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Pattern of Local, Regional and Distant Recurrence of Merkel Cell Carcinoma after Excision with 3D-Histology

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1. INTRODUCTION

1.1. BACKGROUND

1.1.1. Historical aspects

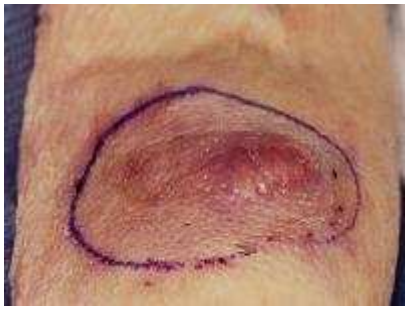
Merkel Cell Carcinoma (MCC) is a rare but highly malignant neoplasm of the skin that was first reported by Toker in 1972 [1]. Several names have been used since then to describe this cutaneous malignancy, ranging from “neuroendocrine carcinoma” to “primary undifferentiated carcinoma of the skin “endocrine carcinoma of the skin”, “cutaneous APUDoma” and “trabecular carcinoma”. Nevertheless the title of Merkel Cell Carcinoma has ultimately prevailed.

Merkel cells are named after Friedrich Merkel, a german anatomist and histiopathologist, who first described and defiened them in 1875 as touch-cells or “Tastzellen” [2]. The Merkel cell is located in or near the basal layer of the epidermis, it is closely associated with terminal axons and the presumed function is that of a slowly adapting type I mechanoreceptor which mediates the sense of touch and hair movement [3,4].

1.1.2. Clinical presentation

The Merkel Cell Tumor arises in the dermis and often extends into subcutaneous fat and muscle. It usually presents at sun exposed areas of the skin as a painless, indurated, solitary dermal nodule with a slightly erythematous to deeply violaceous colour, measuring up to several centimetres in dimension [5,6]. Due to its nonspecific clinical presentation, the diagnosis of Merkel Cell Carcinoma is normally only made after biopsy. A broad differential diagnosis exists that includes squamaous cell carcinoma, basal cell carcinoma, adnexal tumors, lymphoma, malignant melanoma, leukaemia cutis, metastases of small cell lung carcinoma, carcinoid and Ewing’s sarcoma [4].

Picture 1: Large, violaceous nodule of Merkel Cell Carcinoma on the antecubital fossa.



Photograph courtesy of Dr. Jonathan Cook.

1.1.3. Pathology

Histologically, Merkel Cell Carcinoma has been classified into three distinct subtypes: 1. The Trabecular Subtype (cells are arranged in organoid clusters and trabeculae; the cell cytoplasm is comparatively abundant; mitoses are few to moderate; it is the least frequent histologic pattern); 2. The Intermediate Subtype (has a solid and diffuse growth pattern; the cytoplasm is less abundant; mitoses are frequently, clinically more aggressive than the trabecular subtype; the most common histologic subtype); and 3. The Small Cell Subtype (mimics small cell tumors of other sites; areas of necrosis are frequently seen, high mitotic activity, can be as aggressive as the intermediate subtypes) (Table 1) [7].

Table 1: Cellular classification

Subtype	Features	Prognosis
Trabecular Type	- Cells arranged in organoid clusters	Good
	- Few mitoses	
	- Cytoplasm often abundant, well defined	
Intermediate Type	- Diffuse growth pattern	Moderate
	- Frequent mitoses	
	- Cytoplasm less abundant than Trabecular Type	
	- Most frequent histologic subtype	
Small Cell Type	- Solid clusters of cells	Poor
	- Frequent areas of necrosis	
	- Closely mimics small cell tumors of other sites	

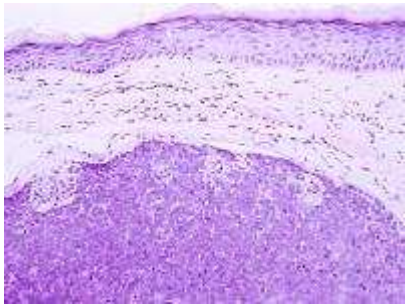
Given the extent of differential diagnosis and possible difficulties to distinguish tumor cells histologically, immunohistochemistry seems to be a useful tool to differentiate Merkel Cell Carcinomas from other malignancies. The low-molecular-weight cytokeratin (CK) 20 has turned out to be one of the most sensitive markers to differentiate MCC from small cell carcinoma of the lung [8]. Nevertheless, adenocarcinomas of the gastrointestinal tract were often stained by antibodies to CK 20. Recent immunohistochemical findings suggest the combined immunostaining with thyroid transcription factor-1 and CK 20 to distinguish between MCC and small cell carcinoma of the lung [9] (see 1.2).

Concerning the diagnosis of Merkel Cell Carcinoma, one should also bear in mind that this cutaneous malignancy may occur due to carcinogenic events. Several chromosomal aberrations and losses have been revealed so far, whereas the loss of chromosome 10, or parts of its long arm, seems to be one of the most frequent ones (occurring in one third of cases) [10]. But also the loss of heterozygosity on chromosome 1q occurs

similarly frequently and trisomy 6 was found in 47% of the Merkel Cell Carcinoma lesions [11]. Furthermore aberrations in oncogenes as well as oncosuppressor genes have been connected to the MCC. For instance mutations in P 53, a suppressor gene that normally helps to repair DNA damage in cells caused by ultraviolet irradiation, have been found in 20% of patients with Merkel Cell Carcinoma [12]

Additionally Merkel Cell Carcinoma has been reported to occur in association with an impaired immune status, either from iatrogenic immunosuppression, neoplasia, organ transplant or human immunodeficiency virus infection [13,14,15].

Picture 2: Histologic appearance of nodular Merkel Cell Carcinoma. Dermal nodule with a cohesive, expansile growth of basophilic cells.



Photograph courtesy of Dr. Jonathan Cook.

1.1.4. Etiology

The etiology of the Merkel Cell Carcinoma is still unknown, although its propensity to occur on the head, neck and extremity suggests that sun exposure may play a role. Nevertheless also MCC arising from, the nasal mucosa, lips and vulvar region have been reported [16].

In general Merkel Cell Carcinoma is a tumor of the elderly (in their seventh decade or older), Caucasians are predominantly affected and only a few cases of MCC among other population groups have been reported [17,18]. Merkel Cell Carcinoma is a rare but highly aggressive neuroendocrine

neoplasm that tends to recur locally and frequently spreads to regional and distant sites [19]. It has a lethal outcome in about 30% of patients and survival at 1, 2, and 3 years has been estimated at 88%, 72% and 55%, respectively [19].

The incidence of the tumor has been lately described with approximately 0.1-0.3 per 100 000 inhabitants per year [20].

1.2. CURRENT DIAGNOSIS, STAGES AND TREATMENT

1.2.1. Diagnosis

The diagnosis of MCC is made with a skin or excision biopsy which is examined under the microscope. The result must always be confirmed by immunohistochemical stains and/or ultrastructural analysis as it is very difficult to differentiate MCC from other neoplasms such as metastatic small cell carcinoma of the lung, lymphoma and amelanotic small cell melanoma [4]. Each of these cancers has a unique profile as defined by special stains:

MCC will stain for low molecular weight cytokeratins (CAM 5.2 or AE1/AE3) CK 20 and neuron-specific enolase (NSE). But MCC will not stain for CK 7 or thyroid transcription factor-1 (TTF-1) which are positive in small cell lung cancer (SCLC). Furthermore MCC will neither stain for leukocyte common antigen (LCA) which is positive in lymphoma, nor for S 100 which is positive in small cell melanoma (Table 2). Tumorthickness evaluated under the microscope is a prognostic factor of a great number of malignant tumors of the skin and have to complete the investigation.

After an immunohistochemical confirmation of MCC an appropriate staging examination should include a clinical examination with palpation and ultrasonography of the draining region and determination of lymph node status. Laboratory examination should include a complete blood cell count, erythrocyte sedimentation rate, liver enzymes and lactate dehydrogenase.

Imaging tests at diagnosis should incorporate chest x-rays, abdominal and regional nodal sonography and, in case of high risk tumors, computed tomography (CT) of the brain and skeletal scintigraphy [21]

Recently, scintigraphic detection of somatostatin receptor positive MCCs has become possible using intravenous administration of Indium-111 pentreotide, a radiolabeled somatostatin analog. The sensitivity of this technique approaches 80% for detection of MCC in Stages I and II, with a specifity of 96% [22]

Table 2: Biopsy characteristics for MCC and resembling tumors

	MCC	SCLC	Lymphoma	Melanoma
CAM 5.2	+	+	-	-
CK 20	+	-	-	-
NSE	+	+	-	+
CK 7	-	+	-	-
LCA	-	-	+	-
S 100	-	-	-	+

(modified from Goessling et al. 2002)

1.2.2. Stages

Concerning the stages of Merkel Cell Carcinoma one must admit that no standardized staging classification based upon prognosis has been widely accepted so far. But there is a commonly used staging system which is based upon clinical presentation [3]:

Stage I of MCC is defined as disease that is localized to the skin at the primary site, whereas this stage is further divided. Stage I A defines a primary lesion with less than or equal to 2 cm, whereas Stage I B defines a primary lesion with greater than 2 cm. Stage II is defined as disease that involves regional lymph nodes but with no evidence of distant metastases.

Finally Stage III defines the presence of systemic metastases beyond the regional lymph nodes (e.g. brain, liver, lung, bones, distant lymph nodes and skin) (Table 3).

Table 3: Stage information for MCC

Stage	Feature	Localized disease	Lymph node	Metastasis
I A	Primary tumor </= 2 cm	+	-	-
I B	Primary tumor > 2 cm	+	-	-
II	Regional nodes involvement	+/-	+	-
III	Systemic metastases	+/-	+/-	+

(modified from Allen et al. 1999)

As Merkel Cell Carcinoma frequently metastasizes to regional lymph nodes (in 27% reported by Voog [18]) the need of finding macro- and micrometastases in lymph nodes are evident. An established and well observed technique to find the first lymph node in which MCC cells can be found is the sentinel lymph node biopsy [23]. A radioactive tracer and a blue dye are injected at the site of the primary lesion before operation. The dye and tracer travel along the same path that cancer cells would spread through the lymphatic vessels and so the sentinel lymph node can be detected.

The importance of staging in MCC patient in respective to the prognosis of this disease is significant and will be discussed in the course of this dissertation.

1.2.3. Treatment

The basic treatment of MCC-Primary sites is the surgical excision with a security margin of 3 cm [24]. However, treatment is generally based on the stage of the disease and beside the surgical excision, lymph node surgery, radiation therapy and chemotherapy are treatment options. The absence of adequately designed prospectively controlled studies has resulted in an absence of acknowledged treatment guidelines. Nevertheless there is some accord in the international literature about treatment options for Merkel Cell Carcinoma.

For Stage I Merkel Cell Carcinoma wide local excision has been recommended whenever possible as well as frozen section control [3,14]. But especially in the head and neck region where wide margins are not amenable, the complete histology of three-dimensional excisional margins (3D-Histology) is advocated [25]. As Merkel Cell Carcinomas are radiosensitive [26], many authors recommend postoperative radiotherapy of the tumor and the draining lymphatics with a total dose between 50-60 Gray [3,4,27,28], however for other authors adjuvant radiation seems unessential to secure local control of primary MCC lesions [29]. Also the relevance of sentinel node biopsy in Merkel Cell Carcinoma at Stage I is still discussed controversially [23,30,31].

The treatment of Stage II includes wide local excision and lymph node dissection followed by post-operative radiation of both primary site and nodal basin in regional lymph node disease [3,33,35]. Some authors believe that adjuvant chemotherapy should be given to prevent progression to Stage III although there are no prospective trials so far that demonstrate that it prolongs survival [20,21,32,34].

In case of distant metastases at Stage III MCC-surgery, radiation and chemotherapy are palliative treatment options, whereas chemotherapy is the treatment most often used [3,4,18,21,34]. Here experience with polychemotherapy schemes especially in SCLC but also in lymphomas

and other tumors has helped to create some commonly utilized regimen. Krasagakis and collegues for example have composed a Merkel Cell Carcinoma Regimen considering results of other authors (Table 4) [21].

Furthermore there are several treating options for Merkel Cell Carcinoma that may become more relevant in future, so for example chemotherapy with TNF-alpha, Interferon-alpha-2a/b or Bcl-2 antisense [36-39].

Table 4: Merkel Cell Carcinoma treatment options

Drugs	Dosage	Repeat	Reference
Inoperable Stage I			
Cyclophosphamide	600 mg/m^2 i.v. day 1	Repeat every 3 weeks	Ferrau *et al.* (1994) [46]
Epidoxorubicin	75 mg/m^2 i.v. day 1		
Etoposide	150 mg/m^2 i.v. days 1 + 2		
Stages II and III			
Cisplatin	50 mg/m^2 i.v. days 1 + 7	Repeat every 3-4 weeks	
Etoposide	170 mg/m^2 i.v. days 3-5		
Cyclophosphamide	600 mg/m^2 i.v. days 1 + 8	Repeat on day 28	Fenig *et al.* (1993) [53] : CR 4/5 patients, PR 1/5 patients
Methotrexate	40 mg/m^2 i.v. days 1 + 8		
5-Fluorouracil	600 mg/m^2 i.v. days 1 + 8		
VP-16	150 mg/m^2 i.v. days 1 + 2	Repeat on day 22	Azagury *et al.* (1993) [54] : CR 1 patient
Cisplatin	150 mg/m^2 i.v. days 1 + 2		
Doxorubicin	150 mg/m^2 i.v. day 1		
Bleomycin	150 mg/m^2 i.v. day 1		

1.3. PROBLEM, QUESTION AND AIM OF THIS STUDY

As it was mentioned earlier on, the current treatment recommendations for Merkel Cell Carcinoma are quite controversial. To begin with, this is a result of the rarity of this malignancy. With an estimated incidence around 600 cases per year in the US in 2003 it is about 100 times less common than melanoma with an incidence of roughly 60 000 [40,41]. With such low numbers there is no way to provide high-quality data from prospective studies on which to base clinical decisions. Secondly until now no single speciality has taken a leading role in managing Merkel Cell Carcinoma which leads to a lack of balanced information. These factors can lead to severe mismanagement of patients suffering from this malignancy and to their physicians this current state is barely sustainable. In consideration of the fact that the incidence of Merkel Cell Carcinoma has tripled since 1986, this study intends to draw the reader's attention to the prevailing importance of this disease [42]. As randomised prospective multicenter studies with two or more therapy options are generally very difficult to implement, this study will neither be able to suggest new guidelines for the treatment of Merkel Cell Carcinoma. So what else can be the aim of this study?

The concept of Profesor Dr. med. Breuninger of the University Hospital of Dermatology in Tuebingen is primary local therapy (surgery) without radiation plus complete histology of three-dimensional excisional margins (3D-Histology) in paraffin-technique called "Tuebingen Cake" that was developed by Breuninger in 1982 [43]. This histologic evaluation with a high sensitivity to detect tumor outgrowths may reduce safety margins in combination with therapy modalities of the lymph node region. This available dissertation targets to provide a small contribution to gain more knowledge about the therapy of this rare malignancy by evaluating the patients with Merkel Cell Carcinoma at the University Hospital of Dermatology in Tuebingen that have been operated with the 3D-Histology. For more than 15 years data about these patients have been collected at

the University Hospital of Tuebingen in collaboration with the Comprehensive Cancer Centre in Tuebingen.

The aim of this study is to describe the course of Merkel Cell Carcinoma Disease in 33 patients including relaps rates and metastases using the gained data. By means of the results this study intends to draw conclusions about adaptation and optimisation of treatment schemes for patients suffering from MCC of the skin.

Therefore relevant international literature will be discussed in reference to the acquired findings of this study.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Patients

The tumor registries of the Comprehensive Cancer Centre were searched for all cases of Merkel Cell Carcinoma and 46 Patients with MCC that have been treated at the University Hospital of Dermatology in Tuebingen between 1989-2004 were identified. From this group 33 patients were qualified for our study, therefrom 19 women and 14 men. The main condition for acceptability was a seamless reconstruction from the date of first diagnosis until the contemporary condition, all re-treatments included. 13 patients with Merkel Cell Carcinoma turned out to be unsuitable as 12 of them didn't return the forwarded questionnaires and one patient was a case of address unknown. In 25 of the eligible patients with Merkel Cell Carcinoma re-excision was performed, in 5 cases the primary tumor was excised and 3 patients were not able to undergo surgery. Among these three patients there were two with widespread metastases of MCC and one with an extremely bad general condition, whereas all the three of them died within one year after appraisal at the University Hospital of Dermatology.

All patients that underwent surgical treatment, both primary and secondary excision, were operated with the complete histology of three-dimensional excisional margins (3D-Histology) in paraffin technique

2.1.2. Origin of data

General patient orientated data were gathered from the following sources:

Archived patient files:

- Check list of medical record
- Surgery dossier
- Referral documents from GPs or other hospitals
- Medical records on CD-Rom and/or microfilm
- Outpatients charts

Comprehensive Cancer Centre:

List of all patients with Merkel Cell Carcinoma treated at the University Hospital of Dermatology in Tuebingen including name, date of birth and death respectively as well as the patient's address.

Histology:

- Review of pathologic material
- Histological findings on the histology application form

2.1.3. Standardised questionnaires

As this study aims to reconstruct each patient's course of Merkel Cell Carcinoma without gap, the necessity of a detailed questionnaire was obvious.

Important facts that had to be obtained were:

- Gender distribution
- Age of first appearance
- Institutions that posed the diagnoses
- Location of the primary site
- Dates and institutions of primary surgical excision and possible re-excisions
- Incidence of locoregional, regional lymph node or distant recurrence
- After-treatments, e.g. radiation or chemotherapy, with dates, locations and name of the institutions
- Aftercare with time intervals, dates, locations and name of the institutions

Given the fact that most of the patients were seniors, it was the adjacency to create an easily comprehensible form that can still provide the study with all necessary information.

Against the background of this thread a four sided multicolour questionnaire was sent to each one of the 33 patients. By distributing primary site, locoregional recurrence, lymph node recurrurence and distant recurrence to different coloured pages while keeping the same strictly arranged scheme, a structural composition was assured. Thus the patients were able to note the for this study important facts effortlessly according to their individual course of Merkel Cell Carcinoma.

In case of a patient’s death his relatives were asked to fill in the questionnaire. Additionally another questionnaire with an extra form concerning the circumstances and details of the patient’s death was sent to his treating GP.

2.2. METHODS

2.2.1. Method of processing tissue

In order to evaluate the results of this study accurately, one must take a close look at the method of processing tissue that has been employed on our patients.

The 3D-Histology technique is an alternative to Mohs Micrographic Surgery and has been an essential element of several publications so far [43-49].

All the patients with Merkel Cell Carcinoma of our study were treated with 3D-Histology. In order to elaborate the differences between Mohs Micrographic Surgery and the 3D Histology it is appropriate to examine the two different techniques separately.

Mohs Micrographic Surgery

In the 1940's Dr. Frederick Mohs invented a technique to remove certain skin cancers with immediate microscopic examination of the removed tissue in a way to insure that the tumor has been completely removed [50]. In former times, during the original procedure, a chemical paste was applied to the tumor for 6-24 hours before the area was surgically removed and examined for residual tumor. This process had to be repeated over days until all margins were found to be free of tumor.

Today Mohs surgery has been simplified. The visible tumor is removed with a scalpel then a thin layer of tissue is excised a few millimeters around and underneath the resulting defect. This layer is divided into pieces and inked, whereas the Mohs surgeon draws a map that shows each piece in relation to the patient. The tissue is freezed immediately in the laboratory, fixed in a waxy substance and very thinly sectioned horizontally to identify potential tumor roots.

If the surgical margins are tumor-free, the defect of the skin can be repaired immediately. But if the Mohs surgeon discovers tumor roots, the whole procedure has to be repeated.

Although Mohs Micrographic Surgery is said to be a reliable and tissue-sparing approach to the management of cutaneous malignancies [25,51,52], this procedure is still discussed controversially and several alternative approaches are described [44,53,54].

3D-Histology ("Tuebingen Cake")

In 1988 Breuninger et al. published an alternative treatment to Mohs Micrographic Surgery [43, 44]. His specified method is about a modified complete histology of three-dimensional excisional margins (3D-Histology) which is performed with a rush-fixed paraffin technique. It comprehends the following steps:

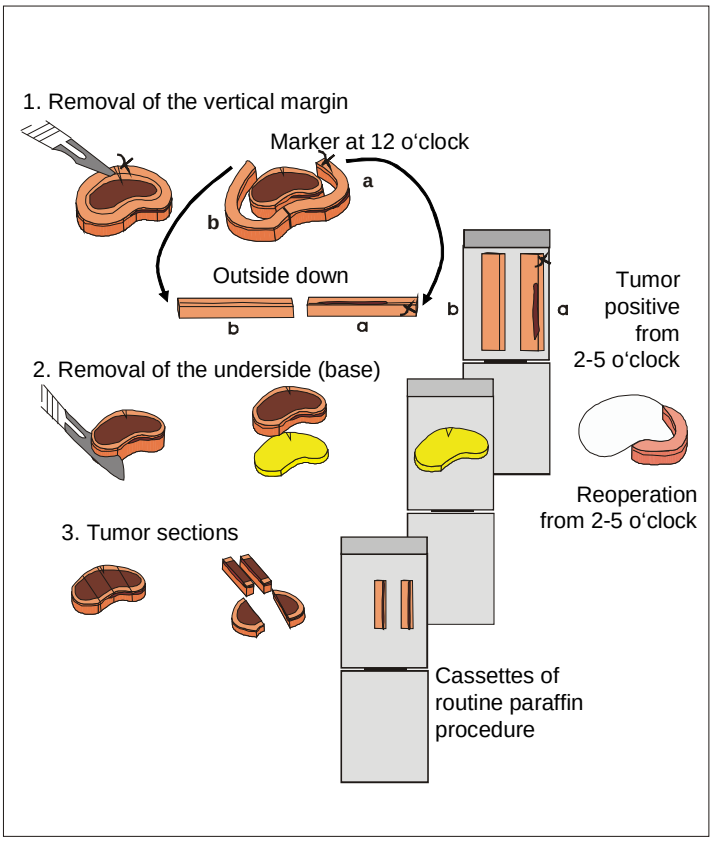
After measuring the skin tumor an area of the clinically healthy skin around the malignancy is defined. This so called safety margin varies depending on the size of the tumor and measures at least 5 mm. A thread is fixed to mark the 12 o'clock position of the excised tissue related to the patient's body-axis. This "12 o'clock rule" is conducted to guarantee a correct orientation during processing tissue and deviations should just be made in very small tumors. Subsequent to this procedure the tissue is located in formalin and sent to the laboratory where a seamless inspection of basis and margins is performed.

A strip of approximately 2 mm is cut from the margin of the tissue starting at the thread and a thin layer is cut off from underneath. Then the strip is divided in two or three parts whereas each part is named in alphabetical order to keep the proper sequence. In case of an excised spindle-shaped tissue, the sample is divided lengthwise.

After that the tissue samples are placed horizontally in a histology box and embedded in paraffin while heated to 60 degree Celsius. In order to obtain optimal preconditions for cutting these paraffin blocks in thin slices they have to be deep-freezed for a short time. With the help of a microtome cuts are subsequently conducted vertically by moving from outside inwardly. Then each of the fine slices is fixed on an object holder, stained with Hematoxylin-Eosin and finally ready for appraisal. It is advisable to start the microscopic inspection of the tissue sample from the middle of the tumor to its margins to ensure the diagnosis.

In the event of tumor-positive margins accurate localization is assured via 12 o’clock position. Even narrow tumor roots can be located exactly and reoperation can be carried out allowing for the position of the tumor on the time’s clockface (Figure 1). Following re-excision histological elaboration must be performed anew until all margins are tumor-free.

Figure 1: 3D-Histology (modified from [45])



During 3D-Histology process the defect of the patient's skin is left open if a difficult closure of the defect is expected.

2.2.2. Histological samples

With Merkel Cell Carcinoma patients whose histological findings were incomplete, a review of pathologic material was conducted. By means of histological numbers on the patient's medical records and the histology application form respectively the required histological samples could be recovered from the hospital's storeroom. Regrettably this procedure could only be accomplished with those samples which have been processed at the laboratories of the University Hospital of Dermatology in Tuebingen. That is why some data had to remain incomplete.

The histological samples were reviewed using a Leitz microscope Laborlux 12 on examination 25-times, 100-times, 250-times and 400-times magnified.

2.2.3. Statistical methods

Due to the small number of patients in this study no statistically significant results were demanded. Additionally this study contains a very heterogenic collective of patients: 25 patients with Merkel Cell Carcinoma had been pretreated and underwent re-excisions at the University Hospital of Dermatology in Tuebingen, in 5 cases primary excision was performed and 3 patients were not operated at the University Hospital of Dermatology. In consideration of the expected limited explanatory power demonstration of the results should be regarded as descriptive.

The statistical analysis and its graphical conversion were mainly achieved by means of Microsoft Excel 2002. Furthermore a simple comparison of sample means and univariate and multivariate regression analysis will be shown under 3.8.

3. RESULTS

3.1. PATIENTS

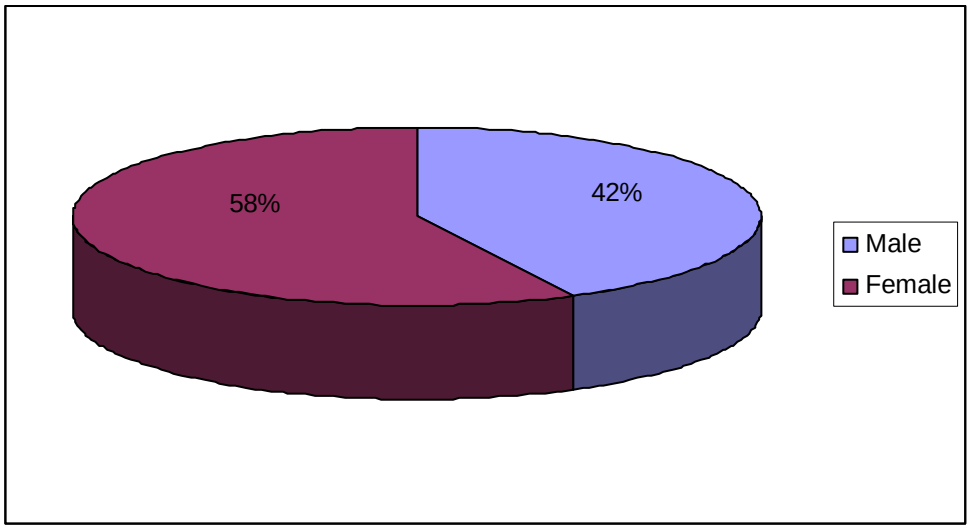
In this descriptive study with retrospective data acquisition 33 patients with Merkel Cell Carcinoma are presented that have been treated at the University Hospital of Dermatology in Tuebingen. In 28 of the cases primary excision has been performed elsewhere, either by other hospitals or by settled GPs, dermatologists or surgeons. These patients were operated at the University Hospital of Tuebingen in different stadiums of re-excision whereas 18 patients had their first re-excision, 5 patients underwent their second re-excision and 2 patients with a forth and a fifth re-excision, respectively. The number of 3 patients were not at all operated at the University Hospital of Tuebingen due to their bad general condition.

3.2. DISTRIBUTION OF GENDER AND AGE

3.2.1. Gender allocation

In this study of 33 observed patients with Merkel Cell Carcinoma 19 were women and 14 were men. This makes a women's ratio of 58% and a men's ratio of 42% (Figure 2).

Figure 2: Distribution of gender



3.2.2. Age allocation

All following specifications refer to the age of the first diagnosis of MCC.

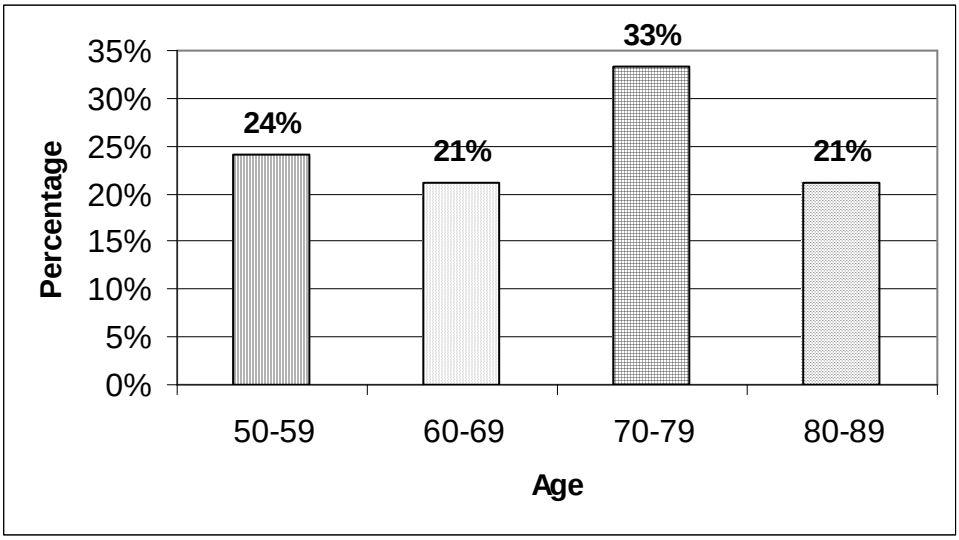
The age limits of the patient's collective were located between 51 and 87 years whereas the mean value was 69.9 years (median = 70). In this connexion the mean age distribution among women and men were relatively close with 71.0 and 69.0 years respectively. The women's age ranged from 51 to 87 and the men's age ranged from 57 to 87 years.

Concerning the frequency of MCC in four different age groups no fundamental variety of incidences could be assessed (Figure 3/Table 5).

Table 5: Age groups and distribution

Age group	Total number of patients	Frequency distribution
50-59 years	8	24.2%
60-69 years	7	21.2%
70-79 years	11	33.3%
80-89 years	7	21.2%

Figure 3: Distribution of age



For the sake of completeness also the age group distribution split into different gender are mentioned in Table 6 and the most important demographic data can be looked up in Table 7.

Table 6: Female-male-ratio

	Female (19)		Male (14)	
Age group	Number	Percentage	Number	Percentage
50-59	5	26.3%	3	21.4%
60-69	5	26.3%	2	14.3%
70-79	6	31.6%	5	35.7%
80-89	3	15.8%	4	28.6%

Table 7: Demographic data

Case	Sex	Age	Site of primary lesion	Primary therapy / where*	First recurrence (in months)	Localization of first recurrence	Further recur-rences	Current clinical outcome*	Follow-up (in months)
1	F	67	face	surgery/e	∅	∅	∅	NED	51
2	M	74	face	surgery/e	0	regional lymph nodes	yes	DID	26
3	F	78	buttock	surgery/e	2	regional lymph nodes	yes	DID	19
4	F	66	face	surgery/e radiation	∅	∅	∅	NED	91
5	M	72	face	surgery/e	∅	∅	∅	NED	119
6	M	87	face	surgery/i	∅	∅	∅	NED	32
7	M	72	face	surgery/i	∅	∅	∅	NED	72
8	F	53	upper leg	surgery/e	8	regional lymph nodes	no	NED	78
9	F	81	face	surgery/i	∅	∅		DID	11
10	F	51	upper arm	surgery/e	5	regional lymph nodes	no	NED	182
11	M	57	forearm	surgery/e	15	local	no	NED	167
12	F	75	upper arm	surgery/e	14	Local	no	NED	67
13	F	68	forearm	surgery/e	∅	∅	∅	NED	42
14	M	65	hand	surgery/i	∅	∅	∅	NED	52
15	M	71	buttock	surgery/e	5	regional lymph nodes	yes	DMCC	14
16	M	59	forearm	surgery/e radiation	∅	∅	∅	DID	29
17	F	64	lower leg	surgery/e	2	regional lymph nodes	no	NED	44
18	M	58	buttock	surgery/e	∅	∅	∅	NED	47

Case	Sex	Age	Site of primary lesion	Primary therapy / where*	First recurrence (in months)	Localization of first recurrence	Further recur-rences	Current clinical outcome*	Follow-up (in months)
19	F	69	lower leg	surgery/e	0	regional lymph nodes	yes	DMCC	23
20	M	84	hand	surgery/e	∅	∅	∅	DID	24
21	F	79	face	surgery/i	1	regional lymph nodes	no	NED	75
22	F	77	hand	surgery/e	4	bones	No	DMCC	9
23	F	70	lower leg	surgery/e	∅	∅	∅	NED	72
24	M	82	capilitium	surgery/e	28	local	Yes	DID	40
25	F	76	face	surgery/e	∅	∅	∅	NED	35
26	M	64	buttock	surgery/e	5	local	Yes	DMCC	15
27	F	58	forearm	surgery/e	∅	∅	∅	NED	28
28	M	80	lower leg	surgery/e	6	regional lymph nodes	No	NED	20
29	M	70	face	surgery/e	∅	∅	∅	NED	3
30	F	87	neck	surgery/e	6	local	No	DID	33
31	F	55	face	surgery/e	1	local	Yes	NED	35
32	F	80	face	surgery/e	∅	∅	∅	NED	3
33	F	57	forearm	surgery/e radiation	∅	∅	∅	NED	15

*i=intern=University Hospital of Dermatology in Tuebingen ; e=extern=private practice / other hospitals ; NED=no evidence of disease ; DID=died of intercurrent disease ; DMCC=died of Merkel Cell Carcinoma

3.3. LOCALIZATION, SIZE AND STAGE OF THE PRIMARY TUMOR

3.3.1. Tumor localization

Merkel Cell Carcinoma was found at nine different sites of the body. The majority was diagnosed in the facial region (36.4%) followed by forearms (15.2%), lower legs and buttock (both 12.1%), hands (9.1%) and the upper

arms (6.1%). Furthermore single appearances were detected elsewhere: one at the thigh of a patient, one at a patient's neck and one at the capillitium (Figure 4 and Figure 5). Interestingly there was not one single appearance of Merkel Cell Carcinoma at the trunk among the patient collective in this study.

With regard to a superordinate classification in which just major regions of the body are described, one gets a better picture of the distribution. In the head and neck region 42.4% (14 out of 33) of the Merkel Cell Carcinomas were located, in the area of the upper limbs the percentage was 30.3% (10 out of 33) the region of the lower limbs had a fraction of 27.3% (9 out of 33) and finally the region of the trunk was not affected at all with 0 % (Figure 6).

Concerning the two different sexes, the location of the tumor was nearly evenly spread. On the patient's female side 8 out of 19 Merkel Cell Carcinomas were diagnosed in the head and neck region (42.1%), 6 at the upper and 5 at the lower limbs respectively (31.6% and 26.3% respectively). On the male side the tumor was found in the head and neck region in 6 out of 14 cases (42.9%) whereas in 4 patients the Merkel Cell Carcinoma was located at the upper and in 4 cases in the lower limbs respectively (each 28.6%).

Figure 4: Tumor localisation

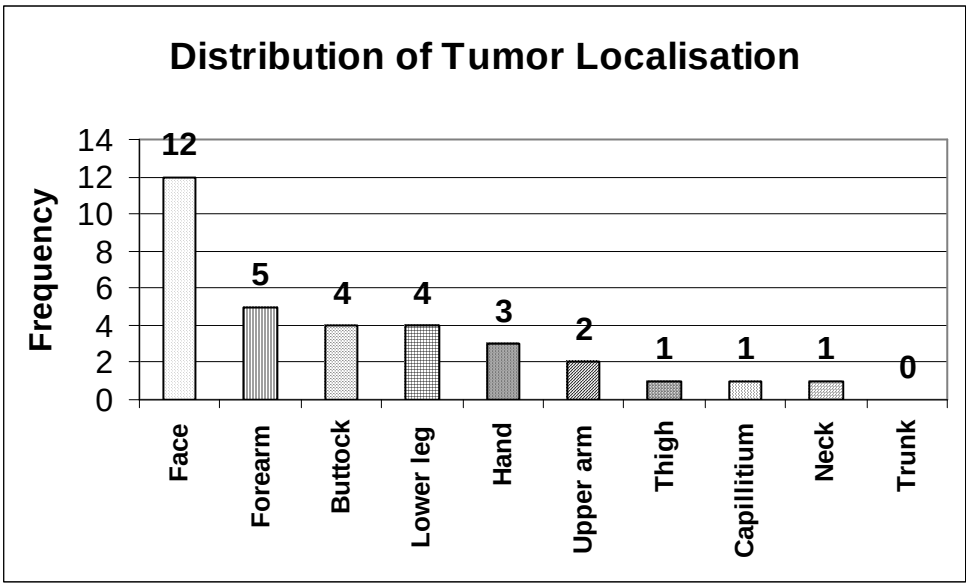
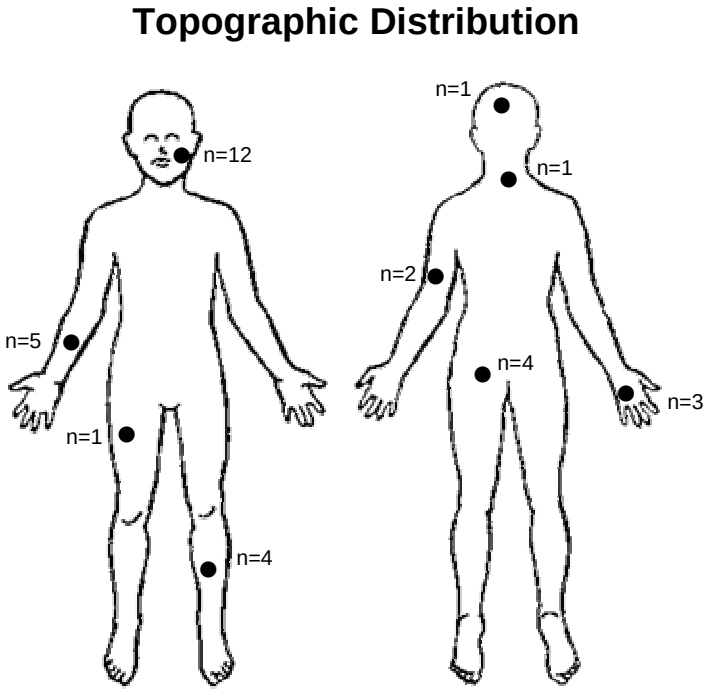
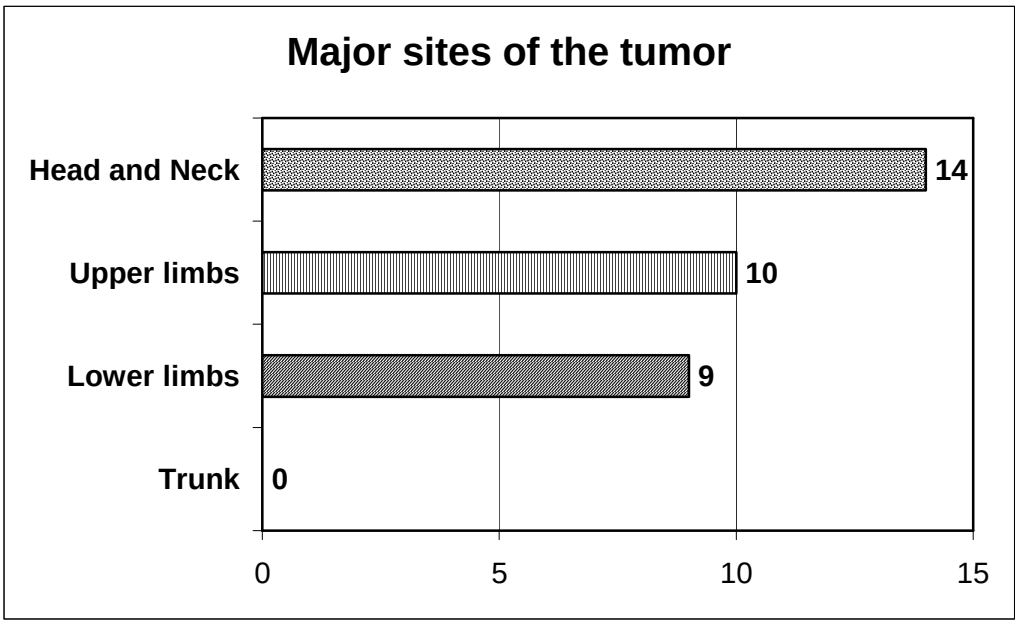


Figure 5: Topographic distribution



n=number of patients with Merkel Cell Carcinoma at the tagged spot of the body.

Figure 6: Major sites of the tumor



3.3.2. Tumor size and stage

Due to the fact that in 28 of the 33 cases of Merkel Cell Carcinoma the primary tumor has been excised either by settled GPs, dermatologists or surgeons (23 of 28) or other hospitals (5 of 28) provision of data was not possible in all circumstances. The complete particulars of length, width and thickness were detected in 14 cases and for another 6 patients just length and width of the primary tumor were available. The average over these data highlights a tumor size of 28.45 mm x 19.4 mm with a thickness of 7.1 mm (median = 17.5 mm x 10 mm with 5 mm thickness) whereas the maximum tumor dimension was 90 mm x 60 mm with 20 mm thickness and the minimum 4 mm x 2,5 mm with 1.5 mm thickness, respectively.

Given the data that were available at presentation at the University Hospital of Dermatology in Tuebingen, all 33 patients must be assigned to Stage I. In order to categorize a conventional staging system was used in which stage was assigned according to the absence (Stage I) or presence of positive lymph nodes (Stage II) or presence of systemic metastases (Stage III). Nevertheless the limitation of this statement must be taken into

consideration because only a few patients were staged in Tuebingen and the external staging was difficult to assess.

A correlation between tumor thickness and recurrence can not be seen from the data of this study (Table 8).

Table 8: Correlation tumor thickness and recurrence

Number of patients with known tumor thickness	Tumor thickness	Recurrence
1	1.5 mm	Ø
2	2 mm	Ø
3	3 mm	3 times local recurrence
4	4 mm	1 lymph node metastasis
5	4mm	1 lymph node metastasis
6	5 mm	Ø
7	5 mm	Ø
8	5 mm	1 distant metastasis
9	6 mm	3 times local recurrence
10	10 mm	Ø
11	10 mm	1 lymph node metastasis
12	12 mm	Ø
13	12 mm	1 lymph node metastasis
14	20 mm	Ø

3.4. TREATMENT OF DISEASE AT PRESENTATION AND RESULTS

As mentioned earlier on, only 5 cases of Merkel Cell Carcinoma underwent primary excision of the tumor at the University Hospital of Dermatology in Tuebingen, whereas the majority of 25 patients had their first re-excision there. It is important to emphasize that all of these patients did get the same surgical treatment and that the excised tissue

was processed in the same way, namely by 3D-Histology, after presenting in Tuebingen. The operations that were carried out differed only in the chosen width of the security margins. Unfortunately it was not possible to gain all specific data about the excision of the primary Merkel Cell Carcinoma from those patients who were pretreated at other hospitals, GPs or settled surgeons and dermatologists. This is why the following explanations do mainly refer to the treatment at the University Hospital of Dermatology in Tuebingen.

3.4.1. Surgical treatment, security margins and patient outcome

All patients that were operated at the University Hospital of Dermatology in Tuebingen were treated with the histological controlled excision (3D-Histology). Herewith two important steps were taken to ensure the success of the surgical treatment:

First of all a clinically healthy looking zone of the skin around the Merkel Cell Carcinoma was fixed and marked. The size of this so called security margin was chosen individually from patient to patient depending on the size and location of the tumor. In this available patient collective, the security margins ranged from 3 mm to 30 mm with a mean of 13.4 mm (median = 10 mm). In most of the cases (n = 15) the security margins were ≤ 10 mm x 10 mm and a security margin of ≥ 30 mm x 30 mm was only performed in one single case (see Table 9).

Secondly the excised tissue of the patients suffering from Merkel Cell Carcinoma was always processed with the help of the 3D-Histology.

Those patients whose primary tumor was not excised at the University Hospital of Dermatology in Tuebingen but who underwent their first re-excision there, were treated with the histological controlled re-excision within on average 1.8 months. In this connection the range between their excision of the primary tumor and the after-excision was 1 to 4 months.

The period of time of the remaining patients who had later re-excisions at the University Hospital of Dermatology in Tuebingen ranged from 1 to 12 months.

Concerning the patient outcome of the surgical treatment, 17 of the patients (51.5%) that have been operated in Tuebingen stayed relapse-free during the period of follow-up. Recurrence rates will be described in section 3.5.

Table 9: Security margins

Security margins max/min in mm	Number of patients	Operated in sano	Operated non in sano
≤ 10/10	15	13	2
≤ 20/20	11	9	2
≤ 30/30	1	1	0

3.4.2. Results of 3D-Histology

When it comes to the histological results of 3D-Histology after tumor excision one is always focusing on the R0-Resection. In this study 30 of the 30 patients with first appearance of Merkel Cell Carcinioma (excluding recurrences) did have tumor-free margins in their histological samples after surgery and 3D-Histology-check. From these 30 cases of R0-Resection, 26 (86.7%) were available after the first operation at the University Hospital of Dermatology in Tuebingen and 4 (13.3%) had to be re-excised one time to achieve tumor-free margins.

Interestingly 2 of the 5 patients with Merkel Cell Carcinoma that underwent their primary tumor excision at the University Hospital of Dermatology had to be re-excised, whereas only another 2 of the remaining 25 patients with re-excision had be re-operated to reach R0-Resection. The tumor of the first 2 patients was located in the facial region and excised with a safety margin of at least 10 mm. Those 2 patients, however, that underwent their

re-excision in Tuebingen did have their tumor localization at the limbs and the Merkel Cell Carcinoma was removed considering safety margins of at least 20 mm.

3.4.3. Radiation and chemotherapy

The primary therapy of Merkel Cell Carcinoma at the University Hospital of Dermatology in Tuebingen usually neither includes radiotherapy nor chemotherapy. Nevertheless 10 persons of our patient collective received radiotherapy and 4 received chemotherapy after surgery due to their recurrences. 3 patients wished radiation after the excision of the primary Merkel Cell Carcinoma as adjuvant therapy although they haven't had any recurrences so far. In 2 cases radiation therapy was applied twice plus a following chemotherapy and in one case a single radiotherapy was combined with a following radiotherapy. It is important to mention that only one of the 14 patients that received either radiation or chemotherapy underwent primary excision of his Merkel Cell Carcinoma in Tuebingen. The majority of this group developed diverse recurrences after having been pretreated at other hospitals, GPs or settled surgeons and dermatologists.

The level of radiation ranged from a dose of 30 to 80 Gray, with a mean of 47.7 Gray (median = 50 Gray), whereas the most common dosage was around 50 Gray. Concerning the applied chemotherapy four different modalities were in use: Interferon Alpha 3 Mio IE, Interferon Beta 3 Mio IE, CMF and local application of Bleomycin.

Concerning the results of radiotherapy alone the responses were: 5 patients with no further recurrences, 1 patient with local- and lymph nodal recurrence and 1 patient dying from Merkel Cell Carcinoma. Radiation plus chemotherapy revealed 1 relapse-free patient, 1 patient with progressive disease (local and lymph nodes) and another patient dying from Merkel Cell Carcinoma. Finally chemotherapy alone (Interferon Beta 3 Mio IE) that

was performed in only one patient showed further relapse-freedom. The mean follow-up after radiation- or/and chemotherapy was 54 months with a maximum of 168 months and a minimum of 3 months respectively.

3.5. RECURRENCES

In this study 16 (48.5%) of the patient collective developed at least one recurrence during the period of after-observation. 2 patients suffered from both local-, lymph node- and distant recurrence. Another 2 patients presented with lymph node- and distant metastases. The mean time to recurrence was 6.4 months (median = 5 months).

Interestingly from these 16 patients only 1 underwent the excision of his primary Merkel Cell Carcinoma at the University Hospital of Dermatology in Tuebingen. The number of 15 patients (93.8%) had the primary tumor excised at other hospitals, GPs or settled surgeons and dermatologists.

The following details about recurrence rates of the patients with Merkel Cell Carcinoma that have been treated at the University Hospital of Dermatology in Tuebingen, embrace a time span of maximum 182 and minimum 3 months.

The mean value lies at about 47.7 months whereas the after-observations are calculated from the individual date of first diagnosis until the fixed deadline January 2005 or the date of death respectively.

Due to the occasional manyfold re-excisions the given recurrence rates are always calculated from the state of R0-Resection after excision of the first appearance of MCC.

3.5.1. Local recurrences

In this study local recurrences occured in 7 patients with the total of 11 appearances, whereas 2 patients suffered from three local recurrences altogether. Referred to the total number of 33 patients in this study, local recurrence appeared in 21.2% of the cases. Furthermore local recurrences accounted for the first site of recurrence in 6 patients that means in 37.5% of all patients with recurrences.

The first local recurrence was diagnosed on average 11.9 months (median = 14 months) after R0-Resection of the primary Merkel Cell Carcinoma. With 4 patients that developed local recurrence the primary tumor was located at the head and neck region, with 2 patients at the upper limbs and with 1 patient at the lower limbs. In the case of those 2 patients with several local recurrences the primary Merkel Cell Carcinoma was located at the head and neck region, whereas the mean time span between the recurrences was 4.5 months. Another 2 patients, whose primary tumor was located at the head and lower limb respectively, developed additional lymph node and distant recurrences within on average 9.5 months.

None of the patients with local recurrence had his primary Merkel Cell Carcinoma excised at the University Hospital of Dermatology in Tuebingen and only 1 of them had his first re-excision there. All the other patients have been pretreated on average 6.7 months at other hospitals, GPs or settled surgeons and dermatologists.

3.5.2. Regional lymph node metastases

The most common site of recurrence was the regional lymph nodes. 10 of all 33 patients in this study (30.3%) presented a total of 15 lymph node metastases. 1 patient suffered from 4 and 2 patients from altogether 2 lymph node recurrences. Regional lymph node metastases accounted for the first site of recurrences in 9 patients (56.3%). The mean time to lymph node metastases was 3.4 months (median = 4.5 months) after

R0-Resection. Furthermore the distribution of the primary Merkel Cell Carcinoma with patients that developed regional lymph node metastases is quite interesting: 7 patients presented the primary tumor at the lower limb region, 2 at the head and only 1 patient at the upper limbs. The one patient who suffered from a total of 4 lymph node recurrences had his primary tumor at the head and neck region, whereas the period of time between the recurrences was on average 4.3 months. The primary tumor´s location of the 2 patients with double lymph node metastases both was the lower leg region and the time between the recurrences was on average 13.5 months. As already mentioned in section 3.5.1 two of the patients with regional lymph node metastases had additional local- and distant recurrences respectively.

From these 10 patients only 1 had his primary excision done at the University Hospital of Dermatology in Tuebingen and 4 underwent the first re-excision there. The rest of patients have been pretreated at other places for on average 3.4 months.

3.5.3. Distant metastases

Distant metastases occurred in 5 patients of the patient collective (15.2%), whereas 1 patient developed two different sites of metastases. The most common location for these metastases were distant cutaneous sites (n = 3), bones (n = 1) and lung (n = 1). Distant recurrences accounted for the first site of recurrence in only 1 of those 5 patients, the mean time between the primary Merkel Cell Carcinoma and the appearance of the different distant metastases was 8.4 months (median = 5 months). Concerning the localization of the primary tumor 3 were found at the lower limbs, 1 at the head and 1 at the upper limb. From these 5 patients all have been pretreated and their primary tumor has been excised by other hospitals, GPs or settled surgeons and dermatologists.

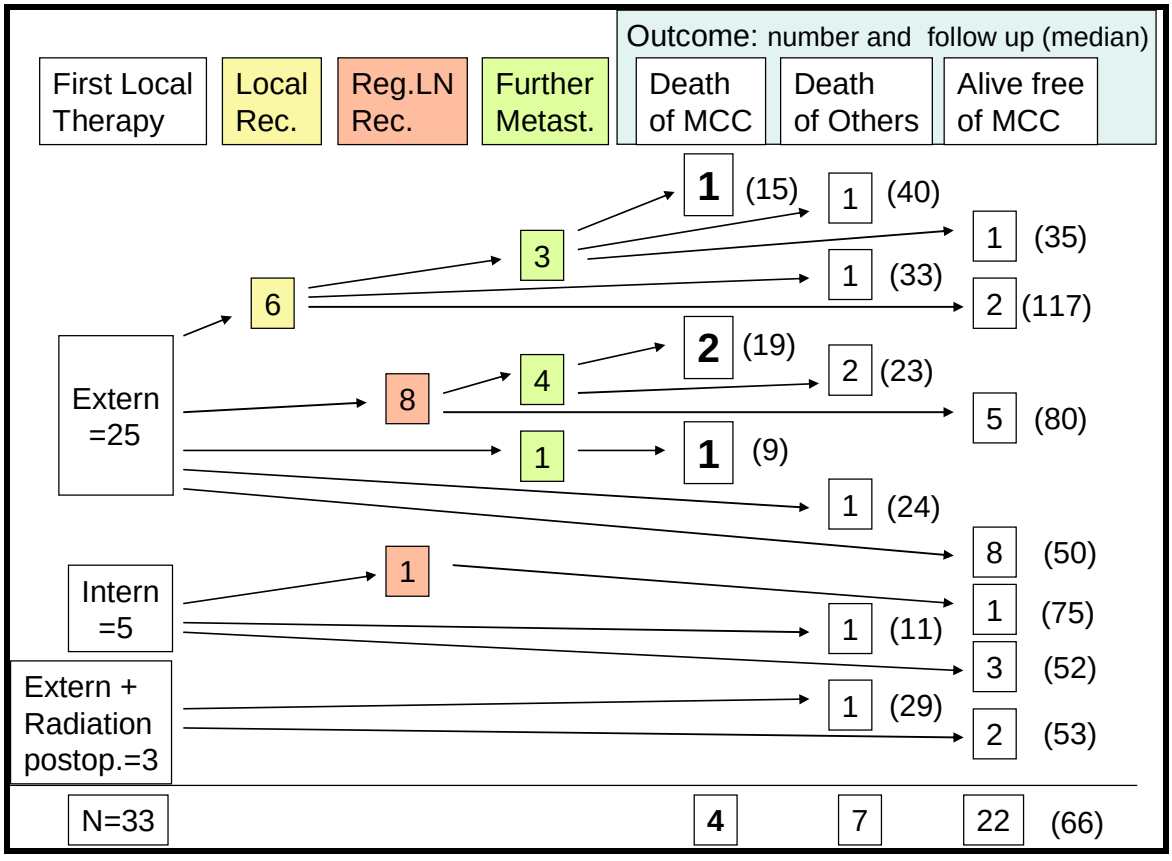
Only 1 of them had the second re-excision of his primary Merkel Cell Carcinoma at the University Hospital of Dermatology in Tuebingen.

3.6. SURVIVAL

During the period of follow-up 11 patients have died among the 33 patients of the patient collective. Out of these 4 (12.1%) have died of Merkel Cell Carcinoma. The mean time from diagnosis to cancer-related death was 15.3 months (median = 14.5 months) with a range of 9-23 months (Figure 7).

A total number of 17 patients (51.5%) stayed relapse-free after their primary Merkel Cell Carcinoma. Interestingly this study reveals several long-term survivors with up to 182 months.

Figure 7: Table of outcome



3.7. INCIDENCE OF OTHER MALIGNANT NEOPLASMS

In some of the international literature the incidence of other malignant neoplasms among patients with Merkel Cell Carcinoma has been described.

So the files of the patients in this study were searched for side-diagnosis and 10 of the 33 patients turned out to have had malignant neoplasms before they were diagnosed with MCC. Interestingly 4 of these patients (40%) had hematological malignancies, 2 patients with CLL and 2 patients with lymphoma, whereas 3 of them underwent chemotherapy. Beside this 2 patients suffered from basal cell carcinoma and among the other patients each one had a different malignancy: prostate cancer, livercell carcinoma, laryngical and endometrial cancer.

3.8. STATISTICAL ANALYSIS OF THE DATA

The limited number of observations that is available in this study does not allow to perform elaborate statistical analyses. Nevertheless some insights can be gained by applying simple methods to the data. Accordingly a simple comparison of sample means and univariate and multivariate regression analysis will be done.

The key question of this study is whether the use of 3D-Histology surgery for primary Merkel Cell Carcinoma treatment is superior to the current standard method of wide local excision. In order to investigate this question with statistical means all information has to be translated into numerical data and a measure that allows to compare and judge both surgery methods has to be defined.

To begin with the latter task a potential performance measure of different surgery methods is the number of recurrences after primary surgery. An alternative measure could be the number of operations performed after primary surgery. This second measure also includes surgery at the same

location even if no recurrence has taken place. The following tables show means and standard deviations for both measures in the whole sample and for each surgery method separately and the graphs give a representation of the sample distributions.

Table 10: Re-excisions

	Sample Mean	Standard Deviation	No. of observations
Full sample	1.91	1.76	33
Tuebingen	0.6	0.55	5
Standard	2.14	1.8	28

Table 11: Recurrences

	Sample Mean	Standard Deviation	No. of observations
Full sample	0.97	1.49	33
Tuebingen	0.2	0.45	5
Standard	1.11	1.57	28

Figure 8: Recurrences

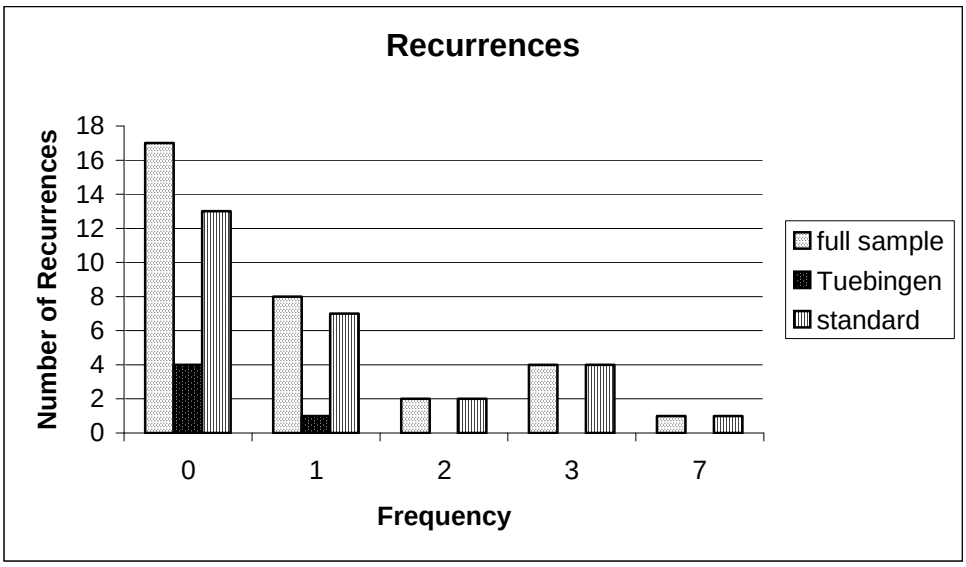
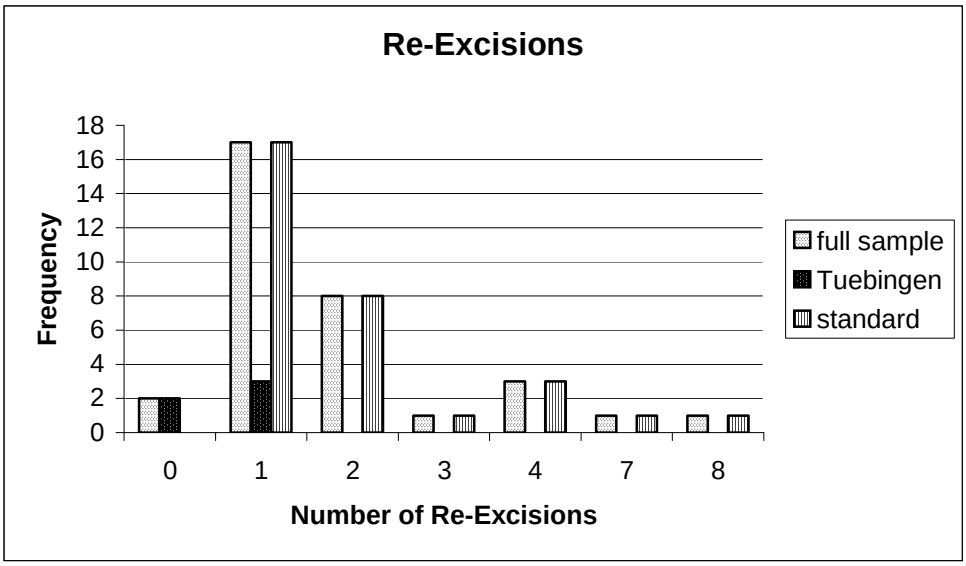


Figure 9: Re-excisions



The point of interest is whether the “Tuebingen-Torte”-method is associated with a lower number of re-excisions and recurrences than the standard method. The natural starting point for such an inquiry is to have a look at the means of the two distributions as in tables 8 and 9. While the

mean of the Tuebingen sample is clearly smaller on both counts, it is difficult to draw immediate conclusions from this as the sample sizes are not only small but also different. In order to obtain a statistical assessment of the difference of these means it will be assumed that both samples are drawn from a normally distributed population. While normality is probably the most common assumption it may not be innocent. Normality allows support values from minus infinity to plus infinity while the given measures can only assume positive values. Therefore a log-normal distribution might be more appropriate. With normality introduced, it is possible to perform a two-sample t-test for equal means [see e.g. Snedecor, George W. and Cochran, William G. (1989), Statistical Methods, Eighth Edition, Iowa State University Press.] It allows to test whether one mean is different or smaller than another taking into account the different sample sizes. Here the question is whether the mean of the Tuebingen-sample is smaller than the mean from the standard-sample. Indexing the Tuebingen-values with 1 and the standard-values with 2 the relevant test statistic is computed as

$$T = \frac{\overline{Y_1} - \overline{Y_2}}{\sqrt{s_1^2 / N_1 + s_2^2 / N_2}}$$

where N1 and N2 are the sample sizes, $\overline{Y_1}$ and $\overline{Y_2}$ are the sample means, and s_1^2 and s_2^2 are the sample variances. The degrees of freedom for the t-distribution is given by

$$\nu = \frac{\left(s_1^2 / N_1 + s_2^2 / N_2\right)^2}{\left(s_1^2 / N_1\right)^2 /(N_1 - 1) + \left(s_2^2 / N_2\right)^2 /(N_2 - 1)}$$

The results from this exercise are shown in table 10.

Table 12: T-Statistic

	T-statistic	95% critical value of t-distribution (degrees of freedom)	P-value of T-statistic
Re-excision	-3.68	-1.717 (22)	0.000
Recurrences	-2.53	-1.711 (24)	0.008

The results clearly reject the null-hypothesis that the means are the same. Hence under the assumption of normality the Tuebingen method appears to be preferable to the standard method.

A simple mean comparison is no strong evidence of course. Any number of other influence factors may drive this result. Multivariate regression analysis is a possible way to examine the effect of the surgery method while accounting for the effect of several other influence factors on the outcomes. It is necessary to assume that the outcomes are a linear function of the explanatory variables for this method to be applicable that is the following model is assumed:

$$y = \alpha + \beta X + \varepsilon$$

where y represents the outcome variable in question, here either re-excisions or recurrences, alpha and beta are the coefficients to be estimated, X is the matrix of explanatory variables and epsilon is an error term. Different specifications are tried out which are shown in tables 11 and 12.

Table 13: Regression re-excisions

	(1)	(2)	(3)	(4)	(5)
Constant	2.14	2.64	2.63	4.22	1.9
	(6.71)	(1.21)	(1.19)	(1.25)	(0.86)
Tuebingen	-1.54	-1.48	-1.5	-1.62	-1.45
	(-1.88)	(-1.7)	(-1.68)	(-1.51)	(-1.45)
Age		-0.007	-0.007	-0.03	0.009
		(-0.23)	(-0.24)	(-0.77)	(0.21)
Sex			0.09	-0.028	-0.64
			(0.14)	(-0.04)	(-0.74)
Localisation dummies	No	No	No	yes	No
Length					-0.006(-0.36)
Adjusted R-squared	0.073	0.044	0.012	-0.17	-0.034
Observations	33	33	33	33	20

Table 14: Regression recurrences

	(1)	(2)	(3)	(4)	(5)
Constant	1.11	0.89	-0.001	2.18	0.88
	(3.97)	(0.05)	(-0.00)	(0.75)	(0.5)
Tuebingen	-0.91	-1.03	-1.12	-0.92	-0.93
	(-1.27)	(-1.36)	(-1.47)	(-1)	(-1.45)
Age		0.14	0.013	-0.01	-0.0006
		(0.54)	(0.48)	(-0.27)	(-0.02)
Sex			0.51	0.39	(0.26
			(0.98)	(0.6)	(0.47)
Localisation dummies	No	No	no	yes	No
Length					0.005
					(0.44)
Observations	33	33	33	33	20
R-squared	0.018	-0.004	-0.006	-0.2	-0.08

Column (1) of tables 11 and 12 show the results from a univariate regression of the outcomes on a constant and a binary indicator that takes on value 1 if surgery was performed in Tuebingen and value 0 otherwise. As can be seen the coefficient on this binary variable is just the difference between the means of the two samples. Different from the two sample mean comparison test, however, the two means computed here are not statistically different at the 5% level. The coefficient on Tuebingen in table 4 is still significant at the 10% level though. In both regressions the adjusted R-squared value shows that only little of the variation in the data can be explained by whether surgery was done in Tuebingen or not. As can be seen by the low t-values of the additional variables and the decreasing adjusted R-squared, adding more variables does not improve the explanatory power of the model. Given the small number of observations this is not surprising.

Thus the simple comparison of means under the assumption that all observations follow a normal distribution does give some support to the hypothesis that patients treated under the Tuebingen method do have to undergo less after excisions and suffer from fewer recurrences. It is unlikely though, that the method of surgery is the only factor influencing the outcomes. The low number of observations does not allow to assess the effect of several factors on the outcomes, however. Moreover, the assumption of normality may be wrong and again the number of observations that is available is too small to argue that normality could be a good approximation to the true underlying distribution. Therefore no definite conclusions can be drawn from this analysis in either direction.

4. DISCUSSION

4.1. AGE AND GENDER DISTRIBUTION

4.1.1. Dispersion of age

As stated by the AWMF [24] and agreed upon among the international literature, Merkel Cell Carcinoma is a malignancy of the elderly. The age limits of the 33 patients in this study were located between 51 and 87 years whereas the mean value was 69.9 years. In this connexion the mean age distribution among women and men were relatively close with 71.0 and 69.0 years respectively. The women's age ranged from 51 to 87 and the men's age ranged from 57 to 87 years. The MCC occurred most frequently among the 70-79 age group with the total of 33%. These results are consistent with recent larger case studies and reaffirm Merkel Cell Carcinoma as a skin tumor that occurs predominantly after the 5th decade [55,25,29].

As noted by others, we also come to the conclusion that age seems to be one of the major risk factors in developing MCC.

4.1.2. Dispersion of gender

Among the observed patients with Merkel Cell Carcinoma 19 (58%) were women and 14 (42%) were men. So more women suffered from this malignancy with a female:male ratio of 1.4:1.

Interestingly this shows exactly the opposite result of Medina-Franco's review of 1024 cases [56]. Also other authors have found that Merkel Cell Carcinoma appears more often in men than in woman [21,25,33,57,58], whereas studies with a higher female percentage have been published as well [32,59]. Some recent studies, however, have not mentioned their gender distribution at all and some authors have revealed a near equal

male to female ratio [19,29,60,61]. So one must possibly reconsider the significance of gender as an explanatory variable within MCC. Furthermore the age as a presumed risk factor to develop Merkel Cell Carcinoma does not occur in all current larger literature reviews [56].

4.2. LOCALIZATION OF THE PRIMARY TUMOR

Merkel Cell Carcinoma is usually developing on sun-exposed skin of Caucasian individuals [4,20,40]. As Goessling et al. has pointed out, most of all tumors occur in the face and neck (50%), followed by the extremities (40%) and the trunk (10%). In our patient collective that consisted solely of Caucasians, we found very similar data. The majority of the primary tumor localizations were diagnosed in the head and neck region with 42.4%. The area of the upper limbs represented 30.3% of all primary sites and the region of the lower limbs had a fraction of 27.3%. It is important to mention that no single patient had a primary affection of the trunk. These figures underline the assumed influence of sun exposure in the emergence of MCC.

Described in detail, 12 patients (36.4%) in this study suffered from Merkel Cell Carcinoma in the facial region, in 5 patients (15.2%) the malignancy occured at the forearms, there were each 4 patients (each 12.1%) with primary affection of the lower leg and buttock, respectively, 3 patients (9.1%) with hands and 2 patients (6.1%) with upper arms as primary site. Additionally other single appearances of Merkel Cell Carcinoma were diagnosed at one patient’s thigh, one patient’s capillitium and one patient’s neck (each 3%).

A review of 1024 cases in 2001 has shown likewise results with high rates of Merkel Cell Carcinoma at sun-exposed areas of the skin: 40% in the head and neck area and 33% in the extremeties [56]. However, Medina-Franco points out that more than 20% were localized to the trunk and other not sun-exposed areas. And primary tumors have also been reported

to occur in the oral mucosa, larynx, esophagus, cervix and vulva [62,63,64,65,66]. So one must state that although Merkel Cell Carcinoma seems to have a predilection for areas of the skin that are exposed to sunlight its occurence is not inevitably connected to ultraviolet light-exposure.

4.3. TUMOR SIZE AND STAGE

As the primary Merkel Cell Carcinoma of many of the patients in this study was excised at private settings, it was not possible to gain all data concerning tumor size from every single patient. The tumor ranged from 90 mm x 60 mm with 20 mm thickness in maximal dimension to 4 mm x 2.5 mm and 1.5 mm thickness in minimal dimension. The average highlightened a tumor size of 28 mm x 19 mm with a thickness of 7 mm and all patients with Merkel Cell Carcinoma in this study were assigned to Stage I based upon the commonly used stage system [3].

Due to the limited number of observations no conclusions could be drawn from the statistical analysis of these data. Mott et al., however, underlines the significance of tumor size as a prognostic factor [58]. He found that among other things tumor size ≥ 5 mm correlates with a poor prognosis in Merkel Cell Carcinoma. Furthermore his study shows that also depth of invasion is important in predicting the biological behaviour of MCC.

Consistent with his results, the average tumor thickness in this study of those patients who suffered from recurrences was 6.8 mm, whereas the mean tumor size amounted 27.8 mm x 17.7 mm. However, the significance of depth of invasion as prognostic factor is not shared by all authors [74].

4.4. TREATMENT OF DISEASE AT PRESENTATION

All of the 30 patients with Merkel Cell Carcinoma that were operated at the University Hospital of Dermatology in Tuebingen were treated with the histological controlled excision, the so called 3D-Histology. As a matter of fact, only 5 of these patients underwent primary excision at the University Hospital of Dermatology, whereas the majority of 25 had their first re-excision there.

Despite this numeral disequilibrium the results of 3D-Histology are significant as more than half of these 25 patients had their first re-excision within the first 2 months of the primary occurence of Merkel Cell Carcinoma. In this context primary excisions at private settings can be considered as skin biopsies in order to confirm an estimated diagnosis. However, the fact that many patients were pretreated elsewhere can be regarded as a disadvantage for them. The figures that are presented under 4.4. will hint the pre-eminence of 3D-Histology compared to mostly used two-dimensional histological analysis at private settings.

The technique of 3D-Histology has proven of value in the treatment of malignant skin tumors since it has been invented by Breuninger in 1982. Several studies that have been conducted at the University Hospital of Dermatology in Tuebingen document the success of this tissue-processing on the basis of local recurrences [43-49,67]. This present study, however, is the first one to describe the use of 3D-Histology in MCC. The following paragraphs aim to endorse the results of the mentioned studies related to 3D-Histology that have been carried out so far.

4.4.1. Security margins and results of 3D-Histology

In this study the size of the security margins were chosen individually from patient to patient depending on the size and localization of the Merkel Cell Carcinoma, whereas they ranged from 3 mm to 30 mm with a mean of 13.4 mm.

As the aim of surgical treatment is complete resection of the tumor in all dimensions of its growth, the results of 3D-Histology were reviewed in respect of R0-Resection. From 30 patients that were operated at the University Hospital of Dermatology in Tuebingen, 26 (86.7%) could be released with R0-Resection after the first operation. Only 4 patients (13.3%) had to be re-excised once to achieve tumor-free 3D-Histology-margins. If one takes a closer look at the exact security margins in this study, an interesting fact can be observed. The majority of 15 patients were successfully treated with security margins of ≤ 10 mm x 10 mm and only two of them had to be re-excised to gain R0-Resection. Another 11 patients had security margins of ≤ 20 mm x 20 mm with another two cases of re-excision to achieve R0-Resection. Only in one single case the security margin had primarily been ≥ 30 mm x 30 mm to secure RO-Resection. These results indicate that with the help of 3D-Histology intact tissue close to Merkel Cell Carcinoma can be saved without a bigger risk of R1- or R2-Resection. Compared to conventional tumor surgery with larger excision margins, the use of 3D-Histology enables the surgeon to save healthy tissue. By avoiding large excision margins 3D-Histology achieves better cosmetic results and is hence a useful treatment option particularly for skin tumors in the head and neck region which represent cosmetically sensitive anatomic areas.

Among international literature about Merkel Cell Carcinoma, however, wide local excision with security margins of ≥ 20 mm is still recommended by some authors [32,33,55,56,60] . Nonetheless micrographic surgery as surgical treatment of Merkel Cell Carcinoma has been recently described as more efficacious than wide excision [20,25,29,61].

Moehrle et al. consolidated the term 3D-Histology in a recent study in the British Journal of Dermatology [49]. The paper underlines the sensitivity of 3D-Histology in detecting tumor outgrowths and states its special significance in tumors which spread per continuitatem. Even though Moehrle et al. focus on histologic procedures in lentigo maligna

melanoma, the findings are also relevant for this present study. They recommend 3D-Histology with reduced excision margins for head & neck as well as acral melanomas for functional and cosmetic reasons and showed lower rates of recurrence with their patient collective in comparison to conventional histology. Given that Merkel Cell Carcinoma occurs predominantly in the head and neck region followed by the extremeties, 3D-Histology suggests itself as a useful treatment option.

4.4.2. Radiation and chemotherapy

Merkel Cell Carcinoma is considered to be sensitive to radiation therapy by several authors and MCC cell lines have been shown to be radiosensitive in vitro [27,28,69,70]. Most of these studies suggest adjuvant radiotherapy plus surgery but Mortier et al. for instance even suggest radiation alone [71]. Allen et al., however, found in the largest recently published single institution study that local recurrence was not more common in patients who didn't receive adjuvant radiotherapy after resection of the primary tumor [55]. Yiengpruksawan et al. in turn considered radiotherapy just in selected patients with advanced disease [33]. So the role of radiation therapy in the treatment of Merkel Cell Carcinoma remains unclear.

The standard primary therapy of Merkel Cell Carcinoma at the University Hospital of Dermatology in Tuebingen neither includes radiation nor chemotherapy. Nevertheless 10 patients received radiotherapy in this present study. In one of the cases radiation was followed by chemotherapy and in another two of these 10 cases radiotherapy was applied twice plus a following chemotherapy. The majority of this group of 10 developed diverse recurrences after having been pretreated at private settings and were hence irradiated. Only 3 of the patients wished radiation after the excision of the primary Merkel Cell Carcinoma as adjuvant therapy although they didn't have had any recurrences so far. The level of

radiation ranged from a dose of 30 to 80 Gray. The follow up of those with radiotherapy alone revealed 5 patients (71.4%) that stayed relapse-free, 1 patient with local- and lymph nodal recurrence and 1 patient dying from Merkel Cell Carcinoma. The responses to radiation plus chemotherapy were 1 patient with no further recurrences, one with progressive disease (local and lymph nodes) and another one dying from Merkel Cell Carcinoma. At first glance the 71.4% relapse-freedom of the patients that received adjuvant radiotherapy after surgery seem to speak in favour of this combined treatment. Regrettably the very small number of observations doesn't allow to draw any conclusions from this finding.

In contrast to radiotherapy there is a rather broad consensus among international literature when it comes to the role of chemotherapy for patients suffering from Merkel Cell Carcinoma. Chemotherapy is hence usually stated to be reserved for those patients with evidence of distant metastases or advanced local or regional disease. Several studies that dealt with adjuvant chemotherapy as treatment option have shown that is has short-lived benefit, that it does not improve survival and even that toxic deaths were not uncommon [18,32,72]. Nonetheless Merkel Cell Carcinoma seems to be partially sensitive to agents such as Doxorubicin and Cisplation. So Tai et al. come to the conclusion that chemo-radiation for locally recurrent disease may be an option for patients with a good performance status [34].

In this present study 4 patients received chemotherapy after surgery at the University Hospital of Dermatology in Tuebingen, whereas different modalities were in use: Interferon Alpha 3 Mio IE, Interferon Beta 3 Mio IE, CMF and local application of Bleomycin. All patients had advanced local or distant disease. Only one patient received solely chemotherapy in the form of local Bleomycin application and stayed relapse-free. The remaining 3 patients all had radiation plus chemotherapy after surgery. Concerning the results, 1 patient remained relapse-free, 1 patient developed progressive disease (local and lymph nodes) and one dying from MCC.

Due to the heterogeneous group of patients receiving very different sorts of chemotherapy, no deeper insights can be gained from these data with respect to the chemotherapeutic treatment of Merkel Cell Carcinoma. Nevertheless chemotherapy as palliative action can be favoured by this study.

4.5. RECURRENCE

In the early 80ies early reports of Merkel Cell Carcinoma qualified it as a low grade malignancy [73]. Succeeding studies, however, have revealed that this tumor often develops an aggressive course [19,20,33,56,60]. In a recent review article Goessling et al. range the overall recurrence rate from 55% to 79%, whereas MCC seems most often to occur in regional lymph nodes [20].

In this study 16 of the 33 patients that have been treated at the University Hospital of Dermatology in Tuebingen developed at least one recurrence. This makes an overall percentage of 48.5% during the period of after-observation which embraces a time span of maximum 182 and minimum 3 months. Due to the enormous significance of recurrence as prognostic factor in Merkel Cell Carcinoma the different forms of recurrence are described in detail separately.

4.5.1. Local recurrence

In their extensive review Haag et al. stated that local recurrence develops in 26-44% whereas the first local recurrence is usually noted within 4 months [3]. This study showed local recurrence in 7 patients which corresponds to a local recurrence rate of 21.2%. In 6 cases local recurrence accounted for the first recurrence.

In this context first local recurrence was diagnosed on average 11.9 months (median = 14 months) after R0-Resection of the primary Merkel Cell Carcinoma. In comparison to Haag's review the mean time of recurrence is thus by all means striking [3]. Given the results of Goessling et al. who describe the majority of recurrences appearing within the first 6 to 12 months after initial diagnosis, this study seems to stand at the further end of the range [20].

Interestingly 2 of the 7 patients suffered from 3 local recurrences altogether with a mean time span between these recurrences of 4.5 months. This fact stresses the potential aggressive nature of MCC.

It is important to stress that none of the patients that have developed local recurrence underwent excision of the primary tumor at the University Hospital of Tuebingen.

Treatment and outcome of local recurrence

From the 7 patients with local recurrence, 4 were treated with excision and 3D-Histology only. Three of them stayed relapse-free after the first excision of the local tumor at the University Hospital of Dermatology in Tuebingen and one developed another 2 local recurrences that were likewise treated with excision and 3D-Histology only. One patient that suffered from three successive local recurrences was treated with excision and 3D-Histology the first two times ensuing excision plus radiation after the third recurrence. The last two patients refused any treatment and were not operated at all whereas both patients died, whereof one due to Merkel Cell Carcinoma as he had further lymph node and distant metastases.

Among international literature no specific guideline is described to handle local recurrence and its significance in predicting survival has been discussed controversially [33,60,75]. The results in this study are too small

to be significant, nevertheless excision alone as treatment option seems justifiable.

4.5.2. Regional lymph node metastases

In comparison to local recurrence the significance of regional lymph node metastases in diminishing survival rates is widely agreed upon. Allen et al. report that regional lymph node was the most common site of first recurrence and a separate study that a majority of patients with distant metastases had had prior nodal recurrence [55,76]. In Medina Franco's review of 1024 cases, 55.5% of the patients with Merkel Cell Carcinoma had lymph node metastases at presentation or developed them during follow-up [56]. In a recent paper that deals with current management of Merkel Cell Carcinoma the use of sentinel lymph node (SLN) mapping has been stressed [68]. In other studies up to 25% of patients with Merkel Cell Carcinoma were found to have metastatic disease in the SLN and up to 30% present pathologic lymph nodes who undergo lymph node dissection [19,33,55,77].

Consistent with Allen et al. regional lymph node metastases was the most common site of recurrence in this study and accounted for the first site of recurrences in 56.3%. On the whole 30.3% of the patients presented with a total of 15 lymph node metastases whereas the mean time was 3.4 months after R0-Resection. This short time span is quite interesting especially in comparison with the mean time of local recurrence of 11.9 months. Beside this, multiple authors have described median times from treatment of the primary tumor until the development of clinically apparent nodal metastases with 7-8 months and thus longer than time spans for local recurrence [33,75,78].

Interestingly from all the 10 patients that developed regional lymph node metastases only 1 patient had his primary excision done at the University Hospital of Dermatology in Tuebingen.

Treatment and outcome of regional lymph node metastases

Concerning treatment modalities of those patients who developed regional lymph node metastases, several different therapies have been applied. Two patients were treated with lymphadenectomy alone and another two patients were not operated at all. In 2 cases lymphadenectomy plus radiation therapy was applied and in 3 patients lymphadenectomy and radiation in combination with chemotherapy. Only one patient was treated with lymphadenectomy and chemotherapy alone.

As described with local recurrences, some patients of this study showed not only one but several lymph node recurrences. One patient suffered from 4 lymph node recurrences and finally died after having developed distant disease. Two further patients were affected from altogether 2 lymph node recurrences whereas one patient died of Merkel Cell Carcinoma. This result characterizes Merkel Cell Carcinoma as a highly aggressive and lethal tumor.

Only 5 patients stayed relapse-free, one with lymphadenectomy alone, one with lymphadenectomy plus radiation, another one with lymphadenectomy plus chemotherapy and two with lymphadenectomy plus radiation and chemotherapy. Due to this very heterogeneous treatment result it is difficult to assess the relevance of the individual therapies.

Similar to the treatment of local recurrence, no guidelines to handle regional lymph node metastases are agreed upon among international literature. Brady specifies that complete lymphadenectomy should be performed when Merkel Cell Carcinoma is found in sentinel lymph node biopsy even though half of those patients will go on to die of distant disease [68]. Recently published papers recommend complete lymph node dissection followed by post-operative irradiation of the nodal basin [21,69], however others have stated no significant difference in recurrence rates

after lymphadenectomy alone and lymphadenectomy plus adjuvant radiotherapy [29,33].

4.5.3. Distant metastases

The most common sites of Merkel Cell Carcinoma metastasis have been described to be the skin followed by distant lymph nodes, liver, lung, bone and other [18].

This study showed distant metastases in 5 patients whereas the most common site was the skin (n = 3) followed by lung and bone (n = 1 each). The median time span between the primary Merkel Cell Carcinoma and the appearance of distant metastases was 5 months. Interestingly in all patients but one, systemic disease was associated with antecedent regional lymph node metastases.

Treatment and outcome of distant metastases

Distant disease in Merkel Cell Carcinoma has a very poor prognosis and treatment is usually palliative as long-term survival of patients with disseminated Merkel Cell Carcinoma is extremely rare [21].

In this study 3 patients were treated palliative (2 with radiation only and one with radiation plus chemotherapy) and 2 refused any further treatment. All 5 patients with distant disease died, thereof 4 from Merkel Cell Carcinoma. The median time from discovery of distant metastasis to cancer-related death was 7 months and thus consistent with Yiengpruksawan’s findings [33].

4.6. SURVIVAL

In this study 12.1% of all patients died of Merkel Cell Carcinoma whereas the median time from diagnosis to cancer-related death was 14.5 months.

Compared to a recently published case study of 36 patients with Merkel Cell Carcinoma where 36.1% of the patient collective died within a median time of 16.4 months, 3D-Histology seems to be a justifiable treatment [79].

A total number of 17 patients (51.5%) stayed relapse-free after primary Merkel Cell Carcinoma. Furthermore this study reveals several long-term survivors with up to 182 months which underlines similar findings of other authors [57,59].

4.7. CONCLUSIONS

Merkel Cell Carcinoma is a malignancy of the elderly and the results of this study are consistent with recent larger studies, which describe age as one of the major risk factors in developing MCC. Also sun exposure is underlined by this study as a risk factor to develop Merkel Cell Carcinoma, as the head and neck was affected in 42.4%, the upper limbs in 30% and none of the patients had a primary affection of the trunk.

When it comes to the outcome of therapy options, this study can clearly recommend primary excision with subsequent processing in 3D-Histology. In this study 50% of the patients could be treated with ≤ 10 mm x 10 mm, whereas only two pf those had to be re-excised to gain R0-Resection, and only one patient needed a security margin of ≥ 30 mm x 30 mm. With the means of 3D-Histology intact tissue can be saved and better cosmetic results can be achieved. Obviously the saving of healthy tissue must not diminish life expectancy, hence recurrence rates have been observed accurately in this study. Concerning local recurrence, Haag et al. stated that it develops in 26-44% whereas the first local recurrence is usually noted within 4 months [3]. This study showed local recurrence in 7 patients

which corresponds to a local recurrence rate of 21.2%. Regional lymph node metastases was the most common site of recurrence in this study and accounted for the first site of recurrences in 56.3%. In Medina Franco's review of 1024 cases, 55.5% of the patients with Merkel Cell Carcinoma developed lymph node metastases [56], so this study does not come out worse. When it comes to the survival rates of this study, 12.1% of all patients died of Merkel Cell Carcinoma whereas the median time from diagnosis to cancer-related death was 14.5 months. And again the comparison with a recently published case study of 36 patients with Merkel Cell Carcinoma, where 36.1% of the patient collective died within a median time of 16.4 months, reveals that 3D-Histology seems to be a good treatment option [79].

Of course, on the one hand statistical analysis of data are not significant due to the small number of patients. But on the other hand, many results of the cited case studies are not significant either, so one must state that the overall results of therapy of Merkel Cell Carcinoma by means of 3D-Histology are satisfying and justify continuation to gain more data.

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CURRICULUM VITAE

Sarah Taha was born on the 29.08.1978 in Boeblingen. She started her school education in Leinfelden in 1985 and finished with a high school degree in June 1998. Ensuing she absolved a social year in South Africa (near Johannesburg) in a home for mentally handicapped people.

In October 1999 Sarah Taha matriculated at the Eberhard-Karls-University of Tuebingen in human medicine. Medical preliminary examination on September the 7th 2001 in Tuebingen, first state exam on August the 29th 2002 in Eningen u. A., second state exam on September the 5th 2005 in Tuebingen and final medical exam on November the 28th 2006 in Stuttgart. Receipt of approbation as medical doctor on December the 5th 2006.

Since January 2007 Sarah Taha is working as assistant physician in the field of anesthesiology at the Robert-Bosch-Hospital in Stuttgart.

Sarah Taha became a member of the German National Scholarship Foundation in February 2000. In 2002 she was accepted as candidate for a doctor's degree by Professor Dr. H. Breuninger at the University Hospital of Dermatology in Tuebingen. She acquired professional experience during several internships in various medical areas like psychiatry, gynaecology, dermatology as well as paediatric surgery and obtained profound medical working experience during her practical year in internal medicine, surgery and anestesiology. Furthermore she gained medical experience abroad by studying one term at the University of Aberdeen in 2003 and another term at the Université René Descartes Paris V in 2004 and by absolving the surgical term of the practical year at the Hôpital Général de Douala (University Hospital of Yaoundé) in Cameroon.