Diplomarbeit

Effect of Patient, Tumor, and Treatment Variables on Outcome in a Cohort of Patients With Merkel Cell Carcinoma - Retrospective Study

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Vorwort

Nach einigen wenigen Semestern im Medizinstudium entwickelte ich bereits ein wachsendes Interesse am Fach der Dermatologie. Mit der Entscheidung eines der speziellen Studienmodule zum Thema Dermatoonkologie zu absolvieren wurde ich letztendlich darin noch weiter bestätigt. Somit wurde mir die Wahl zur Erstellung einer Diplomarbeit auf diesem Gebiet ein großes Anliegen.

Das Thema Merkelzellkarzinom erweckte vor allem in Anbetracht seiner Seltenheit und daher eingeschränkten wissenschaftlichen Datenlage noch weiteres Interesse und Motivation. Dadurch und die damit verbundene Erweiterung meines Wissens auf dem Gebiet der Dermatologie, erwies sich die Erstellung der Diplomarbeit als äußerst lehr- und aufschlussreich.

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Zusammenfassung

Einleitung

Das Merkelzellkarzinom gilt als ein äußerst seltener, aggressiver, neuroendokriner Hauttumor mit steigenden Inzidenzraten und einer hohen Mortalität sowie Rezidivrate. Bekannte Risikofaktoren sind ultraviolette Strahlung, Immunsuppression und das Merkelzellpolyomavirus. Dennoch sind die Kenntnisse bezüglich pathophysiologischer Zusammenhänge und des klinischen Verhaltens noch lückenhaft. Vor allem die Untersuchung von prognostischen Faktoren ist von großer Bedeutung für den klinischen Alltag, um gegebenenfalls die Überlebenschancen von Patientinnen und Patienten zu steigern.

Material und Methoden

Diese retrospektive Studie umfasste 89 Patientinnen und Patienten, die zwischen 1992 und 2016 die Diagnose Merkelzellkarzinom erhalten haben. Die einzelnen medizinischen Aufzeichnungen wurden in Hinblick auf patienten-, tumor-, diagnostik- und behandlungsbezogenen Variablen überprüft. Deskriptive Statistik und Inferenzstatistik wurden verwendet, um die Charakteristiken zusammenzufassen und Zusammenhänge zu Rezidiventstehung, Gesamtüberlebens- und Todesrate am Merkelzellkarzinom.

Ergebnisse

In dieser Studie zeigten sich mehrere statistisch signifikante Zusammenhänge. Eine Abhängigkeit zwischen den Variablen Geschlecht ($p = 0,009$), Tumorausbreitung zum Zeitpunkt der Diagnosestellung ($p = 0,045$), initiale Therapiemodalität ($p = 0,026$), Chemotherapie ($p = 0,048$) und der Rezidiventstehung konnte nachgewiesen werden. Die Gesamtüberlebensrate stand hingegen im Zusammenhang mit Alter ($p = 0,027$), muskuloskelettalen Erkrankungen ($p = 0,04$), Durchführung einer Strahlentherapie ($p = 0,005$), Ergebnissen der pathologischen Lymphknotenuntersuchungen ($p = 0,045$), Rezidiventstehung ($p = 0,001$) und den Zeiträumen von 1 bis 6 ($p = 0,039$) und 7 bis 12 Monaten ($p = 0,003$) bis zum Auftreten von Rezidiven. Die Variablen Alter ($p = 0,031$), Tumorausbreitung zum Zeitpunkt der Diagnosestellung ($p = 0,031$) und Rezidiventstehung ($p = <0,001$) zeigten wiederum eine Koinzidenz mit merkelzellkarzinomspezifischen Todesfällen.

Conclusio

Die Auswirkungen des Merkelzellkarzinoms standen in dieser Studie im Zusammenhang mit Geschlecht, Alter, muskuloskelettalen Erkrankungen, Tumorausbreitung, den Ergebnissen der pathologischen Lymphknotenuntersuchungen, der initialen Therapie, Chemotherapie und Strahlentherapie. Diese Ergebnisse können bei klinischen Entscheidungen hinsichtlich des diagnostischen und therapeutischen Managements, sowie bei der Bewertung von Risikofaktoren und Prognosen helfen. Nichtsdestotrotz sollten für eine genauere Untersuchung der angeführten Assoziationen weiterführende Studien mit größeren Fallzahlen durchgeführt werden.

Abstract

Introduction

Merkel cell carcinoma (MCC) is a rare and aggressive skin cancer with high rates of recurrence and mortality. Known risk factors include ultraviolet radiation, immunosuppression, and Merkel cell polyomavirus (MCPyV). However, knowledge concerning pathophysiology and clinical behavior is lacking. Especially identifying prognostic factors to potentially improve MCC outcomes is of utmost importance.

Material and Methods

This retrospective study involved a cohort of 89 patients diagnosed with MCC between 1992 and 2016. Medical records were reviewed concerning host-, tumor-, diagnostic- and treatment variables. We used descriptive and inferential statistics to summarize characteristics and demonstrate associations with disease recurrence, overall survival and MCC-specific death.

Results

Among 89 patients with MCC, we observed several statistically significant associations. Sex ($p = 0.009$), tumor extent at time of diagnosis ($p = 0.045$) and initial treatment modality ($p = 0.026$) were associated with disease recurrence. Chemotherapy ($p = 0.048$) was further associated with a higher risk of recurrence. Initial treatment modality and pathologic nodal evaluation results ($p = 0.045$), were associated with overall survival. Age ($p = 0.027$), disease recurrence ($p = 0.001$), 1 to 6 months ($p = 0.039$) and 7 to 12 months ($p = 0.003$) to recurrence were associated with reduced overall survival. Musculoskeletal disease ($p = 0.04$) and radiotherapy ($p = 0.005$) were associated with improved overall survival. Tumor extent at time of diagnosis ($p = 0.031$) was further associated with MCC-specific death. Age ($p = 0.031$) and disease recurrence ($p = <0.001$) were associated with a higher risk of MCC-specific death.

Conclusion

MCC outcome was associated with sex, age, musculoskeletal disease, tumor extent, pathologic nodal evaluation results, initial treatment modality, chemotherapy and radiotherapy. These findings may help to assess risk and prognostic factors for MCC and may assist with clinical decisions concerning diagnostic or therapeutic management. Nonetheless, further studies with larger study populations may be useful to examine the statistical relevance of these findings.

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1 Introduction

1.1 Background

Merkel cell carcinoma is known as a rare, aggressive, neuroendocrine-derived skin cancer and has first been described in 1972 by Toker. (1) Since then, terms like APUDoma (Amine Precursor Uptake Decarboxylase) of the skin, anaplastic cancer of the skin (2), cutaneous carcinoma of the skin or small cell primary cutaneous carcinoma have been used as synonyms to describe the tumor. (3) In 1972, Toker established these malignant skin tumors as “trabecular carcinoma of the skin” and assumed them to consist of anastomosing trabeculae and nests of cells located in the dermis, which were further described as relatively small tumors ranging between 1 to 3 cm in diameter, mainly appearing on elderly people with light skin.

Along with Tang, he identified the cells of origin to be derived from the neural crest by conducting electron microscopic studies of three trabecular skin tumors. By proving neurosecretory granules to be present in all three tumors, they supposed them to originate from a neurocrest derivative. After excluding epidermis, sweat glands and hair follicles due to the fact that carcinomatous changes had never been observed in any of these structures, Toker and Tang considered melanoblasts to be one of those neurocrest derivatives the tumor may origin from. However, melanoblasts were excluded, as melanosomes or premelanosomes could not be found in any of the carcinomas. Toker and Tang assumed Merkel cells to be most probably the site of origin. These cells closely resembled the tumor cells at the ultrastructural level, whereby the skin cancer gained its final name. (4)

1.2 Epidemiology

1.2.1 Incidence

Although MCC is classified as a rare skin cancer, incidence rates seem to increase all over the world. The Surveillance of Rare Cancers in Europe (RARECARE) distinguished an incidence rate of 0.13 per 100,000 people between 1995 and 2002. Data from the Surveillance, Epidemiology and End Results (SEER) Program reported an incidence rate of 0.79 per 100,000 people in 2011 in the US. (5) For comparison, annual incidence rate analysed from the SEER Program in 2006 was 0.6 per 100,000 people which demonstrates a significant increase of the MCC incidence rate. (2)

Considering the distribution of incidence rates of MCC all over the world, the highest ones can be found in Australia and New Zealand. Especially Queensland in north-eastern Australia shows the highest rates examined, as subtropical climate and high levels of UV radiation all year around can play a remarkable role in developing MCC. According to the Queensland Cancer Registry, the average annual incidence rate of MCC between 2006 and 2010 accounted 1.6 per 100,000 people, further increasing in elderly people to 20.7 per 100,000 for people older than 80 years. Furthermore, incidence rates in men were much higher than in women, accounting to 2.5 per 100,000 in males and 0.9 per 100,000 in females - a trend that can also be shown considering data from all over the world. (6) Similarly, higher incidence rates can be reported in New Zealand, as the annual incidence rate between 2002 and 2011 was 0.88 per 100,000, again increasing drastically in the group of people at the age of 85 years and older (17.6 per 100,000). (7) In comparison the incidence rate of people of African descent is only 0.01 per 100,000 and is therefore far lower than in Caucasians. (3)

1.2.2 Risk factors

Risk factors strongly associated with the development of MCC include (1) fair skin, (2) a history of extensive sun exposure, (3) chronic immune suppression, and (4) age (older than 50 years). (8) Mean age at diagnosis is 76 years for women and 74 years for men (9). However, patients at younger age with suppressed immune system are at a significantly higher risk of developing MCC. Studies showed that solid-organ transplant recipients had a 24-fold higher risk of MCC compared to individuals with a competent immune system. (10) Further, patients with non-Hodgkin lymphoma, multiple myeloma or malignant melanoma showed a 3- to 7-fold higher risk of MCC. (11)

The incidence rate varies between different ethnicities; people of colour, Asians and Hispanics have a significantly lower risk of developing MCC than Caucasians. Together, MCC is most commonly found on sun-exposed areas of the body (e.g., head, neck, arms) in older Caucasian individuals, who may also have other sun-induced skin cancers. (12)

1.3 Pathogenesis

1.3.1 UV radiation

As mentioned above, many studies have claimed UV radiation exposure to play an important etiologic role for the development of MCC. Not only this sort of skin cancer appears most of the time on sun-exposed areas such as the head and neck and in fair-skinned patients (2), but also the solar UV index can be connected to the occurrence of the tumor as Agelli et al. assumed. (13)

Furthermore, MCC was linked to other skin cancers including melanoma, whose association with sun exposure has already been investigated, concluding that patients who have suffered from melanoma have a 3-fold higher risk to develop MCC. (11)

Additionally, in two studies evidence for sun-exposure as an important factor for pathogenesis of MCC could be provided, since UVB-specific mutations in the p53 gene and typical C-T transitions in Haras genes have been identified. Therefore the importance of UVB as mutagen for MCC has been shown. (14) (15)

The cause of local immunosuppression due to UV radiation could also be linked to pathogenesis, as inflammation and alterations in antigen-presenting dendritic cells can be initiated which subsequently leads to a modulation of the immune system. (12)

1.3.2 Merkel cell polyomavirus

Merkel cell polyomavirus (MCPyV), a DNA virus belonging to the family of Polyomaviridae, has first been discovered by Feng et al. in 2008 (16). MCPyV was detected in eight out of ten MCCs and showed the viral DNA integrated into the genome of MCC cells. Further studies implicated MCPyV to be present in the skin of most healthy individuals and therefore to be a part of the human skin microbiome. Further it is not causing any identifiable symptoms after primary infection. (12)

A meta-analysis performed in 2015 assumed the prevalence of MCPyV to be 79% in MCCs and 12% in control skin samples. (17) Feng et al. discovered the integration of MCPyV into the genome of MCC, which leads to clonal expression of tumor cells. (16) Thus, it can be assumed that the virus already occurs early in tumorigenic process and drives oncogenic mechanisms.

Genetic analysis yielded MCPyV to encode two oncoproteins, the large tumor antigen (LT) and the small tumor antigen (sT). Both of them were associated with

tumorigenesis in MCC. (2) To clarify, the LT protein is truncated due to mutations in MCPyV genomes and therefore the helicase domain, which actually should ensure stable integration of the viral genome, is deleted. (18)

In contrast to the results of recent studies reporting the evidence of MCPyV-DNA in samples of many patients with MCC, a study in Australia found much less MCPyV-positive tumors in patients. In view of the fact that studies recorded the incidence of MCC to be the highest in Australia, UV radiation could also play an even bigger role regarding the development of viral-negative MCCs. (2) Vice versa, studies showed only low levels of UV-induced mutations in viral-positive MCCs. Nonetheless, other studies suggest the contribution of UV radiation to virally induced transformation, as local immunosuppression could ease the viral integration required. (5)

Several studies also showed the evidence of MCPyV in other skin cancers like squamous cell carcinoma and basal cell carcinoma, though clonal integration of the virus and expression of viral proteins could not be detected in any of those cancers. However, MCPyV infection seems to be specific to MCC tumor cells, leading to the assumption of MCPyV being a causal factor of development of MCCs tested MCPyV-positive. (19)

1.3.3 Immunosuppression

As many studies reported, MCC often occurs in immunosuppressed individuals including HIV-infected and organ transplanted patients, as well as patients who suffer from B-cell malignancies. (8) However, knowledge of specific mechanisms driven by immunosuppression and its potential interaction with UV radiation and MCPyV in the pathogenesis of MCC remains limited. Nonetheless, studies demonstrated that immunosuppression could possibly ease the replication of MCPyV and enhance the virus integration in the progenitor cell, as well as survival and proliferation of atypical cells could be supported by insufficient immune competence. Further, immunosuppressants as azathioprine could interact with UV radiation to trigger oncogenesis. (2)

1.4 Diagnosis

1.4.1 Clinical features/suspicion

MCC can macroscopically be described as a rapidly growing, firm, non-tender, intracutaneous nodule ranging from less than 1 cm to 2 cm in size with a colour of flesh to bluish-red. Sporadically, ulceration or crusting can be observed and most of the time the tumor remains painless. Mainly affecting sun-exposed areas, the most common anatomic sites are the head and neck area, the limbs including shoulder and hip and the trunk. (2) A cohort study of 195 MCC cases between 1980 and 2007 summarized the clinical features of MCC as AEIOU with A for asymptomatic/lack of tenderness, E for expanding rapidly, I for immunosuppression, O for older than 50 years and U for ultraviolet-exposed skin to support clinicians in suspecting MCC. Moreover, they suggested the lack of tenderness as a distinctive feature of MCC in contrast to other rapidly growing and reddish lesions like inflamed cysts.

Nonetheless, in some cases the tumor develops on sun-protected areas (8) or the primary site of the tumor remains unknown. In the latter case, spontaneous regression of the tumor, or emergence in lymph nodes can be discussed. (20) Unfortunately, in many cases regional to distant tumor extent can already be observed at time of diagnosis.

For a lack of data regarding dermoscopy, specific features have not yet been fully described. Nonetheless, some investigations refer to milky-red areas and polymorphous or linear irregular vessels. (2)

1.4.2 Histopathology

The histologic examination of the MCC-suspicious lesion presents indispensably and haematoxylin, eosin and immunohistochemical stains are necessitated for diagnosis. The histological preparation typically shows the tumor as a dermal lesion, which commonly extends to the reticular dermis and subcutis, most of the time neither involving the epidermis nor any ulceration. Further, nests of uniform round blue cells with large basophilic nuclei can be observed.

Apart from their clinical significance, three subtypes of MCC can be histologically differentiated. The most frequent, the intermediate type, shows large, solid nodules of basophilic cells with round to oval nuclei. The second subtype, the small cell variant consists of solid sheets or clusters created from round tumor cells with scant cytoplasm and oval hyperchromatic nuclei. The third and least common one, called

the trabecular type, is defined by polygonal cells, abundant cytoplasm, round nuclei and nucleoli arranged in a trabecular pattern. In some cases the expression of squamoid, eccrine, melanocytic or glandular differentiation has been described as well as the combined occurrence of MCC with invasive squamous cell carcinoma or melanoma. (21)

1.4.3 Immunohistochemistry

MCC shows neuroendocrine and epithelial features when it comes to immunohistochemistry. The tumor cells express epithelial markers such as AE1/AE3, CAM 5.2, pan-cytokeratin, epithelial membrane antigen and Ber-EP4. The immunoreactivity for Cytokeratin (CK) 20 distinguishes MCC from other undifferentiated tumors and therefore acts as a sensitive tumormarker for MCC because of its specific paranuclear dotlike positivity. Although most MCCs show CK20 positivity, studies also reported some cases of CK7 positivity. Besides CK20, MCC stains for a large number of neuroendocrine markers like neurofilaments, neuron-specific enolase, chromogranin, synaptophysin, bombesin, somatostatin and vasoactive intestinal peptide. (21) Also, the mouse monoclonal antibody CM2B4 can be used to identify the presence of MCPyV in MCC.

1.4.4 Cytogenetic and molecular analyses

Referring to mutations described in MCC, chromosome abnormalities like gains, losses and rearrangements can be found, however their association with pathogenesis or therapeutic outcome still remains unclear. The most common chromosomes involved are 1, 5, 6, 8 and 13. (2) In case of chromosomal alterations, studies showed an association with larger tumors and therefore higher potential for metastatic spread. Further, MCPyV negative tumors tend to have more genetic alterations than MCPyV positive MCCs.

Regarding the TP53 tumor suppressor gene, studies only identified mutations in up to 28% of the cases, most frequently single nucleotide polymorphisms and silent mutations of unknown clinical significance. Although rare, TP53 protein expression can be found and seems to be associated with MCPyV negativity in MCC. However, mutations that are responsible for inactivating TP53 are unlikely linked to oncogenesis. In contrast, retinoblastoma (RB) inactivation seems to be affiliated to pathogenesis of MCPyV-positive, as well as MCPyV-negative MCCs. Studies detected

an increased genomic instability of virus-negative tumors compared to virus-positive ones with more frequent deletion in the RB1 locus. Additionally, studies reported mutations in genes part of the tyrosin kinase signaling such as PIK3CA. In a study of 60 patients PIK3CA mutations could be found in at least 10% of the cases. (22)

1.5 Differential Diagnosis

In some cases MCC may be difficult to differentiate in clinical praxis as many lesions, whether benign or not, can meet similar criteria like the occurrence on sun-exposed skin. Possible differential diagnoses could be basal and squamous cell carcinoma, keratoacanthoma, amelanotic melanoma, pyogenic granuloma or lipoma. As mentioned before, histological results present a crucial tool to expose MCC whereby conventional light microscopy may not be sufficient. Therefore immunohistochemistry is required for a distinctive diagnosis. To have a closer look on distinguishing potential differential diagnoses, as for example the small cell melanoma stains for HMB-45, Melan-A and S100-protein unlike MCC. Also, small cell carcinoma of the lung does not stain for CK20 as MCC does. (2)

1.6 Prognostic factors

The extent of disease at initial diagnosis as well as the involvement of lymph nodes plays a decisive role in disease outcome. However, many studies examined further factors that could have an impact on the course of disease and eventually on survival.

With this in mind, MCPyV positive tumors seem to be related to a better prognosis compared to those without viral involvement. As studies found, tumors expressing LT antigen and retinoblastoma (RB) tend to occur more frequently on the limbs and metastatic extent seems not to be common. To conclude, MCPyV-positive tumors apparently have better overall and disease specific survival. (23)

In addition, higher titers of Anti-VP1 antibodies, which mark previous exposure to MCPyV, are associated with better progression-free survival as a study of 67 MCC patients reported. (24)

As expected, disease specific mortality rates are higher in patients with a concomitant immunosuppression due to HIV infection, solid organ transplantation or B-cell malignancies. A study of 218 patients reported a significant association of immunosuppression and disease-specific mortality. (25)

Further, prognostic factors negatively affecting disease outcome can also be lymphovascular invasion and infiltrative growth, the absence of intratumoral CD8+ lymphocyte infiltration or the expression of p63 influencing low-stage MCCs by increasing risk of death. (2)

1.7 Staging

1.7.1 TNM Staging System

The eighth edition of the TNM (tumor, node, metastasis) system from 2018 (26), assists with management and prognosis of patients suffering from MCC. Therefore, both the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) suggest using this system to categorize disease severity for optimal management.
To summarize, patients can be divided in 4 prognostic stage groups:

Stage I – the maximum tumor size is 2 cm without any regional lymph nodes involved;
Stage II – the tumor is more than 2 cm in size or it invades into bone, muscle, fascia or cartilage, again without any lymph nodes involved; further division into two subgroups depending on tumor size and level of invasion;
Stage III – regional lymph node involvement, division into three subgroups;
Staging IV – occurrence of distant metastasis; (27)

The detailed description of the TNM staging system from the eighth edition of AJCC can be viewed in Table 1 below.

Table 1: ***TNM staging of merkel cell carcinoma, AJCC eighth edition*** *(26)*

Primary tumor (pT)	
pTX	primary tumor cannot be assessed (eg. curetted)
pT0	no evidence of primary tumor
pTis	in situ primary tumor
pT1	max. clinical tumor diameter ≤2 cm
PT2	max. clinical tumor diameter >2 cm but ≤5 cm
pT3	max. clinical tumor diameter >5cm
pT4	primary tumor invades fascia, muscle, cartilage or bone

Regional lymph nodes (pN)	
pNX	regional lymph nodes cannot be assessed (staging procedure has not been performed or previously removed)
pN0	no regional lymph node metastasis detected
pN1a(sn)	microscopic lymph node metastasis detected by sentinel node biopsy, clinically occult
pN1a	microscopic regional lymph node metastasis following dissection, clinically occult
pN1b	microscopic confirmation of clinically detected regional lymph node metastasis
pN2	in-transit metastasis **without** lymph node metastasis
pN3	in-transit metastasis **with** lymph node metastasis
Distant metastasis (pM)	
pM0	no distant metastasis detected
pM1	distant metastasis detected
pM1a	microscopic confirmation of metastasis to skin, distant subcutaneous tissue, or distant lymph node(s)
pM1b	microscopic confirmation metastasis to lung
pM1c	microscopic confirmation metastasis to all other sites
Prognostic stage groups	
0	Tis,N0, M0
I	T1, N0, M0
IIA	T2-3, N0, M0
IIB	T4, N0, M0
IIIA	T1-4,N1a(sn) or N1a,M0 T0, N1b, M0
IIIB	T1-4,M1b-N3,M0
IV	T0-4, N0-N3,M1

1.7.2 Initial staging procedure and regional lymph nodes

If histological and immunohistochemical examination has proven presence of MCC, cross-sectional imaging should check the presence of distant metastases. If evidence of lymph node involvement is neither given clinically nor through diagnostic imaging, sentinel lymph node biopsy (SLNB) should follow due to the high likelihood of occult lymph node metastases. (28) Further, lymph node status plays an important role on planning further treatment and evaluating prognosis. (27)

However, the impact of SLNB is less given for individuals with a tumor occurring on the head and neck area due to higher rates of lymphatic metastatic spread and false

negative results as well as due to the variability of lymphatic drainage in this area. In this case the performance of a neck dissection should be considered. (28)
If results of SLNB are negative, wide local excision should be performed. In case that surgical intervention is not possible, radiation therapy to the primary MCC is indicated.
If results of SLNB are positive, the presence of distant metastasis should be examined again. In case of exclusion of any metastasis, regional lymph node dissection or nodal radiation therapy are suggested. (27)
If SLNB is not performed, whether due to anatomic site of occurrence, or fear of lymph node metastasis in case of a tumor size of ≥2 cm, radiation therapy is suggested. (29)

1.8 Therapy

1.8.1 Primary tumor

Until now, MCC in initial stages is mainly treated with surgery and radiation therapy. If possible, wide surgical excision of the tumor should be performed (3) and margins should be at least 1 to 2 cm. If margins are positive or close to the tumor mass in histological examination, patients should undergo postoperative radiation therapy as clear margins are crucial for long-term disease control. (27)
To accomplish clear margins, excision deep into the muscle fascia or Mohs micrographic surgery (MMC) can be discussed. (3) However, adjuvant radiotherapy seems to be essential regardless of the safety margin. (28) Anyway, radiation therapy in addition to wide surgery is said to have a positive impact on disease control as several studies showed. If surgery of the primary tumor is not feasible, isolated radiation therapy presents a reasonable alternative. (3) However, if radiation therapy is performed alone, the in-field control rate only amounts to about 75% and many patients may receive systemic relapse. (30)

1.8.2 Adjuvant therapy

As mentioned above, radiation therapy (RT) seems to be particularly important according to the success of treatment, as MCC is assumed to be radiosensitive. As an adjuvant therapy, RT ought to prevent recurrence and improve overall survival at least for patients in initial stages of I-II. (31) Whether RT serves as an adjuvant or

isolated treatment module, all regional lymphatics should be irradiated to avoid the development of recurrence in any untreated region.
In contrast, the impact of chemotherapy or immunotherapy is not well examined yet. (27) Unlike radiation therapy, chemotherapy is not suggested to be used as a routine in adjuvant therapy (31) concerning its collateral immunosuppressive effects. Therefore, studies assumed that if chemotherapy was performed isolated as the only adjuvant therapy after surgery, it is not beneficial to the overall probability of survival. (32)

1.8.3 Metastatic disease

Chemotherapy has been used as treatment modality for patients with distant site of extent for many years and carboplatin-etoposide presents the initial treatment option. By adding carboplatin to the treatment regime, toxicity can be reduced (27), though studies identified several treatment-related deaths. (33) Further, response rates seem to be high in patients with metastatic disease, but the response duration is short. (34) Outcomes in patients with metastatic MCC have historically been poor; median time to progression with cytotoxic chemotherapy is only three months.
PD-1 axis checkpoint inhibitors are now regarded as the preferred first line systemic therapy in eligible patients, with impressive durability and objective response rates. Avelumab, Pembrolizumab and Nivolumab are antibodies targeting PD-L1 and PD-1, a protein for programmed cell death. (27) Recent studies, which examined successful usage of Avelumab (an anti-programmed cell death ligand 1 (PD-L1) antibodyanti-PD-L1), showed durable responses in patients with metastatic MCC and a progression-free survival, as well as an overall survival rate of 40-69% after six months. (35) Additionally, studies found better response in patients undergoing immunotherapy as first-line therapy for patients with metastatic MCC. (5) Further, Pembrolizumab and Nivolumab also showed durable activity in patients with distant extent of disease.

1.9 Surveillance after initial treatment

Due to MCC's aggressiveness and therefore the high risk of recurrences, patients diagnosed with MCC should undergo a strict and frequent follow-up based on the individual situation, including risk factors and therapeutic options. (27) The German AWMF (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesell-

schaften) guideline suggests a follow-up every three months for two years and every six months afterwards.

The surveillance should contain a qualitative regimen of physical examination including skin examination as well as palpation and sonography of the lymph nodes. Concerning imaging techniques, such as magnetic resonance imaging, computed tomography or positron emission tomography, individual clinical indications should be used analysing its risks and benefits. Patients with a negative SLNB should get positron emission tomography or computed tomography and as magnetic resonance imaging of the head once a year. Due to the worse prognosis for patients with a positive SLNB, imaging examinations can be performed every three months within the first two years of follow-up and every six months until five years of surveillance. Additionally, patients should be instructed to examine their locoregional skin beyond the surveillance period. (28)

1.10 Prognosis

Known as an aggressive skin cancer, MCC-specific mortality is considered to range between 33 and 46% and is therefore assumed to be higher than in patients with malignant melanoma. As the incidence of cases increased from 1986 to 2011, so did the amount of disease-specific mortality increase by 333% as indicated by the SEER registry data. (5)

The progression of disease seems to be variable in MCC. However, several prognostic factors beneficial for overall survival were identified including female sex, age less than 65 years, absence of any comorbidities and initial local extent of disease. Special emphasis should be placed on the involvement of lymph nodes at initial diagnosis, as this is a meaningful factor for disease progression. (27) Regarding MCC-specific survival, studies showed survival rates of 90% for patients with local disease compared to 52% for patients with nodal involvement and only 10% maximum for patients with metastatic disease at time of diagnosis. (8)

As expected, studies also described a reduced stage-independent MCC-specific survival in immunosuppressed patients. This means not only an increased risk of developing MCC for patients with immunosuppression, but also a poorer prognosis if they suffer from MCC. (36)

Regarding site of extent, research indicated a worse prognosis for patients with MCC occurring on the head and neck area compared to those occurring on other

anatomical sites. One probable explanation may be the difficulty of wide surgical excision (37) as well as the high variability of lymphatic drainage in this area. Furthermore, truncal lesions seem to have poorer prognosis as well, probably due to late detection. Similarly, MCCs arising on the legs seem to have worse prognosis since wide surgical excision seems to be difficult regarding poor blood circulation in older patients and poorer tolerance of radiation therapy in this area. (27)

Further, studies found improved prognosis for patients with a tumor of unknown primary site regarding to overall survival and risk of metastatic recurrences. (25)

Concerning diagnostic modalities, pathologic nodal evaluation helps obtaining prognosis and was found to be associated with improved outcome. Survey showed radiation therapy to be linked to a reduced probability for local recurrences, but not to metastatic recurrences or overall survival. (25)

Referring to MCPyV, studies demonstrated controversial results regarding its impact on prognosis of MCC. Some studies shared the prognosis of MCPyV-positive and -negative tumors, (18) although worse overall response in virus-positive MCCs was described. Regarding immune status, investigation found an improved overall survival rate in patients with high levels of MCPyV-specific antibodies among MCPyV-positive tumors. (5)

Additionally, studies revealed an increased risk of secondary cancers after MCC, especially of the salivary gland, brain (mostly in males) and biliary sites other than liver or gallbladder (11) and also a greater chance of developing malignant melanoma. (5)

2 Material and Methods

2.1 Study Setting

The medical documentation and communication network “openMEDOCS” was used for acquisition of data needed for the study. Introduced from 2002 to 2006 after a pilot stage from 1999 to 2001, the network is now being used in every health institution of KAGes, the styrian company of hospitals. (38) “openMEDOCS” provides clinical electronic data about every patient treated at any hospital using the system. Additionally, doctor’s letters and other important information of a patient’s medical history including individual treatment modalities or test results can be uploaded and allocated for medical research. Information on each patient including demographic characteristics, clinical results, diagnosis, treatments, lab data, pathologic and radiologic evaluation and medication is provided for employees of the styrian company of hospitals made accessible via the input of a corresponding medical research number.

2.2 Study Population

The cohort of this study consists of the patients who were treated at the Department of Dermatology and Venerology at the LKH University Clinic Graz and received initial diagnosis of MCC between 1992 and 2016. Within this time period, 99 patients were suspected to suffer from MCC. Patients were excluded if the diagnosis of MCC proved to be false or if the majority of information needed, was missing. Due to the fact that “openMEDOCS” was only introduced in 2005, information about some variables examined in our study was lacking, especially in patients treated prior to the introduction of “openMEDOCS”, which led to the exclusion of four patients due to false diagnosis, as well as the exclusion of another six because of too little information. Therefore, the final study cohort consists of 89 patients.

2.3 Covariates

For better illustration, we distinguished five groups of covariates concerning host, tumor, diagnostic, treatment and outcome. Relating to previous studies, we included all variables whose statistical analysis could improve knowledge of MCC including risk factors, treatment modalities, optimal staging or prognosis.

2.3.1 Host Variables

Firstly, we identified important information about each patient including age at diagnosis, sex and race. We categorized age at diagnosis into the following five groups to outline correlations more easily: <60 years, 60 to 69 years, 70 to 79 years, 80 to 89 years and ≥90 years.

Secondly, we determined if patients were immunosuppressed due to organ transplantation, B-cell malignancies or any other medical immunosuppression at time of diagnosis or if they had been immunosuppressed at some point before the diagnosis of MCC. In the course of this procedure, we documented the different reasons for immunosuppression.

Thirdly, we recorded comorbidities from patients with MCC. Comorbidities were grouped as cardiovascular disease, post cardiovascular event, respiratory disease, gastrointestinal (GI) disease, metabolic disease, thyroid disease, urogenital disease, musculoskeletal disease, hepatobiliary disease, ophthalmological disease, neurological disorders, psychological disorders, hormonal imbalance (male), hematologic disease, hematologic malignancies, dermatological disease, skin cancer, and cancer (other than skin).

“Cardiovascular disease” included coronary heart disease, heart failure, peripheral artery disease, and cerebrovascular disease. “Post cardiovascular event” included thrombosis, myocardial infarction, cardiac pacemaker implantation and cardiac valve replacement. “Respiratory disease” included chronic obstructive pulmonary disease (COPD), chronic bronchitis, emphysema, asthma, tuberculosis and sarcoidosis. “Gastrointestinal disease” included gastritis, gastric or duodenal ulcers, malabsorptions, celiac disease, colitis, Crohn’s disease, and gastroesophageal reflux disease. “Metabolic disease” included diabetes, hypertension, hyperlipidaemia and obesity. “Thyroid disease” included hypothyroidism, hyperthyroidism, thyroiditis, and other unspecified thyroid conditions. “Urogenital disease” included chronic or recurrent urinary tract infections, urolithiasis, chronic renal insufficiency, polycystic kidney disease, nephritis, proteinuria, and other unspecified kidney conditions. “Musculoskeletal disease” included arthropathies, osteopathies, chondropathies and systemic connective tissue disorders. “Hepatobiliary disease” included hepatitis, cirrhosis, and other unspecified hepatobiliary conditions. “Ophthalmological disease” included glaucoma and cataract. “Neurological disorders” included Parkinson disease, dementia, epilepsy, multiple sclerosis, brain tumors, neuropathy and

meningitis. “Psychological disorders” included depression and anxiety. “Hormonal imbalance in male” included prostate hyperplasia. “Hematologic disease” included anaemia, pancytopenia, haemophilia, sickle-cell disease, and other non-specified hematologic conditions. “Hematologic malignancy” included leukemias and lymphomas. “Dermatologic disorders” included panniculitis, psoriasis, rosacea, among others. “Skin cancer” included melanoma, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). “Cancer other than skin and blood cancer” included endometrial cancer, prostate cancer, bronchial cancer and laryngeal cancer.

For better understanding in regard to the impact of comorbidities we used the Charlson Comorbidity Index (CCI) and allocated every patient a specific index as shown in Table 2.

Table 2: ***Charlson Comorbidity Index*** (39)

Comorbidity	**Valuation**
Myocardial infarction	1
Congestive heart failure	1
Peripheral vascular disease	1
Cerebrovascular disease	1
Dementia	1
Chronic pulmonary disease	1
Connective tissue disease	1
Peptic ulcer disease	1
Mild liver disease	1
Diabetes mellitus (without end-organ damage)	1
Hemiplegia	2
Moderate to severe renal disease	2
Diabetes mellitus (with end-organ damage)	2
Tumor (without metastases)	2
Leukaemia	2
Lymphoma	2
Moderate to severe liver disease	3
Metastatic solid tumor	6
Acquired Immune Deficiency Syndrom (AIDS)	6

Further, we categorized CCIs into four groups to demonstrate correlations more easily: CCI of 0 (none disease), CCI of 1-2 (mild disease), CCI of 3-4 (moderate to severe disease) and ≥5 (very serious disease).
In addition, we assigned every patient an age-adjusted Charlson Comorbidity Index (ACCI) by adding an age-specific score to the CCI we had previously determined. We allocated an age-specific score of 1 to patients who were between 50 and 59 years old at time of diagnosis, a score of 2 to patients between 60 and 69 years old, a score of 3 to patients between 70 and 79 years old, a score of 4 to patients between 80 and 89 years old and a score of 5 to patients who were 90 years or older. Finally, we allocated a value of 0 to patients younger than 50.
Regarding lab values, we equally divided parameters into different groups. We categorized C-reactive protein (CRP) values into CRP of <5, 5-20, 20-100 and >100 mg/dl, LDH (lactate dehydrogenase) values into non-elevated and elevated with a cut-off value of 240 U/l, Albumin values into low (<3.5 g/dl) and normal range and the neutrophil to lymphocytes ratio (NLR) into <2.5, 2.5-5 and >5.

2.3.2 Tumor Variables

The characteristics of MCC composed of anatomic site, size and extent. In regard to anatomic site, we distinguished unknown primary site, tumors that occurred on the head and neck area, tumors that arose on the limbs whether they developed on upper or lower extremity and tumors that primarily occurred on the trunk. Regarding size, we divided between tumors that were smaller or exactly 2 cm in diameter and tumors that were larger than 2 cm in diameter. Finally, we examined tumor extent at time of diagnosis and distinguished the following three groups: local, if the tumor had not spread and was still restricted to the area it occurred in, regional, if the tumor had spread into lymph nodes close to the origin and distant, if metastases were already present at time of diagnosis.
Finally, we ascertained if MCC occurred combined with another sort of skin cancer like SCC or Bowen's disease and recorded results under "collision lesion".

2.3.3 Diagnostic Variables

In regard to diagnostic variables, we distinguished three groups of characteristics including imaging, type of pathologic nodal evaluation and pathologic nodal evaluation results. We examined the type of diagnostic imaging patients underwent after

initial diagnosis including computed tomography, magnetic resonance tomography, positron emission tomography, scintigraphy, sonography and X-ray.
Further, we examined if patients underwent any pathologic nodal evaluation including sentinel lymph node biopsy (SLNB), lymphadenectomy (LAD) or LAD after a positive finding of SLNB. We distinguished results of SLNBs and LADs into positive SLNB, positive LAD and negative findings of SLNB or LAD.

2.3.4 Treatment Variables

Concerning treatment variables, we investigated the treatment modalities performed within three months of initial diagnosis of MCC. We documented seven groups of treatment modalities including monotherapies as surgery alone, radiotherapy alone or chemotherapy alone, combination therapies as surgery and radiotherapy, surgery and chemotherapy, radio- and chemotherapy and a combination therapy of all three modalities (surgery, radio- and chemotherapy). For better illustration, we categorized the initial tumor therapy and determined whether any surgery, any radiotherapy or any chemotherapy had been performed in a patient.
Regarding surgery, we also examined results concerning margins and we distinguished whether margins had been clear or positive after first surgical excision.

2.4 Outcomes

Disease recurrence, time to recurrence, overall survival and MCC-specific death were the main outcomes of interest. We categorized information about recurrence into “no recurrence”, “locoregional recurrence” (at/near the primary tumor or the regional lymph nodes), and “distant metastatic recurrence”. We recorded time to recurrence in months after first reference in “openMEDOCS”. For easier demonstration, we categorized time to diagnosis into six groups including <1 month if doctors had recorded recurrence within 1 month after initial diagnosis, 1 to 6 months, 7 to 12 months, 13 to 18 months, 19 to 24 months and >24 months. Further, we examined whether patients were still alive and if not, if MCC was the cause of death. Associations between host-, tumor-, diagnostic-, treatment-variables and the outcome of MCC were our main objectives.

2.5 Statistical analysis

For statistical analysis we used descriptive statistics to summarize host-, tumor-, diagnostic-, treatment-characteristics and our main outcomes of interest including disease recurrence, overall survival and death due to MCC. In addition, we investigated associations between host-, tumor-, diagnostic-, treatment-variables and the outcomes of MCC using the Pearson Chi-Square test, Yates correction and Fisher's exact test. If information on any variables was missing, values were excluded from statistical analysis. All statistical analyses were performed using statistical software (IBM SPSS statistics, version 25.0, International Business Machines Corporation, Armonk, NY). For all analysis, p-value of <0.05 was accepted as significant.

3 Results

From 1992 to 2016, 99 patients treated at the Department of Dermatology and Venerology were suspected of MCC. We excluded a total of ten patients from the study due to false diagnosis in four cases and lack of information about the majority of variables examined in six cases. The variables analysed in the cohort are shown in Tables 3, 4, 5, 6 and 7. Due to the fact that identification of all relevant characteristics in every single case of MCC was not possible, the number of patients shown does not always tally to the full 89 examined.

3.1 Frequencies

3.1.1 Host Variables

In our cohort, age ranged between 41 and 97 with a mean age of 74.99 (SD 9.4) years and a median age of 76 years. Most patients were between 70 and 79 (43.8%) years old, followed by the age group of 80 to 89 with 33.7%. The age distribution by categories is shown in Table 3.

Slightly more men were diagnosed with MCC in our study cohort. We identified overall 46 (51.7%) men and 43 (48.3%) women, who were all white.

The group of patients who were immunosuppressed at time of diagnosis of MCC and the group who have had immunosuppression before diagnosis were about the same size of four (4.5%) patients. Additionally, we ascertained the different reasons for immunosuppression and whether patients were still immunosuppressed or not. Three (3.4%) patients were immunosuppressed due to organ transplantation, two (2.2%) suffered from chronic lymphatic leukaemia, two (2.2%) got other medical immunosuppression and one (1.1%) had Non-Hodgkin lymphoma.

In regard to comorbidities, the biggest group, sufferers of metabolic disease, were 59 (66.3%) patients. Second most comorbidity, cardiovascular disease, afflicted 35 (39.3%) patients. Equally, 26 (29.2%) patients had experienced a cardiovascular event of sorts before diagnosis of MCC. We each observed the following diseases in approximately a fifth of the cohort: thyroid disease in 19 (21.3%) patients, urogenital disease in 18 (20.2%) patients and other sorts of skin cancer in 20 (22.5%) patients.

Concerning skin cancer, we found 17 (19.1%) patients suffering from BCC, which presents the most frequent skin cancer besides MCC in patients of our cohort. Additionally, melanoma arose on five (5.6%) patients and eight (9%) patients had SCC. Staying with cancer as a subject, seven (7.9%) patients suffered from another type of carcinoma besides skin cancer or hematologic malignancies. In each case, two (2.2%) patients had endometrial, prostate and pulmonary cancer, and one (1.1%) had cancer of the larynx. Moreover, six patients had a hematologic malignancy such as B-cell lymphoma (n = 3, 3.4%), Non-Hodgkin lymphoma (n = 1, 1.1%) and chronic lymphatic leukaemia (n = 2, 2.2%).

Musculoskeletal disease was found in 15 (16.9%) patients, hepatobiliary disease in 12 (13.5%) patients and neurological disorders in 14 (15.7%) patients. In regard to the remaining comorbidities, we determined all of them in only a little number of patients. Respiratory disease was found in ten (11.2%), gastrointestinal disease in seven (7.9%), ophthalmological disease in six (6.7%), psychological disorder in five (5.6%) and hormonal imbalance in four (4.5%) patients.

To conclude, 31 (31.8%) patients had a Charlson Comorbidity Index of 0, 21 (23.6%) patients had a CCI of 1, 17 (19.1%) patients had a CCI of 2, eight (9%) patients had a CCI of 3, five (5.6%) patients had a CCI of 4, three (3.4%) patients had a CCI of 5, and two (2.2%) patients had a CCI of 6. We allocated an Index of 7 and 8 to one (1.1%) patient each. Our mean CCI was low at 1.58 (SD 1.8) with a median CCI of 1.

For better illustration, we divided CCIs into four groups: no comorbid disease (CCI of 0), mild disease (CCI of 1-2), moderate to severe disease (CCI of 3-4) and very serious disease (≥5). Therefore, 31 (34.8%) patients had no notable disease, 38 (42.7%) patients had mild disease, 13 (14.6%) patients had moderate to severe disease and seven (7.9%) patients had very serious disease. Age adjusted CCI ranged between 0 and 11 with a mean ACCI of 4.6 (SD 2.03) and a median ACCI of 4.

Reviewing lab data at time of diagnosis, we found a median lab value for CRP of 4.1 mg/dl. Values for CRP ranged between 0.6 and 140 mg/dl. Most patients, which amounted 37 (41.1%), showed non-elevated CRP values (<5 mg/dl) at time of diagnosis. 18 (20%) showed a CRP of 5-20 mg/dl, 9 (10%) showed a CRP of 20-100 mg/dl and only one (1.1%) patient showed a CRP value of >100 mg/dl. The median value for LDH was 211 U/l. LDH values ranged between 144 and 5115 U/l. Almost

twice as many patients showed non-elevated LDH compared to elevated LDH (>240 U/l), which means 39 (43.3%) patients had non-elevated and 22 (24.2%) patients had elevated LDH values.

The median value for Albumin was 4.1 g/dl and values ranged between 0 and 5.3 g/dl. 42 (46.7%) patients had an Albumin value within normal range. Only five (5.6%) patients showed low (<3.5 g/dl) Albumin values. The median value for neutrophils was 4.25 $x10^9$/l and the median value for lymphocytes was 1.5 $x10^9$/l. The median value for the neutrophil to lymphocyte ratio (NLR) was 3.03. Most patients had a NLR of 2.5-5, which were 31 (34.4%) patients, followed by those with a NLR of <2.5, which were 23 (25.6%) patients. Only ten (15.6%) patients had a NLR of >5.

Table 3: ***Host Characteristics***

Variables	Frequency (%)
Age	
<60	7 (7.9)
60-69	12 (13.5)
70-79	39 (43.8)
80-89	30 (33.7)
≥90	1 (1.1)
Sex	
male	46 (51.7)
female	43 (48.3)
Race	
white	89 (100)
other	0 (0)
Immunosuppression	
Yes	4 (4.5)
No	85 (95.5)
post immunosuppression	
Yes	4 (4.5)
No	85 (95.5)
History of Immunosuppression	
Yes	8 (9)
No	81 (91)
Reason for Immunosuppression	
NHL	1 (1.1)
CLL	2 (2.2)
organ transplantation	3 (3.4)

other medical immunosuppression	2 (2.2)
Comorbidities	
cardiovascular disease	35 (39.3)
post cardiovascular event	26 (29.2)
respiratory disease	10 (11.2)
GI disease	7 (7.9)
metabolic disease	59 (66.3)
thyroid disease	19 (21.3)
urogenital disease	18 (20.2)
musculoskeletal disease	15 (16.9)
hepatobiliary disease	12 (13.5)
ophthalmological disease	6 (6.7)
neurological disorders	14 (15.7)
psychological disorders	5 (5.6)
hormonal imbalance (male)	4 (4.5)
hematologic disease	4 (4.5)
hematologic malignancies	6 (6.7)
type of hematologic malignancy	
none	83 (93.3)
B-cell lymphoma	3 (3.4)
NHL	1 (1.1)
CLL	2 (2.2)
dermatological disease	9 (10.1)
skin cancer	20 (22.5)
melanoma	5 (5.6)
BCC	17 (19.1)
SCC	8 (9)
cancer (other than skin and blood cancer)	7 (7.9)
type of cancer	
none	82 (92.1)
endometrial	2 (2.2)
prostate	2 (2.2)
pulmonary	2 (2.2)
laryngeal	1 (1.1)
CCI	
CCI of 0	31 (34.8)
CCI of 1-2	38 (42.7)
CCI of 3-4	13 (14.6)
CCI of ≥5	7 (7.9)
ACCI	
ACCI of 0-2	11 (12.4)

ACCI of 3-5	53 (59.6)
ACCI of >5	25 (28.1)
Lab Values	
CRP (mg/dl)	
CRP <5	37 (41.1)
CRP 5-20	18 (20)
CRP 20-100	9 (10)
CRP >100	1 (1.1)
LDH	
non-elevated	39 (43.3)
elevated (>240 U/l)	22 (24.4)
Albumin	
low (<3.5 g/dl)	5 (5.6)
normal	42 (46.7)
N:L Ratio	
<2.5	23 (25.6)
2.5-5	31 (34.4)
>5	10 (15.6)

Abbreviations: NHL, Non-Hodgkin lymphoma; CLL, chronic lymphatic leukaemia; GI, gastrointestinal; BCC, basal cell carcinoma; SCC, squamous cell carcinoma, CCI, Charlson Comorbidity Index; ACCI, age-adjusted Charlson Comorbidity Index; CRP, C-reactive protein LDH, lactate dehydrogenase; N:L Ratio, neutrophil to lymphocyte ratio;

3.1.2 Tumor Variables

Most tumors arose on the head and neck and on the limbs. More precisely, 35 (39.3%) patients had a tumor on the limbs and 34 (38.2%) patients had a tumor on the head and neck area. In 15 (16.9%) patients MCC arose on the trunk, while the primary site of four (4.5%) patients remained unknown. In regard to size, 33 (37.1%) tumors were smaller or exactly 2 cm, 27 (30.3 %) tumors were larger than 2 cm. With a quantity of 52 (58.4%), more than half of MCCs were of local extent at time of diagnosis and eleven (12.4%) tumors of regional extent. Noteworthy enough, 24 (27%) tumors of MCCs had already spread and were of distant extent at time of diagnosis.

Furthermore, the fact that at least five (5.6%) MCCs examined in this study showed up as collision lesions with another sort of skin cancer such as SCC or Bowen's disease is worth mentioning.

Table 4: ***Tumor Characteristics***

Variables	Frequency (%)
Anatomic site	
unknown primary site	4 (4.5)
head and neck	34 (38.2)
limbs	35 (39.3)
trunk	15 (16.9)
Size	
≤2 cm	33 (37.1)
>2 cm	27 (30.3)
Extent at time of diagnosis	
local	52 (58.4)
regional	11 (12.4)
distant	24 (27)
Collision lesion	5 (5.6)

3.1.3 Diagnostic Variables

76 (85.4%) patients had sonography, 72 (80.9%) patients had an X-Ray, 40 (44.9%) patients underwent computed tomography (CT), ten (11.2%) patients underwent magnetic resonance imaging (MRI), six (6.7%) underwent scintigraphy and, only five (5.6%) underwent positron emission tomography (PET). SLNB as pathologic nodal evaluation was performed in nine (10.1%) patients, LAD in 20 (22.5%) patients and SLNB followed by LAD in seven (7.9%) patients. Thus, 48 (53.9%) patients had no pathologic nodal evaluation. Of those who underwent SLNB, seven (7.9%) showed positive findings in at least one node and of those who underwent LAD whether after SLNB or without SLNB, 20 (22.5%) showed positive findings. Accordingly, two (2.2%) SLNBs and seven (7.9%) LADs showed no lymph node involvement.

Table 5: ***Diagnostic Characteristics***

Variables	Frequency (%)
Diagnostic imaging	
CT	40 (44.9)
MRI	10 (11.2)
PET	5 (5.6)
scintigraphy	6 (6.7)
X-Ray	72 (80.9)

Sonography	76 (85.4)
Pathologic nodal evaluation	
none	48 (53.9)
SLNB	9 (10.1)
LAD	20 (22.5)
LAD after SLNB	7 (7.9)
Pathologic nodal evaluation results	
none	48 (53.9)
positive SLNB finding	7 (7.9)
positive LAD finding	20 (22.5)
negative finding	9 (10.1)

Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; SLNB, sentinel lymph node biopsy; LAD, lymphadenectomy;

3.1.4 Treatment Variables

Research on initial treatment modalities, which had been performed within three months of diagnosis, revealed that 45 (50.6%) patients underwent surgery alone and 32 (36%) patients received a combination of surgical excision and radiotherapy. Chemotherapy as a monotherapy was performed in three (3.4%) and a combination therapy of chemotherapy and surgery was performed in four (4.5%) patients. Radiotherapy alone and the combination of radiotherapy and chemotherapy without surgery were not administered to any of the patients in the cohort whereas two patients (2.2%) received the triple combination therapy of surgery, radiotherapy and chemotherapy.

To conclude, a total of 84 (94.4%) patients received surgical excision within three months of diagnosis, 35 (39.3%) received radiotherapy and only ten (11.2%) underwent chemotherapy whether in a combination regimen or as a single treatment.

Clear surgical margins (R0, microscopically margin-negative resection) were achieved in 25 (28.1%) patients while 37 (41.6%) patients had no R0 resections. Of note, 23 (25.3%) histopathological reports lacked information about margins.

Table 6: ***Treatment Characteristics***

Variables	**Frequency (%)**
Initial treatment modality	
surgery alone	45 (50.6)
radiotherapy alone	0 (0)
chemotherapy alone	3 (3.4)

surgery and radiotherapy	32 (36)
surgery and chemotherapy	4 (4.5)
radio- and chemotherapy	0 (0)
surgery, radio- and chemotherapy	2 (2.2)
Initial tumor treatment	
any sugery	84 (94.4)
any radiotherapy	35 (39.3)
any chemotherapy	10 (11.2)
Histopathological margins	
clear	25 (28.1)
positive	37 (41.6)

3.1.5 Outcome Variables

23 (25.8%) patients had distant metastatic and 16 (18%) locoregional recurrences. However, about half of the patients (n = 45, 50.6%) stayed disease-free and no recurrences were discovered.

Time to recurrence ranged between <1 to 32 months with a mean time to recurrence of 10.87 months (SD 8.78) and a median time to recurrence of 8 months. Most of the recurrences developed within one year. More specifically, in 13 (14.6%) patients disease returned within 1 to 6 months and in 12 (13.5%) patients disease returned within 7 to 12 months. In four (4.5%) patients time to recurrence amounted to 13 to 18 months, in five (5.6%) patients to 19 to 24 months and in three (3.4%) patients recurrences developed not until two years after first diagnosis. In one (1.1%) patient first recurrence was discovered within the first month after initial diagnosis of MCC. Relating to survival variables, 26 (29.2%) patients of the study cohort were still alive. 36 (40.4%) were already dead, and 19 (21.3%) died due to MCC.

Table 7: ***Outcome Characteristics***

Variables	**Frequency (%)**
Disease recurrence	
none	45 (50.6)
locoregional	16 (18)
distant metastatic	23 (25.8)
Time to recurrence	
<1 month	1 (1.1)
1-6 months	13 (14.6)
7-12 months	12 (13.5)

13-18 months	4 (4.5)
19-24 months	5 (5.6)
>24 months	3 (3.4)
Overall survival	
yes	26 (29.2)
no	36 (40.4)
unknown	27 (30.3)
MCC-specific death	
yes	19 (21.3)
no	28 (31.5)
unknown	42 (47.2)

Abbreviations: MCC, Merkel cell carcinoma;

3.2 Associations with outcomes

3.2.1 Disease Recurrence

Overall, 16 (18%) patients had locoregional and 23 (25.8%) patients had distant metastatic recurrence. In five (5.6%) patients, information regarding localisation of recurrence was missing.

Age. Patients under 60 years had locoregional recurrence in one (14.3%) and distant metastatic recurrence in three (42.9%) cases. Patients between 60 and 69 years, had locoregional recurrence in one (8.3%) and distant metastatic recurrence in four (33.3%) cases. Patients between 70 and 79 years had locoregional recurrence in six (15.8%) and distant metastatic recurrence in ten (26.3%) cases. Patients between 80 and 89 years, had locoregional recurrence in eight (30.8%) and distant metastatic recurrence in six (23.1%) cases. All p-values are shown in Table 8.

Sex. Males had significantly higher recurrence rates than females (p = 0.009). In detail, seven (16.3%) men had locoregional and 18 (41.9%) men distant metastatic recurrence versus nine (22%) women with locoregional and five (12.2 %) women with distant metastatic recurrence.

Immunosuppression. We summarized groups of patients (1) being immunosuppressed at time of diagnosis and (2) who were immunosuppressed before diagnosed with MCC (“history of immunosuppression”). Equal parts of this group did or did not receive recurrence (each n = 4, 50%) whereby we could not find any relationship between disease recurrence and immunosuppression (p = 0.314).

Comorbidities. Disease recurrence occurred in 13 (40.4%) cases of patients with a cardiovascular disease, 15 (60%) patients with a cardiovascular event in their medical history, three (30%) patients with respiratory disease, 26 (45.6%) people with metabolic disease, eight (44.5%) people with thyroid disease, six (40%) patients with musculoskeletal disease, two (40%) people with ophthalmological disease, five (35.1%) people with neurological disorders, one (25%) patient with hematologic disease, two (40%) patients with hematologic malignancy, three (42.9%) patients with cancer other than skin and blood cancer, and nine (45%) people with skin cancer other than MCC.

However, in the groups with gastrointestinal (n = 5, 71.3%), urogenital (n = 9, 52.9%), hepatobiliary (n = 7, 58.3%), dermatological disease (n = 5, 62.5%), hormonal imbalance in male (n = 3, 75%) more patients had a relapse of MCC than those who did not.

In regard to patients with cancer other than skin and blood cancer, those with endometrial cancer (n = 2, 100%) or laryngeal cancer (n = 1, 100%) did not have any relapse whereas those with prostate cancer had either locoregional (n = 1, 50%) or distant metastatic recurrence (n = 1, 50%). If patients with melanoma had a relapse of MCC, they were all distant metastatic (n = 2, 40%). Most patients with BCC, who had disease recurrence, had distant metastatic occurrence (n = 6, 35,3%) as well. All patients in the cohort with psychological disorders (n = 5, 100%) did not receive any recurrence.

To summarize, we could not identify any statistical significance in all groups of different comorbidities in regard to disease recurrence. The individual p-values can be viewed in Table 8.

CCI and ACCI. Approximately equal parts of patients in any group of CCI had a relapse or not. Almost the same is applicable for the ACCI, whereby just ten (40%) patients with an ACCI over 5 showed disease recurrence (p = 0.742). Therefore we could not determine any statistically significant relationship between either CCI or ACCI and recurrence of MCC.

Lab values. We could not find any statistically significant relationship between any laboratory parameter and disease recurrence. Only concerning NLR, results showed that noticeably more patients with an NLR of >5 (n = 8, 80%) received disease recurrence.

Tumor variables. Most of the MCC patients with unknown primary (n = 3, 75%) had distant metastatic recurrences, but no locoregional recurrence. Half of the patients with a MCC of the head and neck area (n = 16, 50%) and half of the patients with a MCC on the trunk (n = 7, 50%) had a relapse, whereas only 13 (38.2%) patients with a MCC of the limbs had a recurrence. However, we could not show any statistically significant associations between anatomic site of MCC and recurrence (p = 0.238). In regard to size, 13 (40.6%) patients with a tumor equal or less than 2 cm and ten (38.5%) with a tumor over 2 cm received recurrences. Noticeably more patients with a tumor over 2 cm had distant metastatic recurrence (n = 8, 30.8%, locoregional: n = 2, 7.7%), but our analysis did not show any statistically significant relationship between size and disease recurrence (p = 0.627).
Regarding tumor extent, we found a relationship to disease recurrence (p= 0.045). Most people with regional extent (n = 7, 70%) suffered from a relapse and most of those who initially had distant extent (n = 10, 43.5%) received distant metastatic recurrence again. Nevertheless, almost equal parts of the last-mentioned group had a relapse or not (n = 11, 47.8 % versus n = 12, 52.2%) and slightly more patients with local extent at time of diagnosis (n = 30, 58.8%) of MCC had no recurrence at all. The majority of patients with collision lesion (n = 4, 80%) received recurrence of MCC.

Diagnostic variables. In terms of pathologic nodal evaluation, noticeably more patients with a LAD after SLNB (n = 5, 71.4%) did not receive any recurrence. However, we could not find any significant association between pathologic nodal evaluation (p = 0.724) and disease recurrence.

Treatment variables. We identified an association between treatment modalities and disease recurrence (p = 0.026). All patients who underwent surgery and chemotherapy (n = 4, 100%) received distant metastatic recurrence, although two (66.7%) patients who underwent chemotherapy as single modality treatment had no relapse. As mentioned before, most patients underwent either only surgery (n = 45, 50.6%), or surgery and radiotherapy (n = 32, 36%). Of those who underwent surgery and radiotherapy, 20 (62.5%) patients had no relapse at all. If patients in this particular group received any recurrence, there were 3-fold more patients with distant metastatic (n = 9, 28.1%) than with locoregional recurrence (n = 3, 9.4%). Of those

who underwent surgery as single modality treatment, 22 (51.2%) patients had no relapse, whereas the groups of locoregional (n = 12, 27.9%) and distant metastatic recurrence (n = 9, 20.9%) were almost of the same size.
For a closer look concerning the treatment modalities, almost equal parts of those who got any surgery received recurrence (n = 38, 46.3%), or not (n = 44, 53.7%) (p = 0.678). Most patients of those who underwent any radiotherapy (n = 22, 62.9%), stayed recurrence-free (p = 0.240) and altogether seven (70%) patients who underwent any chemotherapy, had a relapse with most of them having distant metastatic recurrence (n = 6, 60%). Therefore, we could identify a significant relationship between chemotherapy and disease recurrence (p = 0.048).
In regard to margins, most of the information, whether they were clear or not, was missing, but slightly more patients with clear ones did not receive recurrence (15, 60%). Nonetheless, we could not find any association.

Outcome variables. Concerning time to recurrence compared to the extent of recurrence, more recurrences were locoregional in the group of 1 to 6 months to recurrence (n = 9, 69.2%). In the group of 7 to 12 months (n = 8, 66.7%), 13 to 18 months (n = 3, 75%) and 18 to 24 months (n = 3, 60%) to recurrence more patients had distant metastatic recurrence. In our group ≥24 months to recurrence, all recurrences were distant metastatic (n = 3, 100%). All p-values can be viewed in Table 8.

3.2.2 Overall Survival

26 (29.2%) patients of the cohort survived, 36 (40.4%) did not survive and on 27 (30.3%) patients we could not find any information regarding overall survival.
Age. We discovered a statistically significant relationship between increasing age and overall survival. In the age group of 80 to 89 years, only four (21.1%) patients (p = 0.027) and seven (70%) patients in the age group of 60 to 69 years survived (p = 0.079).
Sex. Slightly more women (n = 14, 46.7%) than men (n = 12, 37.5%) survived after diagnosis of MCC.
Immunosuppression. In the group of patients with a history of immunosuppression, only one (16.7%) patient survived after diagnosis of MCC. However, we could not show any statistically significance (p = 0.387).

Comorbidities. One third of the patients suffering from cardiovascular disease (n = 7, 33.3%; p = 0.326), with a cardiovascular event in their past (n = 6, 31.6%; p = 0.272), with respiratory disease (n = 2, 33.3%; p = 1.0), suffering from metabolic disease (n = 15, 36.6%; p = 0.233), urogenital disease (n = 4, 36.4%; p = 0.748) or hematologic malignancy (n = 1, 33.3%; p = 1.0) survived. Only one (14.3%) of the patients with a gastrointestinal disease (p = 0.222), about the half of the people with thyroid disease (n = 5, 50%; p = 0.729), neurological disorders (n = 7, 53.8%; p = 0.328), psychological disorders (n = 2, 50%; p = 1.0) and dermatological disease (n = 3, 50%; p = 0.689), one quarter of patients having hepatobiliary disease (n = 2, 25%; p = 0.45), two thirds of patients with hematologic disease (n = 2, 66.7%; p = 0.567) survived. Most of the patients (n = 4, 80 %) suffering from cancer other than skin and blood cancer survived (p = 0.152), only the patient with pulmonary cancer passed away (p = 0.163). Among patients with skin cancer the group of people who survived (n = 8, 53.3%) and those who did not (n = 7, 46.7%), were about the same size (p = 0.304). About two thirds of those with melanoma (n = 2, 66.7%) survived (p = 0.567). Most of those with BCC (n = 8, 61.5%; p = 0.775) or SCC (n = 3, 60%; p = 1.0) along with MCC did not survive.

All patients suffering from ophthalmological disease (n = 5, 100%; p = 0.068), or hormonal imbalance (n = 3 men, 100%; p = 0.258) did not survive. Only in the group of patients having musculoskeletal disease, a relationship could be detected as eight (72.7%) patients survived after suffering from MCC (p = 0.04).

CCI and ACCI. Most of those who had a CCI of 1-2 (n = 18, 62.1%; p = 0.549) and a CCI of >5 (n = 3, 75%; p = 0.633) did not survive. Among those with an ACCI of 0-2, six (75%) patients survived (p = 0.059), whereas 25 (65.8%) patients with an ACCI of 3-5 did not survive (p = 0.121). About half of the patients with an ACCI of >5 (n = 7, 43.8%) did survive (p = 0.864).

Lab values. We could not find any association between lab values and overall survival. However, the majority of patients with higher NLR (2.5-5: n = 15, 65.2% and >5: n = 4, 66.7%) did not survive in comparison with those with a NLR <2.5 (n = 5, 31.3%; p = 0.088).

Tumor variables. Most of those the patients with MCC with unknown primary (n = 3, 75%), or MCC on the trunk (n = 8, 88.9%) did not survive. Those with a MCC on the head and neck area (each n = 10, 50%) or on the limbs (each n = 14, 50%) did

survive and die in equal parts. The same applies to the size of the tumor, since roughly the half of patients who had tumors under (n = 10, 52.6%), equal or over 2 cm in size (n = 11, 45.8%) survived. Patients with tumors, whose extent was regional (n = 4, 80%) or distant (n = 12, 66.7%), mostly died during observation period. All patients who had a collision lesion (n = 4, 100%) did not survive (p = 0.132). Together, we could not find any significant relationship between survival and anatomic site (p = 0.156), size (p = 0.892) or extent (p = 0.245).

Diagnostic variables. Regarding pathologic nodal evaluation, 18 (64.3%) patients of the cohort who did not undergo any sort of nodal evaluation did not survive and six (75%) patients who received SLNB did survive. Among those with LAD, the majority (n = 11, 68.8%) passed away and two thirds of those with LAD after SLNB (n = 4, 66.7%) did survive. Nonetheless, no association between pathologic nodal evaluation and overall survival could be detected (p= 0.102). However, we found a correlation between pathologic nodal evaluation results and overall survival (p = 0.045). Most of those with a positive SLNB finding (n = 4, 66.7%) or a negative finding (n = 7, 77.8%) survived whereas most of those with a positive LAD finding (n = 11, 73.3%) did not.

Treatment variables. Only if patients underwent surgery and radiotherapy the majority of them survived (n = 17, 63%). All other patients, including those who underwent only chemotherapy (n = 1, 100%), surgery and chemotherapy (n = 3, 100%), or surgery, radio- and chemotherapy (n = 1, 100%), did not survive. If only surgery was performed, most of the patients (n = 19, 67.9%) did not survive. However, we could not find any find a borderline statistically significant correlation between initial treatment modality and overall survival (p = 0.05).
Taking a closer look, if any surgery was performed no major effect on the survival rate could be shown (p = 1.0). However, if patients underwent any radiotherapy most of them (n = 18, 62.1%) survived and if they did not undergo any radiotherapy 23 (74.2%) patients did not survive (p = 0.005), which shows a relationship between overall survival and undergoing any radiotherapy. In addition, all patients who underwent chemotherapy (n = 5, 100%) did not survive (p = 0.063). Regarding margins, we could not show any relationship to overall survival (p = 0.818).

Outcome variables. Most patients with either locoregional (n = 9, 81.8%) or distant metastatic recurrence (n = 14, 82.4%) did not survive, resulting in a statistically significant association between disease recurrence and overall survival (p = 0.001). In general, we could not identify a statistically significant relationship between overall survival and time period to recurrence. Specifically, eight (80%) patients with recurrences within 1 to 6 months (p = 0.160), two (100%) patients with recurrences within 13 to 18 months to recurrence (100%), four (80%) patients with recurrences within 18 to 24 months (p = 0.367), and seven (87.5%) patients with recurrences within 7 to 12 months to recurrence did not survive (p = 0.063). Whereas only half of the patients with recurrences after >24 months (n = 1, 50%) did not survive (p = 1.0).

3.2.3 MCC-specific death

According to our records, 19 (21.3%) patients died due to MCC. However, in almost half of the cohort the cause of death is not clear (n = 42, 47.2%).

Age. Three (60%) patients under 60 years at time of diagnosis died due to MCC (p =0.345). In the groups of patients between 60 and 69 (n = 2, 22.2%; p = 0.278) and 70 and 79 years (n = 5, 26.3%; p = 0.187), the minority died due to MCC. However, we found a statistically significant association between the age group of 80 to 89 and MCC-specific death: nine (69.2%) patients died due to MCC (p = 0.031).

Sex. Six (30%) females and 13 (48.1%) males died due to MCC (p = 0.341). Nonetheless, we could not show any statistically significant relationship between sex and MCC-specific death.

Immunosuppression. The same applies to those with a history of immunosuppression as only three (60%) patients died due to MCC (p = 0.381).

Comorbidities. About half of the patients with cardiovascular disease (n = 7, 46.7%; p= 0.781), a cardiovascular event in their medical history (n = 7, 50%; p = 0.384), metabolic disease (n = 13, 44.8%; p = 0.635), thyroid disease (n = 4, 44.4%; p = 1.0) or dermatological disease (n = 3, 50%; p = 0.674) died due to MCC. The minority of those with respiratory disease (n = 2, 40%; p = 1.0), musculoskeletal disease (n = 2, 20%; p = 0.168), neurological disorders (n = 1, 11.1%; p = 0.064), hematologic disease (n = 1, 33.3%; p = 1.0) or skin cancer (n = 5, 38.5%; p = 1.0) died due to MCC. In contrast, the greater part of those with gastrointestinal disease (n = 4, 80%; p = 0.142), urogenital disease (n = 6, 60%; p = 0.276), hepatobiliary disease (n = 4, 57.1%; p = 0.417) died because of MCC.

Furthermore, all patients with ophthalmological disease (n = 2, 100%) died due to MCC (p = 0.158). No patient with psychological disorders (p = 0,262) or cancer other than skin and blood cancer (p = 0,072) died due to MCC. Among men with hormonal imbalance, we found information regarding cause of death in only one patient. This patient died due to MCC (p = 0,404). Regarding patients with hematologic malignancy, we could not identify any patient, who died due to the skin cancer (p = 1,0).

CCI and ACCI. The minority of those with a Charlson Comorbidity Index of 0 (n = 6, 37.5%; p = 1.0), 1-2 (n = 8, 40%; p = 1.0) and 3-4 (n = 3, 42.9%; p= 1.0) did not survive MCC. Half of the patients with a CCI >5 (n = 2, 50%) also died due to MCC (p = 1.0). Regarding ACCI, the minority of all groups died due to MCC. In conclusion, we could not identify any relationship between MCC-specific death and neither any comorbidity nor any Comorbidity Index.

Lab values. We could not show any significant association with MCC-specific death. All p-values are shown in Table 8.

Tumor variables. Anatomic site (p = 0.148) and size (p = 0.566) did not indicate any association with death due to MCC. To have a closer look, most of the patients with unknown primary site (n = 2, 66.7%), or with a tumor appearance on the trunk (n = 4, 80%) died due to MCC, whereas only seven (38.9%) of the patients with a tumor occurring on the head and neck area and six (28.6%) patients with a tumor on the limbs died of MCC. Regarding size, four (26.7%) patients with a tumor equal or less than 2 cm died due to MCC. In comparison, eight (42.1%) patients with a tumor over 2 cm died of MCC. However, we could examine a relationship between tumor extent and death due to MCC (p = 0.031), since only few patients (n = 8, 26.7%) with local extent died of MCC. In comparison, most of those with distant extent at time of diagnosis (n = 8, 57,1%) and the two (100%) patients with regional extent and identified information on cause of death, died due to MCC. The majority of those with collision lesion (n = 2, 66.7%, p = 0.557) also died because of MCC.

Diagnostic variables. We could not show any statistically significant relationship between pathologic nodal evaluation and MCC-specific death (p = 0.403) or pathologic nodal evaluation results and MCC-specific death (p = 0.145). The minority of patients undergoing SLNB (n = 2, 28.6%), LAD after SLNB (n = 1, 20%) or no nodal evaluation at all (n = 8, 38.1%) died due to MCC. Also only few patients with a

positive SLNB finding (n = 2, 33.3%) or negative finding (n = 2, 25%) did not survive MCC, whereas most of the patients with a positive LAD finding (n = 6, 60%) died due to the tumor.

Treatment variables. Everyone who underwent chemotherapy alone (n = 1, 100%), surgery and chemotherapy (n = 2, 100%) or any chemotherapy at all (n = 3, 100%) and whose cause of death could be identified died due to MCC. In contrast, few of those who underwent surgery and radiotherapy (n = 7, 30.4%), any surgery (n = 17, 37.8%) or any radiotherapy (n = 7, 29.2%) died of MCC. No association between initial treatment modality (p = 0.145), any surgery (p = 0.391), any radiotherapy (p= 0.253) or any chemotherapy (p = 0.054) and MCC-specific death could be shown. Also, results of margins did not reveal any association with MCC-specific death (p = 1.0).

Outcome variables. Six (75%) patients with locoregional recurrence and eleven (73.3%) patients with distant metastatic recurrence died due to MCC, whereas almost all of the patients (n = 22, 95.7%) who stayed relapse-free did not die due to MCC (p < 0.001). Referring to time to recurrence, most of the patients with 1 to 6 months (n = 6, 75%; p = 0.039), 7 to 12 months (n = 7, 87.5%; p = 0.003) and 18 to 24 months (n = 2, 66.7%; p = 0.378) to recurrence died due to MCC, which allows associations with two of the groups of time to recurrence.

Table 8: ***Associations between Host, Tumor, Diagnostic, Treatment Characteristics and Outcomes***

	Outcomes						
	Disease Recurrence			**Overall Survival**		**MCC-specific death**	
Characteristics	**locoreg.**	**metastatic**	**p-value**	**events (no)**	**p value**	**events (yes)**	**p-value**
Host							
Age							
<60	1 (14.3)	3 (42.9)	0.630§	3 (60)	0.927*	3 (60)	0.381*
60-69	1 (8.3)	4 (33.3)	0.582§	3 (30)	0.079*	2 (22.2)	0.278*
70-79	6 (15.8)	10 (26.3)	0.720§	15 (55.6)	0.725§	5 (26.3)	0.187+
80-89	8 (30.8)	6 (23.1)	0.187§	15 (78.9)	0.027§	9 (69.2)	0.031+
Sex			0.009§		0.465§		0.341+
male	7 (16.3)	18 (41.9)		20 (62.5)		13 (48.1)	
female	9 (22)	5 (12.2)		16 (53.3)		6 (30)	
History of Immunos.			0.314§		0.387*		0.381*
Yes	3 (37.5)	1 (12.5)		5 (83.3)		3 (60)	
No	13 (17.1)	22 (28.9)		31 (55.4)		16 (38.1)	

Reason for immunos.			0.431§		0.489§		0.632§
NHL	1 (100)	0 (0)		0 (0)		0 (0)	
CLL	1 (50)	0 (0)		1 (100)		1 (100)	
organ transpl.	1 (33.3)	0 (0)		2 (66.7)		1 (50)	
other *medical is.*	0 (0)	1 (50)		2 (100)		1 (50)	
Comorbidities							
cardiovascular dis.	4 (12.1)	9 (27.3)	0.392§	14 (66.7)	0.326§	7 (46.7)	0.781+
post cardio. event	6 (24)	9 (36)	0.266§	13 (68.4)	0.272§	7 (50)	0.384§
respiratory dis.	1 (10)	2 (20)	0.526§	4 (66.7)	1.0*	2 (40)	1.0*
GI dis.	1 (14.3)	4 (57.1)	0.178§	6 (85.7)	0.222*	4 (80)	0.142*
metabolic dis.	13 (22.8)	13 (22.8)	0.260§	26 (63.4)	0.233§	13 (44.8)	0.635+
thyroid dis.	5 (27.8)	3 (16.7)	0.388§	5 (50)	0.729*	4 (44.4)	1.0*
urogenital dis.	4 (23.5)	5 (29.4)	0.808§	7 (63.6)	0.748*	6 (60)	0.276*
musculoskeletal dis.	2 (13.3)	4 (26.7)	0.796§	3 (27.3)	0.040*	2 (20)	0.168*
hepatobiliary dis.	3 (25)	4 (33.3)	0.666§	6 (75)	0.450*	4 (57.1)	0.417*
ophthalmol. dis.	1 (20)	1 (20)	0.928§	5 (100)	0.068*	2 (100)	0.158*
neurological dis.	4 (28.6)	1 (7.1)	0.159§	6 (46.2)	0.328§	1 (11.1)	0.064*
psychological dis.	0 (0)	0 (0)	0.100§	2 (50)	1.0*	0 (0)	0.262*
hormonal imbalance (male)	2 (50)	1 (25)	0.251§	3 (100)	0.258*	1 (100)	0.404*
hematologic dis.	0 (0)	1 (25)	0.557§	1 (33.3)	0.567*	1 (33.3)	1.0*
hematologic malignancies	1 (20)	1 (20)	0.928§	2 (66.7)	1.0*	0 (0)	1.0*
type			0.344§		0.677§		1.0*
none	15 (19)	22 (27.8)		34 (57.6)		19 (41.3)	
B-cell lymphoma	0 (0)	0 (0)		1 (50)		0 (0)	
NHL	1 (100)	0 (0)		0 (0)		0 (0)	
CLL	0 (0)	1 (50)		1 (100)		0 (0)	
dermatological dis.	1 (12.5)	4 (50)	0.320§	3 (50)	0.689*	3 (50)	0.674*
skin cancer	3 (15)	6 (30)	0.860§	7 (46.7)	0.304§	5 (38.5)	1.0+
melanoma	0 (0)	2 (40)	0.506§	1 (33.3)	0.567*	1 (33.3)	1.0*
BCC	3 (17.6)	6 (35.3)	0.712§	8 (61.5)	0.775§	6 (54.5)	0.312*
SCC	2 (25)	1 (12.5)	0.603§	3 (60)	1.0*	2 (50)	1.0*
cancer (not skin)	1 (14.3)	2 (28.6)	0.945§	1 (20)	0.152*	0 (0)	0.072*
type of cancer			0.667§		0.163§		0.434§
none	15 (19.5)	21 (27.3)		35 (61.4)		19 (45.2)	
endometrial	0 (0)	0 (0)		0 (0)		0 (0)	
prostate	1 (50)	1 (50)		0 (0)		0 (0)	
pulmonary	0 (0)	1 (50)		1 (100)		0 (0)	
laryngeal	0 (0)	0 (0)		0 (0)		0 (0)	
CCI							
CCI of 0	3 (10.7)	9 (32.1)	0.372§	11 (52.4)	0.516§	6 (37.5)	1.0+
CCI of 1-2	9 (25)	8 (22.2)	0.408§	18 (62.1)	0.549§	8 (40)	1.0+
CCI of 3-4	4 (30.8)	3 (23.1)	0.504§	4 (50)	0.710*	3 (42.9)	1.0*
CCI of ≥5	0 (0)	3 (42.9)	0.343§	3 (75)	0.633*	2 (50)	1.0*
ACCI							
ACCI of 0-2	1 (9.1)	4 (36.4)	0.596§	2 (25)	0.059*	2 (25)	0.445*
ACCI of 3-5	11 (22.9)	13 (27.1)	0.563§	25 (65.8)	0.121§	12 (46.2)	0.554+
ACCI of >5	4 (16)	6 (24)	0.742§	9 (56.3)	0.864§	5 (38.5)	1.0+
Lab Data							

CRP (mg/dl)			0.528§		0.263§		0.628§
CRP <5	6 (16.2)	10 (27)		13 (43.3)		9 (36)	
CRP 5-20	4 (22.2)	3 (16.7)		8 (72.7)		5 (55.6)	
CRP20-100	1 (11.1)	4 (44.4)		2(40)		2 (40)	
CRP >100	0 (0)	1 (100)		1(100)		0 (0)	
LDH			0.368§		0.198+		0.153*
non-elevated	7(17.9)	8 (20.5)		11 (40.7)		7 (30.4)	
elevated (>240 U/l)	4 (18.2)	8 (36.4)		10 (66.7)		7 (58.3)	
Albumin			0.592§		1.0*		0.520*
low (<3.5 g/dl)	1 (20)	2 (40)		1 (50)		9 (39.1)	
normal	7 (16.7)	9 (21.4)		13 (46.4)		0 (0)	
N:L Ratio			0.125§		0.088§		0.225§
<2,5	2 (8.7)	7 (30.4)		5 (31.3)		4 (25)	
2.5-5	7 (22.6)	6 (19.4)		15 (65.2)		8 (50)	
>5	3 (30)	5 (50)		4 (66.7)		3 (60)	

Tumor							
Anatomic site			0.238§		0.156§		0.148§
unknown prim. site	0 (0)	3 (75)		3 (75)		2 (66.7)	
head and neck	9 (28.1)	7 (21.9)		10 (50)		7 (38.9)	
limbs	5 (14.7)	8 (23.5)		14 (50)		6 (28.6)	
trunk	2 (14.3)	5 (35.7)		8(88.9)		4 (80)	
Size			0.627§		0.892+		0.566+
≤2 cm	5 (15.6)	8 (25)		9 (47.4)		4 (26.7)	
>2 cm	2 (7.7)	8 (30.8)		13 (54.2)		8 (42.1)	
Extent at diagnosis			0.045§		0.245§		0.031§
local	11 (21.6)	10 (19.6)		18 (48.6)		8 (26.7)	
regional	4 (40)	3 (30)		4 (80)		2 (100)	
distant	1 (4.3)	10 (43.5)		12 (66.7)		8 (57.1)	
Collision Lesion	2 (40)	2 (40)	0.265§	4 (100)	0.132*	2 (66.7)	0.557*

Diagnostic							
Pathol. nodal eval.			0.724§		0.102§		0.403§
none	10 (21.3)	11 (23.4)		18 (64.3)		8 (38.1)	
SLNB	1 (11.1)	3 (33.3)		2 (25)		2 (28.6)	
LAD	5 (26.3)	6 (31.6)		11 (68.8)		7 (58.3)	
LAD after SLNB	0 (0)	2 (28.6)		2 (33.3)		1 (20)	
Path. nodal ev. results			0.763§		0.045§		0.465§
pos. SLNB finding	1 (14.3)	3 (42.9)		2 (33.3)		2 (33.3)	
pos. LAD finding	3 (15.8)	7 (36.8)		11 (73.3)		6 (60)	
neg. finding	2 (22.2)	1 (11.1)		2 (22.2)		2 (25)	

Treatment							
Initial treatment mod.			0.026§		0.05§		0.145§
surgery alone	12 (27.9)	9 (20.9)		19 (67.9)		8 (40)	
radioth. alone	0 (0)	0 (0)		0 (0)		0 (0)	
chemoth. alone	0 (0)	1 (33.3)		1 (100)		1 (100)	
surgery + radioth.	3 (9.4)	9 (28.1)		10 (37)		7 (30.4)	
surgery + chemoth.	0 (0)	4 (100)		3 (100)		2 (100)	
radio- and chemoth.	0 (0)	0 (0)		0 (0)		0 (0)	

surgery, radio- and chemotherapy	1 (50)	0 (0)		1 (100)		0 (0)	
Initial tumor treatment							
any sugery	16 (19.5)	22 (26.8)	0.678§	33 (55.9)	1.0*	17 (37.8)	0.391*
any radiotherapy	4 (11.4)	9 (25.7)	0.240§	11 (37.9)	0.005§	7 (29.2)	0.253+
any chemotherapy	1 (10)	6 (60)	0.048§	5 (100)	0.063*	3 (100)	0.054*
Histopathol. margins			0.345§		0.818§		1.0+
clear	4 (16)	6 (24)		10 (55.6)		6 (42.9)	
positive	10 (27.8)	11 (30.6)		13 (52)		8 (40)	
Disease recurrence					0.001§		<0.001§
none	-	-	-	10 (32.3)		1 (4.3)	
locoregional	-	-	-	9 (81.8)		6 (75)	
distant metastatic	-	-	-	14 (82.4)		11 (73.3)	
Time to recurrence							
<1 month	0 (0)	1 (100)	0.246§	0 (0)	-	0 (0)	-
1-6 months	9 (69.2)	4 (30.8)	<0.001§	8 (80)	0.160*	6 (75)	0.039*
7-12 months	4 (33.3)	8 (66.7)	<0.001§	7 (87.5)	0.063*	7 (87.5)	0.003*
13-18 months	1 (25)	3 (75)	0.048§	2 (100)	0.497*	1 (100)	0.378*
18-24 months	2 (40)	3 (60)	0.042§	4 (80)	0.367*	2 (66.7)	0.547*
>24 months	0 (0)	3 (100)	0.013§	1 (50)	1.0*	0 (0)	0.519*

§ Chi-square test

+ Yate's correction for continuity

* Fisher's exact test

Abbreviations: NHL, Non-Hodgkin lymphoma; CLL, chronic lymphatic leukaemia; GI, gastrointestinal; BCC, basal cell carcinoma; SCC, squamous cell carcinoma, CCI, Charlson Comorbidity Index; ACCI, age-adjusted Charlson Comorbidity Index; CRP, C-reactive protein LDH, lactate dehydrogenase; N:L Ratio, neutrophil to lymphocyte ratio; SLNB, sentinel lymph node biopsy; LAD, lymphadenectomy;

4 Discussion

Here, we demonstrate several significant associations between the observed variables and our main outcomes of interest: disease recurrence, overall survival, and MCC-specific death. Patients diagnosed with MCC at the age between 80 and 89 years were significantly more likely to die overall ($p = 0.027$) and from MCC ($p = 0.031$) than other age groups. With respect to comorbidities, our study showed that patients with musculoskeletal disease were more likely to survive ($p = 0.04$), whereas we could not find any further significant associations between outcome characteristics and any other comorbidity.

Additionally, overall survival was statistically significantly associated with pathologic nodal evaluation results ($p = 0.045$) as patients with positive LAD were less likely to survive. Further, receiving radiotherapy was associated with higher overall survival rates ($p = 0.005$). The initial treatment modality ($p = 0.026$) and receiving chemotherapy ($p = 0.048$) could be related to disease recurrence as well. Patients undergoing any chemotherapy were more likely to develop recurrences. In terms of tumor extent at time of diagnosis, this study reports an association with disease recurrence ($p = 0.045$) and MCC-specific death ($p = 0.031$). Patients with regional extent at time of diagnosis had higher odds to develop recurrences and to die due to MCC. Patients with distant extent of disease at time of diagnosis were more likely to develop distant metastatic recurrences and to die due to MCC as well. Sex was associated with disease recurrence ($p = 0.009$) as males were more likely to receive recurrences. Patients with disease recurrence were more likely to die overall ($p = 0.001$) and due to MCC ($p < 0.001$). Lastly, patients having disease recurrence within 1 to 12 months had significantly higher odds to die (1 to 6 months: $p = 0.039$; 7 to 12 months: $p = 0.003$).

4.1 Host Variables

With a mean age of about 75 (SD 9,4) and a median age of 76, the results of this study are comparable to those from earlier studies investigating age at diagnosis (25). The same applies to contribution of sex and race in this cohort, since slightly more men than women and only white individuals were diagnosed with MCC, as preceding studies have already suggested. (8) Concerning outcomes, older patients (age group 80 to 89) were understandably more likely to die overall (n = 15, 78.9%; $p = 0.027$), but also more likely to die due to MCC (n = 9, 69.2%; $p = 0.031$)

compared to the younger age groups. Noticeably, males had significantly higher odds of disease recurrence (n = 25, 58.2%) than women (n = 14, 34.2%; p = 0.009). Also, recurrences were mainly distant metastatic in males (n = 18, 41.9%). Regarding overall survival and MCC-specific death, women had a higher survival rate (n = 14, 46.7%) than men (n = 12, 37.5%), although we could not identify any statistical significance. Together, this study supports the assumption of male sex as a risk factor for worse prognosis of MCC as previous studies have already suggested. (5) (40)

4.1.1 Immunosuppression

As MCC is known to develop more likely and progressing worse in immunosuppressed individuals including predominantly patients with B-cell malignancies, organ transplantation and HIV-infection, an association between MCC and immunocompetence is assumed. (8) (41) (42) Although we could not show any statistical significances, eight (9%) patients with a history of immunosuppression due to organ transplantation, chronic lymphatic leukaemia, Non-Hodgkin lymphoma or other medical immunosuppression were recorded. Notably, only one (16.7%) of them survived. The impossibility of showing any significant relation, particularly between immunosuppression and overall survival or MCC-related death, could either be explained due to the small cohort of this study or the lack of information especially on disease-specific death.

Nonetheless, the results considering the frequency of immunosuppressed individuals among patients with MCC compare well to those from previous studies investigating the relation of MCC and immunosuppression. For example, Heath et al. found 7.8% of their cohort having some sort of immunosuppression, which were described as a 16-fold overrepresentation compared to the estimated frequency of the general US population. (8)

Asgari et al. reported a frequency of 6% of immunosuppressed patients in their study and found immunosuppression significantly associated with MCC-specific death. (25) Further, Paulson et al. described immunosuppression as a stage-independent prognostic factor regarding survival of patients with MCC as they showed an almost twice as good survival rate among immune competent MCC-patients. (36)

Although we could not demonstrate any significant association between immunosuppression and outcome, the results of our study support the assumption of

immunosuppression acting as an important risk factor for MCC. Therefore, close surveillance of immunosuppressed patients and consideration of adapting immunosuppressive therapy, if any modification is conceivable regarding the individual clinical situation, is worth considering.

4.1.2 Comorbidities

As previous studies have already suggested to consider comorbidities in future research on MCC (25), our study intended the identification of further factors contributing to disease outcome. Due to the advanced age of most patients at time of diagnosis, the frequency of pre-existing and accompanying diseases is probably elevated. As immunosuppression was already reported to have an impact on developing MCC and worsening prognosis, the probability of other comorbidities affecting disease outcome as well can be assumed.

In this study, the most frequent comorbidities examined were metabolic (n = 59, 66.3%) and cardiovascular disease (n = 35, 39.3%), which clearly can be attributed to the advanced age of the cohort. Compared to the other comorbidities, recurrences occurred more frequently in patients with gastrointestinal disease (n = 5, 71.4%), dermatological disease (n = 5, 62.5%) and hepatobiliary disease (n = 7, 58.3%). The group of patients with gastrointestinal disease showed a high percentage of deaths related to MCC (n = 4, 80%) as well. Regarding overall survival, all patients suffering from ophthalmological disease (n = 5, 100%). If information on cause of death was given, patients with hormonal imbalance (one, 100%) did not survive and patients with ophthalmological disease (n = 2, 100%) died due to MCC. Regarding psychological disorders, we could not examine any recurrences or MCC-related deaths. However, numbers of patients in these groups were little and we could not find any significant association.

Regarding cancer other than skin and blood cancer, attention was especially paid to cancers prior to MCC. We found six (6.7%) patients with haematologic malignancies like Non-Hodgkin-lymphoma and seven (7.9%) patients with cancer other than skin and blood cancer including prostate cancer, lung cancer, laryngeal cancer and endometrial cancer. Regarding the examination on outcome, the two (100%) patients suffering from prostate cancer and one (50%) with lung cancer showed recurrences of MCC, however no death due to MCC could be recorded in any patient with cancer other than skin and blood cancer.

Several previous studies have already discussed the increased risk of primary cancers prior or following MCC. Youlden et al. found patients with primary MCC at more than 2-fold risk of developing subsequent cancers and patients with cancers other than MCC at 2.5-fold risk of developing subsequent MCC. These risks applied to melanoma, lip cancer, head and neck cancer (such as laryngeal cancer), lung cancer, myelodysplastic diseases and cancer of unknown primary site. If patients had MCC prior to other cancer, risks for kidney cancer and breast cancer in females were increased. Conversely, patients with colorectal cancer, prostate cancer, non-Hodgkin lymphoma and lymphatic leukaemia had higher risks of MCC.

Regarding etiology, studies suggested the involvement of MCPyV in other cancers, UV radiation and certain genetic links as possible explanations. Especially regarding melanoma, the shared association with sun exposure and immunosuppression presents evidence of relation. In addition, Youlden and Baade found a high number of head and neck cancer, female breast cancer and kidney cancer among patients suffering from melanoma in their past, although knowledge about etiological links between these cancers, melanoma and MCC is lacking. (43)

In this study, we found five (5.6%) patients with melanoma. If patients suffering from melanoma had recurrences, they were always distant metastatic (n = 2, 40%). However, most patients with melanoma survived (n = 2, 66.7%), contrary to expectations, considering the aggressiveness of melanoma in advanced stages. Comparing MCC and melanoma, rates of regional metastases are higher and prognosis seems to be worse for MCC, possibly due to the characteristic of commonly affecting elder individuals compared to melanoma. (44) More specifically, studies reported MCC being 2-fold more lethal than melanoma. (5)

As previous studies have already suggested the association of melanoma, BCC, SCC and MCC regarding the shared etiological role of UV radiation, Reinau et al. reported an association between immunosuppression and BCC as well. Equally to melanoma and MCC being reported to be associated due to its similar aetiologies including UV radiation and immunosuppression, Reinau et al. reported a relation of BCC and immunosuppression-related diseases like organ transplantation which are also associated with MCC. (45) Paulson et al. similarly suggested the relation of immunosuppression and melanoma, BCC and SCC in conjunction with worse outcomes. (36) However, we could not find any significant association between outcome of MCC and any skin cancer.

Furthermore, we ascertained a significant correlation between musculoskeletal disease and overall survival as most patients did survive (n = 8, 72.7%). Studies of MCC in patients with musculoskeletal diseases are lacking. Considering rheumatoid arthritis (RA) as an example for musculoskeletal disease, higher incidence and worse prognosis of MCC can be assumed. As outcome and development of MCC are associated with chronic inflammatory disorders, studies reported connective tissue diseases like rheumatoid arthritis to act equally in affected patients (46), opposing the results of this study.

Comparing comorbidities of BCC and MCC, patients with BCC were more likely to suffer, inter alia, from RA or inflammatory bowel disease, explained by the vulnerability of immunosuppressed patients. (45)

Regarding the Charlson Comorbidity Index, most patients with a low ACCI (0-2: n = 6, 75%) were more likely to survive compared to those with higher ACCIs (3-5: n = 13, 34.2%; >5: n = 7, 43.8%). However, we could not find any statistically significant association of either CCI or ACCI and disease recurrence, survival or MCC-related death.

Concerning lab values, our study did not show any significant associations between outcome and neither CRP, LDH nor Albumin values. Although especially NLR previously has been found to be linked to the prognosis of many malignancies (47), this study could not demonstrate a significant relation to disease outcome. However, patients with higher values of NLR (2.5-5: n = 15, 65.2% and >5: n = 4, 66.7%) showed higher likelihood to die. Previous studies especially reported an association of NLR and poor survival rates. Attempts of explanation led to the assumption of an increased recurrence rate due to a relative lymphocytopenia and decreased leukocyte response in patients with elevated NLR. Further, higher values of neutrophils could lead to the release of the vascular endothelial growth factor and therefore to an accelerated progression of the tumor. However, the exact mechanism how NLR can act as a prognostic factor for malignancies is still unclear. (48)

Nonetheless, the results of this study should be viewed critically regarding scientific relevance due to the small size of our cohort and respectively of each group. Therefore, conclusion should be drawn cautiously and further examinations regarding the impact of comorbidities on MCC-specific outcome are worth considering. However, due to the findings concerning other cancers prior or in addition to MCC, a strict

surveillance of patients with oncological diseases, especially hematologic malignancies should be considered.

4.2 Tumor Variables

Most MCCs in this cohort occurred on the head and neck area and on the limbs, similar to results of previous studies. (8) (25) Although we could not find any statistically significant association regarding anatomic site, the higher likelihood of distant metastatic recurrences (n = 3, 75%) and worse overall survival (n = 1, 25%) of patients suffering from MCC with unknown primary site stood out. However, statistical significance could not be reached. In contrast, Asgari et al. found improved prognosis for overall survival and smaller chance of developing distant metastases in patients with a tumor of unknown primary site. (25)

Regarding tumor size, our study could not demonstrate any statistically significant association either. However, metastatic recurrences were more frequent among patients with a tumor size of more than 2 cm (n = 8, 25%). Previous findings, whether tumor size has an impact on relapse-free and overall survival rates, are controversial. Nonetheless, larger studies suggested that tumor size presents a weak predictor for MCC outcome. (34)

In terms of tumor extent at time of diagnosis, most tumors occurred local (n = 52, 58.4%) followed by distant site of extent (n = 24, 27%). Only eleven (12.4%) patients showed regional extent at time of diagnosis. In this study, tumor extent was significantly associated with recurrences (p= 0.045) and death due to MCC (p = 0.031). More specifically, patients with initial regional disease suffered more likely from recurrences after primary MCC (n = 7, 70%). If patients had distant disease at time of diagnosis, they were more likely to develop distant metastatic recurrences (n = 10, 43,5%). All patients with regional disease (n = 2, 100%) and the majority of those with initial distant extent of disease (n = 8, 57.1%) died due to MCC, whereas patients with local extent of disease at time of diagnosis were more likely to survive MCC (n = 8, 26.7%). Therefore, data supports findings from previous studies considering site of extent as a significant and independent prognostic factor for MCC outcome. (5) (25) (49) (50) However, in many cases information on overall survival and MCC-related death was missing.

4.3 Diagnostic Variables

Although results regarding pathologic nodal evaluation were not significant, patients who underwent SLNB or LAD after SLNB showed improved outcome (overall survival and death due to MCC) in comparison to those only undergoing LAD or no pathologic nodal evaluation at all. In depth, only two (25%) patients with SLNB and two (33.3%) with LAD after SLNB did not survive, whereas 18 (64.3%) patients who did not undergo any pathologic nodal evaluation and eleven (68.8%) with LAD died. Regarding MCC-specific death, one (20%) patient with LAD after SLNB and two (28.6%) patients with SLNB died due to MCC, whereas eight (38.1%) patients without any pathological nodal evaluation and seven (58.3%) with LAD died because of the tumor. These results equal previous findings of studies highlighting the benefits of pathologic nodal evaluation including SLNB as it contributes to improved prognostic accuracy. (25) (51) Gupta et al. recommended the performance of SLNB in patients diagnosed with MCC as radiologic imaging failed in some cases and patients were understaged. Therefore, they suggested SLNB even if patients did not show any clinical sign of nodal involvement. (34) Further, Iyer et al. recommended the consideration of pathologic nodal evaluation independent from tumor size and found the amount of lymph nodes involved as an important predictive factor for survival. In addition, results of SLNB were found to improve the decision-making process concerning treatment modalities leading to an explanation for the relation to survival rates. (49)

In addition, a previous study found improved prognosis (overall survival) in patients with negative SLNB compared to those with positive SLNB. (12) In this study, we also found a statistically significant association between pathologic nodal evaluation results and survival ($p = 0.045$). Specifically, patients with negative findings ($n = 7$, 77.8%) were most likely to survive, followed by patients with positive SLNB findings ($n= 4$, 66.7%). In contrast, only about a quarter of patients with positive LAD findings ($n = 4$, 26.7%) survived.

4.4 Treatment Variables

Finally, regarding treatment, data showed an association between recurrence rate and the initial treatment modality ($p= 0.026$). Further, any chemotherapy was associated with a higher risk for recurrences ($p = 0.048$) and any radiotherapy ($p = 0.005$) was associated with improved overall survival.

More specifically, all patients who received chemotherapy in combination with surgery developed recurrences, which were all distant metastatic (n = 4, 100%). However, patients who received chemotherapy as single treatment modality did not show higher recurrence rates (n = 1, 33.3%) compared to the other groups. If we had the required information regarding death, all patients who received chemotherapy did not survive (n = 5, 100%) and died due to MCC (n = 3, 100%).

Although MCC is thought to be sensitive to chemotherapy, studies reporting benefits regarding outcome of MCC are lacking. In the literature, chemotherapy is frequently attributed to increased morbidity and short survival duration. Therefore, the advantage of chemotherapy as treatment modality of MCC is questionable (5) and may only be considered in terminal stages of disease. (25)

As recent studies demonstrated, immunotherapy is assumed to be a reasonable alternative to systemic chemotherapy. The usage of antibodies targeting PD-L1 like Avelumab showed durable responses and improved overall and MCC-specific survival rates in patients with metastatic disease. Kaufman et al. therefore stated immunotherapy to become standard of care in advanced MCC. (35) As immunotherapy had not been introduced until 2016 (12), studies concerning its potential as treatment modality of metastatic MCC had been lacking and checkpoint inhibitors had not been admitted for clinical use, we could not identify any patient in our cohort undergoing immunotherapy since only patients treated between 1992 and 2016 were included in our study. (5)

As a further alternative to chemotherapy, Iyer et al. discussed the usage of single-fraction radiotherapy in case of metastatic disease and managed to obtain durable responses and a reduction of tumor burden without as high numbers of side effects as seen in chemotherapy. (52)

Especially in comparison with surgery as single modality treatment, patients of our cohort receiving radiotherapy (in combination with surgery) showed only few locoregional recurrences (n = 3, 9.4%), but three times as much distant metastatic recurrence (n = 9, 28.1%). Previous studies have already supported the assumption that recurrences occurring after radiotherapy were more likely distant. (53) Similarly, Asgari et al. found patients receiving radiotherapy at reduced risk for developing locoregional, but not metastatic disease. However, their results do not support the finding of this study that receiving any radiotherapy improves overall survival rates. (25) Another study reported improved overall and MCC-specific survival in patients

receiving adjuvant radiotherapy, although results were not statistically significant. (54) Ghadjar et al. stated improved outcome including local recurrence-free, distant metastasis-free survival, disease-free survival and MCC-specific survival in patients receiving radiotherapy compared to patients only undergoing surgery. (55) Similar findings regarding the benefit of radiotherapy whether as adjuvant treatment or as single modality treatment in inoperable cases have been reported in the literature. Not only the advantages of radiotherapy compared to surgery as single modality treatment, but also the improved outcome compared to adjuvant chemotherapy have been highlighted. (3) (5) Therefore, radiotherapy should be considered as an essential component of the treatment of MCC.

4.5 Outcome Variables

As expected, MCC recurrences whether locoregional or distant metastatic were associated with worse outcome (overall survival: $p = 0.001$ and MCC-specific death: $p < 0.001$). However, there could not be found any major differences between locoregional and distant metastatic recurrences concerning survival rates. Indeed, opinions on the impact of site of recurrences regarding survival differ. (27)

As reported in previous studies (43), early metastases develop frequently within 12 months after initial diagnosis. Most locoregional recurrences are thought to develop within the first 12 months with a median time of four months. (27) In this study, most locoregional recurrences did indeed develop within the first 1 to 6 months (n = 9, 69.2%). Distant metastatic recurrences were more likely to develop later with the highest number after 7 to 12 months (n = 8, 66.7%). In fact, the median time to nodal recurrence ranges between 7 and 8 months. (27) The groups of 1 to 6 months (n = 6, 75%; $p = 0.039$) and 7 to 12 months (n = 7, 87.5%; $p = 0.003$) until recurrence died significantly more often due to MCC. However, this finding can be explained by the greater number of patients in these groups compared to the other groups. Concerning overall survival, statistical analysis did not show any significant association, although patients of the groups of 1 to 6 (n = 8, 80%), 7 to 12 (n = 7, 87.5%) and 18 to 24 months (n = 4, 80%) until recurrence were more likely to die.

4.6 Limitations

One of the main limitations of this study is clearly the small number of the cohort due to the rareness of MCC. Furthermore, we only included patients treated at the LKH University Clinic Graz. In some cases, patients had been initially diagnosed at the LKH University Clinic Graz, but further treatment was provided at other hospitals or at established doctors. Therefore, some characteristics could not be included in the study as information was missing.

Additionally, in many cases information on death and cause of death was missing. If patients did not die at the LKH University Clinic Graz, access to data concerning death was not available or data was difficult to collect. Since this study included patient data from 1992 onward, medical records, especially prior to the introduction of “openMEDOCS” in 2005, were lacking.

Further, data regarding immunotherapy as treatment modality was lacking as only patients treated between 1992 and 2016 were included in our study. Since immunotherapy had not been introduced until 2016, we therefore could not identify any patient undergoing immunotherapy. Hence, we can neither approve nor disapprove the ability of immunotherapy as beneficial treatment modality in advanced MCC.

Therefore, the findings of our study should be considered cautiously as the significance of the results is probably reduced. Hence, this study suggests the performance of further examination concerning the impact of the different characteristics on MCC outcome. Especially regarding comorbidities, studies with larger study populations would be useful to explore further risk and prognostic factors.

4.7 Conclusion

Our study demonstrates several associations between host (age at diagnosis, sex and musculoskeletal disease), tumor (site of extent at diagnosis), diagnostic (pathologic nodal evaluation results), treatment (initial treatment modality, any chemotherapy, any radiotherapy) variables and outcomes of MCC. Male sex and site of extent at time of diagnosis are prognostic factors for MCC outcome. The usage of pathologic nodal evaluation should be performed to increase prognostic accuracy. Radiotherapy was associated with improved outcome and should therefore be concerned as preferred adjuvant treatment. In contrast, chemotherapy was associated with reduced outcome.

Patients with musculoskeletal diseases showed improved overall survival, compared to patients without musculoskeletal disease. However, further examinations with larger sample sizes may be advised. Although results were not statistically significant, the importance of a mindful surveillance of immunosuppressed patients concerning MCC should be considered. Future studies may investigate the impact of comorbidities on MCC outcome with larger study cohorts and may help elucidating further risk and prognostic factors of MCC.

5 References

1. Toker C. Trabecular carcinoma of the skin. Arch Dermatol [Internet]. 1972 Jan 1 [cited 2019 Nov 9];105(1):107–10. Available from: http://archderm.jamanetwork.com/article.aspx?doi=10.1001/archderm.1972.01620040075020
2. Tai P, Editors FS, Corona R. Official reprint from UpToDate ® www.uptodate.com ©2017 UpToDate ® Pathogenesis, clinical features, and diagnosis of Merkel cell (neuroendocrine) carcinoma. 2017;1–21.
3. Duprat JP, Landman G, Salvajoli JV, Brechtbühl ER. A review of the epidemiology and treatment of Merkel cell carcinoma. Clinics (Sao Paulo) [Internet]. 2011 [cited 2019 Oct 12];66(10):1817–23. Available from: http://www.scielo.br/pdf/clin/v66n10/23.pdf
4. Tang C, Toker C. Trabecular carcinoma of the skin. An ultrastructural study. Cancer [Internet]. 1978 Nov 1 [cited 2019 Nov 9];42(5):2311–21. Available from: http://doi.wiley.com/10.1002/1097-0142%28197811%2942%3A5%3C2311%3A%3AAID-CNCR2820420531%3E3.0.CO%3B2-L
5. Schadendorf D, Lebbé C, zur Hausen A, Avril MF, Hariharan S, Bharmal M, et al. Merkel cell carcinoma: Epidemiology, prognosis, therapy and unmet medical needs [Internet]. Vol. 71, European Journal of Cancer. Elsevier; 2017 [cited 2019 Sep 25]. p. 53–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27984768
6. Youlden DR, Soyer HP, Youl PH, Fritschi L, Baade PD. Incidence and Survival for Merkel Cell Carcinoma in Queensland, Australia, 1993-2010. JAMA Dermatology [Internet]. 2014 Aug 1 [cited 2019 Sep 25];150(8):864. Available from: http://archderm.jamanetwork.com/article.aspx?doi=10.1001/jamadermatol.2014.124
7. Robertson JP, Liang ES, Martin RCW. Epidemiology of Merkel cell carcinoma in New Zealand: a population-based study. Br J Dermatol [Internet]. 2015 Sep [cited 2020 Jan 3];173(3):835–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25801524
8. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, Peñas PF, et al. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients:

the AEIOU features. J Am Acad Dermatol [Internet]. 2008 Mar 1 [cited 2019 Sep 25];58(3):375–81. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0190962207023894

9. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol [Internet]. 2010 Jan [cited 2020 Jan 4];37(1):20–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19638070
10. Clarke CA, Robbins HA, Tatalovich Z, Lynch CF, Pawlish KS, Finch JL, et al. Risk of Merkel Cell Carcinoma After Solid Organ Transplantation. JNCI J Natl Cancer Inst [Internet]. 2015 Jan 8 [cited 2019 Mar 4];107(2):dju382–dju382. Available from: https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/dju382
11. Howard RA. Merkel Cell Carcinoma and Multiple Primary Cancers. Cancer Epidemiol Biomarkers Prev [Internet]. 2006 Aug 1 [cited 2019 Oct 12];15(8):1545–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16896047
12. Becker JC, Stang A, DeCaprio JA, Cerroni L, Lebbé C, Veness M, et al. Merkel cell carcinoma. Nat Rev Dis Prim [Internet]. 2017 Oct 26 [cited 2020 Jan 4];3:17077. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29072302
13. Agelli M, Clegg LX. Epidemiology of primary Merkel cell carcinoma in the United States. J Am Acad Dermatol [Internet]. 2003 Nov [cited 2020 Jan 7];49(5):832–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14576661
14. Popp S, Waltering S, Herbst C, Moll I, Boukamp P. UV-B-type mutations and chromosomal imbalances indicate common pathways for the development of Merkel and skin squamous cell carcinomas. Int J Cancer [Internet]. 2002 May 20 [cited 2020 Jan 7];99(3):352–60. Available from: http://doi.wiley.com/10.1002/ijc.10321
15. Van Gele M, Kaghad M, Leonard JH, Van Roy N, Naeyaert JM, Geerts ML, et al. Mutation analysis of P73 and TP53 in Merkel cell carcinoma. Br J Cancer. 2000;82(4):823–6.
16. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus

in human Merkel cell carcinoma. Science [Internet]. 2008 Feb 22 [cited 2020 Jan 7];319(5866):1096–100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18202256

17. Santos-Juanes J, Fernández-Vega I, Fuentes N, Galache C, Coto-Segura P, Vivanco B, et al. Merkel cell carcinoma and Merkel cell polyomavirus: a systematic review and meta-analysis. Br J Dermatol [Internet]. 2015 Jul 1 [cited 2020 Jan 7];173(1):42–9. Available from: http://doi.wiley.com/10.1111/bjd.13870
18. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, Eib S, et al. Merkel Cell Polyomavirus Status Is Not Associated with Clinical Course of Merkel Cell Carcinoma. J Invest Dermatol [Internet]. 2011 [cited 2019 Oct 12];131(8):1631–8. Available from: http://www.jidonline.org/article/S0022-202X(15)35381-1/pdf
19. Reisinger DM, Shiffer JD, Cognetta AB, Chang Y, Moore PS. Lack of evidence for basal or squamous cell carcinoma infection with Merkel cell polyomavirus in immunocompetent patients with Merkel cell carcinoma. [cited 2020 Jan 8]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309897/pdf/nihms-1000563.pdf
20. Deneve JL, Messina JL, Marzban SS, Gonzalez RJ, Walls BM, Fisher KJ, et al. Merkel Cell Carcinoma of Unknown Primary Origin. [cited 2020 Jan 9]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4504007/pdf/nihms706627.pdf
21. Wong HH, Wang J. Merkel Cell Carcinoma [Internet]. [cited 2020 Jan 9]. Available from: https://www.archivesofpathology.org/doi/pdf/10.1043/2009-0165-RSR2.1
22. Erstad DJ, Cusack JC. Mutational Analysis of Merkel Cell Carcinoma. Cancers (Basel) [Internet]. 2014 [cited 2020 Jan 10];6:2116–36. Available from: www.mdpi.com/journal/cancers
23. Sihto H, Kukko H, Koljonen V, Sankila R, Böhling T, Joensuu H. Clinical Factors Associated With Merkel Cell Polyomavirus Infection in Merkel Cell Carcinoma. JNCI J Natl Cancer Inst [Internet]. 2009 Jul 1 [cited 2020 Jan 10];101(13):938–45. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19535775

24. Touzé A, Le Bidre E, Laude H, Fleury MJJ, Cazal R, Arnold F, et al. High Levels of Antibodies Against Merkel Cell Polyomavirus Identify a Subset of Patients With Merkel Cell Carcinoma With Better Clinical Outcome. J Clin Oncol [Internet]. 2011 Apr 20 [cited 2020 Jan 10];29(12):1612–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21422439
25. Asgari MM, Sokil MM, Warton EM, Iyer J, Paulson KG, Nghiem P. Effect of Host, Tumor, Diagnostic, and Treatment Variables on Outcomes in a Large Cohort With Merkel Cell Carcinoma. JAMA Dermatology [Internet]. 2014 Jul 1 [cited 2019 Sep 25];150(7):716. Available from: http://archderm.jamanetwork.com/article.aspx?doi=10.1001/jamadermatol.2013.8116
26. Pathology Outlines - TNM staging of Merkel cell carcinoma [Internet]. [cited 2020 Jan 21]. Available from: https://www.pathologyoutlines.com/topic/skintumornonmelanocyticmerkelstaging.html
27. Tai P. Staging and treatment of Merkel cell carcinoma. 2017 [cited 2020 Jan 15];1–8. Available from: https://www.uptodate.com/contents/staging-and-treatment-of-merkel-cell-carcinoma
28. Becker JC, Eigentler T, Frerich B, Gambichler T, Grabbe S, Höller U, et al. S2k-Leitlinie-Merkelzellkarzinom (MZK, MCC, neuroendokrines Karzinom der Haut)-Update 2018 #, § S2k-Guideline Merkel Cell Carcinoma (MCC, Neuroendocrine Carcinoma of the Skin)-Update 2018 [Internet]. [cited 2020 Apr 16]. Available from: https://www.awmf.org/uploads/tx_szleitlinien/032-023l_S2k_Merkelzellkarzinom_2018-12.pdf
29. Lebbe C, Becker JC, Grob J-J, Malvehy J, del Marmol V, Pehamberger H, et al. Diagnosis and treatment of Merkel Cell Carcinoma. European consensus-based interdisciplinary guideline. Eur J Cancer [Internet]. 2015 Nov 1 [cited 2019 Nov 13];51(16):2396–403. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0959804915006619
30. Veness M, Foote M, Gebski V, Poulsen M. The role of radiotherapy alone in patients with merkel cell carcinoma: reporting the Australian experience of 43 patients. Int J Radiat Oncol Biol Phys [Internet]. 2010 Nov 1 [cited 2020 Jan 15];78(3):703–9. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19939581

31. Bhatia S, Storer BE, Iyer JG, Moshiri A, Parvathaneni U, Byrd D, et al. Adjuvant Radiation Therapy and Chemotherapy in Merkel Cell Carcinoma: Survival Analyses of 6908 Cases From the National Cancer Data Base. J Natl Cancer Inst [Internet]. 2016 [cited 2020 Jan 15];108(9). Available from: http://www.ncbi.nlm.nih.gov/pubmed/27245173
32. Chen MM, Roman SA, Sosa JA, Judson BL. The Role of Adjuvant Therapy in the Management of Head and Neck Merkel Cell Carcinoma: An Analysis of 4815 Patients. JAMA Otolaryngol Neck Surg [Internet]. 2015 Feb 1 [cited 2020 Jan 15];141(2):137–41. Available from: https://jamanetwork.com/journals/jamaotolaryngology/fullarticle/2020430
33. Voog E, Biron P, Martin JP, Blay JY. Chemotherapy for patients with locally advanced or metastatic Merkel cell carcinoma. Cancer [Internet]. 1999 Jun 15 [cited 2020 Jan 16];85(12):2589–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10375107
34. Gupta SG, Wang LC, Peñas PF, Gellenthin M, Lee SJ, Nghiem P. Sentinel lymph node biopsy for evaluation and treatment of patients with Merkel cell carcinoma: The Dana-Farber experience and meta-analysis of the literature. Arch Dermatol [Internet]. 2006 Jun 1 [cited 2019 Nov 25];142(6):685–90. Available from: http://archderm.jamanetwork.com/article.aspx?doi=10.1001/archderm.142.6.685
35. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D SP, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncol [Internet]. 2016 [cited 2020 Jan 16];17(10):1374–85. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5587154/pdf/nihms897262.pdf
36. Paulson KG, Iyer JG, Blom A, Warton EM, Sokil M, Yelistratova L, et al. Systemic Immune Suppression Predicts Diminished Merkel Cell Carcinoma–Specific Survival Independent of Stage. J Invest Dermatol [Internet]. 2013 [cited 2019 Nov 25];133(3):642–6. Available from: http://www.nature.com/authors/editorial_policies/license.html#terms
37. Morand GB, Madana J, Da Silva SD, Hier MP, Mlynarek AM, Black MJ.

Merkel cell carcinoma of the head and neck: poorer prognosis than non-head and neck sites. J Laryngol Otol [Internet]. 2016 Apr 15 [cited 2020 Jan 16];130(4):393–7. Available from: https://www.cambridge.org/core/product/identifier/S0022215116000153/type/journal_article

38. openMEDOCS erfolgreich eingeführt - G'sund.net [Internet]. [cited 2019 Nov 6]. Available from: http://www.gsund.net/cms/beitrag/10073293/2052790/
39. Lau T, Fang C, Leung F. Assessment of postoperative short-term and long-term mortality risk in Chinese geriatric patients for hip fracture using the Charlson comorbidity score. Hong Kong Med J [Internet]. 2015 Dec 8 [cited 2020 Apr 1]; Available from: http://www.hkmj.org/abstracts/v22n1/16.htm
40. Smith VA, Camp ER, Lentsch EJ. Merkel cell carcinoma: Identification of prognostic factors unique to tumors located in the head and neck based on analysis of SEER data. Laryngoscope [Internet]. 2012 Jun [cited 2020 Feb 6];122(6):1283–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22522673
41. Miller RW, Rabkin CS. Merkel cell carcinoma and melanoma: etiological similarities and differences. Cancer Epidemiol Biomarkers Prev [Internet]. 1999 Feb 1 [cited 2020 Mar 15];8(2):153–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10067813
42. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. Merkel cell carcinoma and HIV infection. Lancet (London, England) [Internet]. 2002 Feb 9 [cited 2020 Mar 15];359(9305):497–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11853800
43. Youlden DR, Youl PH, Peter Soyer H, Fritschi L, Baade PD. Multiple Primary Cancers Associated with Merkel Cell Carcinoma in Queensland, Australia, 1982–2011. J Invest Dermatol [Internet]. 2014 Dec 1 [cited 2019 Nov 25];134(12):2883–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24991966
44. Grabowski J, Saltzstein SL, Sadler GR, Tahir Z, Blair S. A comparison of merkel cell carcinoma and melanoma: results from the california cancer registry. Clin Med Oncol [Internet]. 2008 [cited 2019 Nov 25];2:327–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21892294
45. Reinau D, Surber C, Jick SS, Meier CR. Epidemiology of basal cell

carcinoma in the United Kingdom: incidence, lifestyle factors, and comorbidities. Br J Cancer [Internet]. 2014 Jul 8 [cited 2019 Oct 17];111(1):203–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24874476

46. Li N, Wang G, Jiang X, Huang M, Tian H, Xuan F, et al. Merkel cell carcinoma metastatic to cervical lymph node in a patient with rheumatoid arthritis: a case report. Onco Targets Ther [Internet]. 2019 [cited 2020 Mar 15];12:1395–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30863106
47. Khoja L, Atenafu EG, Templeton A, Qye Y, Chappell MA, Saibil S, et al. The full blood count as a biomarker of outcome and toxicity in ipilimumab-treated cutaneous metastatic melanoma. Cancer Med [Internet]. 2016 Oct [cited 2019 Nov 25];5(10):2792–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27683208
48. Demirci NS, Erdem GU. Prognostic role of neutrophil-to-lymphocyte ratio (NLR) in patients with operable ampullary carcinoma. Bosn J basic Med Sci [Internet]. 2018 Aug 1 [cited 2020 Mar 30];18(3):268–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29131967
49. Iyer JG, Storer BE, Paulson KG, Lemos B, Phillips JL, Bichakjian CK, et al. Relationships among primary tumor size, number of involved nodes, and survival for 8044 cases of Merkel cell carcinoma. J Am Acad Dermatol [Internet]. 2014 Apr [cited 2020 Mar 19];70(4):637–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24521828
50. Allen PJ, Bowne WB, Jaques DP, Brennan MF, Busam K, Coit DG. Merkel Cell Carcinoma: Prognosis and Treatment of Patients From a Single Institution. J Clin Oncol [Internet]. 2005 Apr 1 [cited 2020 Mar 19];23(10):2300–9. Available from: http://ascopubs.org/doi/10.1200/JCO.2005.02.329
51. Lemos BD, Storer BE, Iyer JG, Phillips JL, Bichakjian CK, Fang LC, et al. Pathologic nodal evaluation improves prognostic accuracy in Merkel cell carcinoma: analysis of 5823 cases as the basis of the first consensus staging system. J Am Acad Dermatol [Internet]. 2010 Nov [cited 2020 Mar 19];63(5):751–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20646783

52. Iyer JG, Parvathaneni U, Gooley T, Miller NJ, Markowitz E, Blom A, et al. Single-fraction radiation therapy in patients with metastatic Merkel cell carcinoma. Cancer Med [Internet]. 2015 Aug [cited 2020 Mar 21];4(8):1161–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25908228
53. Sundaresan P, Hruby G, Hamilton A, Hong A, Boyer M, Chatfield M, et al. Definitive Radiotherapy or Chemoradiotherapy in the Treatment of Merkel Cell Carcinoma. Clin Oncol [Internet]. 2012 Nov [cited 2020 Mar 20];24(9):e131–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22626522
54. Lewis KG, Weinstock MA, Weaver AL, Otley CC. Adjuvant Local Irradiation for Merkel Cell Carcinoma. Arch Dermatol [Internet]. 2006 Jun 1 [cited 2020 Mar 20];142(6):693–700. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16785371
55. Ghadjar P, Kaanders JH, Poortmans P, Zaucha R, Krengli M, Lagrange JL, et al. The Essential Role of Radiotherapy in the Treatment of Merkel Cell Carcinoma: A Study From the Rare Cancer Network. Int J Radiat Oncol [Internet]. 2011 Nov 15 [cited 2020 Mar 21];81(4):e583–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21775069