was the trunk (55.1%).

One explanation for this discrepancy of anatomic distribution in women among different studies could be changing trends in clothing and attitudes towards recreational sun exposure [88]. Studies including differently aged patients, and analyzing retrospective data should adjust for the variation in lifestyle and awareness. Based on these grounds, the analysis of clinical and epidemiological data in defined cohorts seems more plausible. In our study, a subdivision of women and men into age groups was not feasible because of resulting limited case numbers and loss of statistical power.

Consecutive melanoma. Subsequent melanomas showed a steady decrease of the mean Breslow thickness from the initial lesion. Furthermore, the rate of melanoma in situ increased significantly. This is concordant with previous reports [51,55,66,68,74,90,91]. Regular surveillance of pigmented lesions by a clinician and a heightened awareness of the patients most likely account for this development. Consecutive lesions were most likely diagnosed within the first year (44.3%) or after 5 years from the last diagnose (22.7%). This is concordant to the MPM study of *Savoia et al*. [66], with the majority of occurrences (42.6%) in the first year and a second peak (29.3%) after the fifth year. Nearly half (49.3%) of our patients were diagnosed with a second melanoma within the first two years. 12.6% of these CM were spotted at the same time as the initial lesion. Likewise, *Menzies et al*. [68] reported 70% of the second melanoma in their study were diagnosed within the first two years, with 13% presenting synchronously. Furthermore, the frequency of synchronicity increased for subsequent melanoma in our study. While in our patients the median time between diagnoses decreased, no statistically significant differences between the average time periods to consecutive lesion were shown.

The gathered data highlights the importance of follow-up care and long-term surveillance of CM patients.

Genetics. Additionally to germline mutation in CDKN2A and CDK4 [92] various somatic oncogenic alteration have been described for cutaneous melanoma and especially in MPM patients. A positive CDKN2A mutation status, almost exclusively found in familial malignant melanoma, has been identified to have a negative impact on the survival outcome in MPM patients [93]. The most extensively studied genes

BRAF, NRAS and KRAS, all part of the MAPK/ERK signaling pathway, have shown diagnostic value in past studies with the research paving the way for new therapeutic options [94–97]. Recently Egberts et al. [98] investigated the concordance of mutation pattern in subsequent melanoma in a MPM study collective. Their findings support the view that MPM show distinct heterogeneity with only 23.9% presenting with concordant genotypes of the studied mutations in BRAF, NRAS and TERT. This is supported by data published by Pellegrini et al. [99], moreover stating that these findings were not influenced by MC1R genotype. Considering this, genetic reassessment for subsequent melanoma would provide valuable information to insure ideal treatment.

Limitations. The lack of complete survival data and eligible control groups limited an in-depth analysis of clinical outcome. Furthermore, there was no general data accessible on family history of melanoma, phenotypical characteristics or information about average sun exposure. The planed analysis of digital dermatoscopic pictures had to be dropped because of limited or incomplete archived data.

It should be noted that the impact of the statistical significance of our findings is constricted due to the retrospective nature of the study. As a single-center study with a limited case number, no extensive predictions on the entirety of patients with MPM can be made.

Thoughts. Nevertheless, the described trends provide relevant information and objectives for further research. A more extensive multicenter study with appropriate control groups should be contemplated. By incorporating phenotypical, genetical and clinical data in a multivariate analysis, more valid conclusions could be drawn. Variables to include may be skin type, eye color, number of nevi, duration of occupational and recreational sun exposure, and count of blistering sunburns. Information on genetic alterations in subsequent melanomas would be complementary. Furthermore, data obtained through clinical examination like dermoscopy or confocal laser microscopy could be added [100,101].

Conclusion. The data yielded in our study suggests that women and men are equally affected by MPM. These findings are supported by comparable studies from various countries. However, the age at first diagnosed CM differed significantly between the sexes. Women were younger and, moreover, presented with lower AJCC stage. Furthermore, female patients have shown a survival benefit in past studies. Surprisingly, the reduction of Breslow thickness in subsequent lesions was only consistent in men. While more favorable staging parameters including a lower Breslow thickness implicate a better outcome, it is undetermined if they are the sole factors for the difference in mortality.

On the basis of the concept of immunosenescence and the observed preponderance of female patients aged less than 65 years, we put forward the view that protective mechanisms in older aged patients, with a majority of men, may be less capable to defend against the rise and spread of malignant cells. Another interesting finding is that the impact of DN on the age at diagnose is more pronounced in women. Undeniably, multiple DN are a major risk factor for CM and should be recorded and kept under surveillance. Furthermore, consecutive melanomas in women tend to develop within shorter time intervals. In the light of our findings, one can consider even more frequent screenings for young women with identified DN or a history of CM. In contrast, the male patients in our study showed a high percentage of melanoma arising after 5 years from the initial diagnose. The instruction and guidance of the patients are crucial to ensure qualitative self-examination and retain awareness for pigmented lesions throughout the years. Current research appears to validate the view that the count of previous melanoma is an indicator for a worse clinical outcome. Hence, a reevaluation of global data from cancer registries with focus on this question would be appealing. In conclusion, young women with DN are at high risk of developing MPM. In contrast to previous studies, the most common body site was the trunk. Sex could, to some extent, represent an indicator for predictions on the epidemiological and clinical course of CM. Furthermore, the count of CM in MPM patients most likely alters the clinical outcome and should be respected when estimating DFS or overall survival. Further research into the field of sex-specific differences is warranted, in order to gain insight into the influences and connections with MPM, and validate our findings.

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