Merkel Cell Carcinoma treatment and survival at Helsinki University Hospital 2010 – 2018 - Special emphasis on adjuvant radiation therapy

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Thesis

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Tiivistelmä – Referat – Abstract

Bakgrund: Merkelcellkarcinom (MCC) är en mycket ovanlig men väldigt dödlig hudcancer som främst förekommer hos äldre individer med ljus hudton. Exponering för UV-strålning eller infektion med Merkelcell polyomavirus är centralt för patogenesen. MCC kommer oftast till uttryck som en rosa-röd lokal nodul på UV-exponerade hudområden i hals- och huvudområdet eller extremiteterna. Den rekommenderade behandlingen utgörs av en kirurgisk tumörresektion, portvaktskörtelbiopsi och adjuvant strålbehandling, vid metastaserad sjukdom även av cytostatikabehandling eller immunterapi. Femårsöverlevnaden är 40 - 70%. Studiens syfte var att analysera MCC-patienter gällande patient- och sjukdomskarakteristika och behandlingar samt inverkan de har på sjukdomsprognosen med en specifik betoning på adjuvant strålbehandling.

Metoder: Data gällande patient- och sjukdomskarakteristika, behandlingar, sjukdomsprogression och överlevnad samlades in ur patientjournaler gällande alla patienter diagnostiserade med MCC inom HUCS-området 2010-2018. Statiska analyser gjordes gällande oberoende variablers inverkan på överlevnaden, med grundligare kalkyleringar gällande inverkan av adjuvant strålbehandling.

Resultat: 47 patienter inkluderades i studien. Medelåldern bland patienterna var 79 år, 59,6% var kvinnor, primärtumören var oftast i huvud-halsområdet och sjukdomen lokal vid diagnostidpunkten. 43 patienter genomgick en kirurgisk tumörresektion, 28 patienter fick adjuvant strålbehandling, en patient behandlades med cytostatika, och en med immunoterapi. En liten tumörstorlek, låg tumörklass, lägre ålder vid diagnostidpunkten och behandling med adjuvant strålbehandling var fördelaktiga för överlevnaden. 2-årsöverlevnaden var 57,1%. Den sjukdomsspecifika 3-årsöverlevnaden för patienter som behandlades med strålbehandling var 74,0%, och för de som inte behandlades med strålbehandling 51,8%.

Slutsats: Patientgruppen motsvarade till sina egenskaper det som beskrivits i tidigare litteratur. En liten primärtumör, lokal sjukdom vid diagnos, lägre ålder vid insjuknande samt behandling med adjuvant strålbehandling var fördelaktigt för prognosen. MCC är fortfarande en aggressiv cancer med hög mortalitet och behandlingarna varierar märkbart mellan patienter inom HUCS-området.

Avainsanat – Nyckelord – Keywords
Merkel Cell Carcinoma, Radiotherapy, Survival, Skin Cancer, Non-melanoma Skin Cancer, Surgery

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

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1 Introduction

Merkel Cell Carcinoma (MCC) is a rare but particularly aggressive cancer of the skin. It is commonly associated with local and regional recurrence, distant metastases and a high mortality. (5, 6, 16, 19, 24) The disease most commonly presents in elderly patients, with only a fraction of the cases being reported in patients under 60 years of age. The disease is more common in patients with fair skin. (15 – 18, 24 - 26) MCC develops either through long term exposure to UV-radiation or through infection with the Merkel Cell Polyoma Virus (MCPyV), and prior immunosuppressive treatments or diseases are risk factors for MCC. The incidence varies between 0,1 – 1,6 /100 000/year in different countries, and the incidence is generally increasing with some exceptions in the Nordic countries (14 - 23).

MCC presents as a pink-red or violet cutaneous nodule, most commonly in the head and neck area or the limbs and is often primarily mistaken for a benign lesion. The acronym AEIOU can be used as a helpful tool in identifying MCC, the letters standing for Asymptomatic, Expanding rapidly, Immunosuppressed, Over 50 years of age and UV-exposure. (26) The definitive diagnosis is made by a histopathological evaluation of a tumour or a tumour biopsy (5). Patients present with a local disease at time of diagnosis in around half of the cases (2, 17, 20, 24).

Generally, the recommended treatment for MCC is a histologically controlled wide margin excision, a sentinel lymph node biopsy and adjuvant radiation therapy of the primary tumour site and in some cases of the nearby lymph node beds as well. In recurring MCC the treatment options are chemotherapy or the relatively new PD-L1/PD-1-inhibitor immunotherapies such as avelumab. (31, 32) The existing studies regarding the benefits of adjuvant radiation therapy in MCC treatment have shown promising results, however there is a lack in high quality randomised controlled trials. (34 - 36)

Although MCCs are rare, the aggressive nature of MCCs and the associated high mortality rates makes for a strong incentive to develop more efficient treatments for MCC. The existing national guidelines for MCC treatment could still be improved and

followed more consistently, which could lead to a more streamlined treatment process for MCC patients, with shorter delays between diagnosis and treatment.

The aims for this study were to analyse the patients treated for Merkel Cell Carcinomas at Helsinki University Hospitals during the years 2010–2018. The baseline patient and tumour characteristics, as well as the given treatments and survival periods were analysed to evaluate the effect of the different variables on patient survival. The main focus was on investigating how the treatment with adjuvant radiotherapy as well as the delay between diagnosis and treatments affects patient survival and disease relapse, while simultaneously analysing how different patient and tumour characteristic affected survival.

2 Literature review

2.1 History

The Merkel Cell Carcinoma was first described in 1972 by Cyril Toker as a trabecular carcinoma of the skin and has since then been known by many names (1, 2). Nowadays, the most commonly used name is Merkel Cell Carcinoma due to its histological resemblance of the Merkel Cells of the skin. It has been theorised that the lesion originates from Merkel Cells, which are tactile epithelial neuroendocrine cells of epidermal origin located in the basal layer of the epidermis. Clusters of Merkel cells together with afferent nerve fibres form mechanoreceptors for touch sensation. (3) However, some studies have challenged the hypothesis of MCC originating from Merkel Cells, and instead offered a hypothesis that MCCs originate from pluripotent stem cells of the basal layer (2, 4).

2.2 Pathogenesis

The pathogenesis of MCC is thought to be either through infection with Merkel Cell Polyoma Virus (MCPyV) or chronic exposure to UV-radiation (2, 5, 6, 12, 14). MCPyV is largely prevalent in the general population, the prevalence increases with age and 60-80% of adult are seroposite for MCPyV. There seem to be no significant clinical symptoms related to MCPyV infection. (44) The connection between MCC and MCPyV was discovered in 2008 when a research group led by professor Patrick Moore discovered the presence of genome from a previously unknown polyomavirus in MCCs and went on to naming this virus the Merkel Cell Polyoma Virus. They further proved that MCPyV was present in a majority (80%) of Merkel Cell Carcinomas, and that MCPyV was present only in 26% of control samples from the skin and 8% of control samples from various tissues. (7) Later similar findings have been made in numerous studies, for example a Finnish study published in 2009 showed that MCPyV was present in 80% of tested MCC samples (8, 9, 10). It has also been shown that the same virus with identical mutations was found both in primary MCC tumours and in metastases, suggesting that its presence is crucial in the initiation of the carcinogenesis (13). However, an Australian study found MCPyV to be present in only 18.3% of Australian MCCs, giving rise to a theory that MCPyV infection is more important in the MCC pathogenesis in areas with little UV-exposure, whereas its significance for the MCC pathogenesis is lower in areas with high

UV-exposure (11). Both MCPyV positive and negative MCCs are thought to be immunogenic. The mutation burden is generally larger in MCPyV negative MCCs due to the UV-damage, with highly recurrent inactivation of tumour suppressor genes, and expression of neoantigens. The mutation burden is lower in MCPyV positive MCCs, and findings suggest that the mutations occur later in the tumour development. The mutations do not affect the same tumour suppressor genes as in MCPyV negative MCC, but instead lead to T antigen expression. Both MCPyV positive and negative MCCs have been treated successfully with immune checkpoint inhibitors. (44)

2.3 Incidence

MCC incidence varies globally from 0.1 to 1.6 per 100 000, with lower incidences recorded in Europe and the highest incidence in Australia (14 - 23). Even lower incidences have been recorded in some countries, but the population coverage in the studies have not been 100% (14). The incidence has generally been increasing globally, with somewhat stable figures only in a few countries (14-23). The incidences and incidence trends of recent years for some countries are presented in Table 1, however the time periods from which incidence data was available varies, as well as the presentation on the incidences in different studies. Noticeably, in Denmark and Norway where the incidences seem to be stable, the available incidence data is fairly old. MCC mainly presents in elderly patients, with a median age at time of diagnosis varying between 75 and 81 years in several studies (15 - 18, 24, 25). Only a small fraction of the cases are reported in patients under 50 years of age (2, 16, 26). Globally MCC incidence is mostly higher in men than in women, however in Finland the numbers are consistently the opposite (2, 15, 18, 20, 24). MCC is more common in patients with fair skin, with Caucasian patients presenting with MCC far more often than Black, Hispanic or Asian patients (15, 26). Around half to three quarters of the patients present with a local disease at time of diagnosis (2, 17, 20, 24).

Country	Earlier time period		More recent time period		Incidence trend	Source
	Years	Incidence / 100 000/ year	Years	Incidence/ 100 000/ year		
The United States	2000	0.5	2013	0.7	Increasing	*Paulson K. 2018* (15)
Sweden	1993	0.11	2012	0.19	Increasing	*Zaar O. 2016* (16)
The Netherlands	1993	0.17	2016	0.59	Increasing	*Uitentuis S. 2019* (17)
Finland	1989 - 2008	0.12	2016	0.40	Increasing	*Kukko H. 2011* (24), *Sahi H. 2020* (18)
Denmark	1995 - 1999	0.22	2000 - 2006	0.22	Stable	*Kaae J. 2010* (22)
Australia (Queensland)	1993	1.0	2006 - 2010	1.6	Increasing	*Youlden D. 2014* (19)
France*	2006	0.24-0.26	2010	0.38-0.43	Increasing	*Fondain M. 2018* (23)
Norway	1993	1.1	2007	1.1	Stable	*Stang A, 2017* (14)
Germany*	1998 - 2000	0.1-0.2	2008 - 2010	0.3-0.4	Increasing	*Eisemann N.2012* (21)

Table 1. MCC incidence and incidence trends in recent years in some countries. *Incidence in men and women reported separately.

2.4 Risk factors and clinical manifestation

Aside from MCPyV infection and UV-radiation exposure, risk factors for MCC are high age, fair skin, haematological malignancies and immunosuppression, either due to infections such as HIV-infection or medically induced immunosuppression for example after organ transplants (2, 15, 26, 29, 30). Other previous cutaneous malignancies are common among MCC patients, however the common denominator for these malignancies and MCC is UV-exposure, so it is generally not thought to signify that previous cutaneous malignancies would create an independent risk for MCC (2).

MCC presents clinically as a red-pink or blue-violet cutaneous or subcutaneous nodule, most commonly solitary, asymptomatic and rapidly growing. It is often mistaken for a benign lesion. (5, 26) The acronym AEIOU was presented in 2008 by Heath et al. to describe the clinical findings associated with MCC, the letters standing for Asymptomatic, Expanding rapidly, Immunosuppression, Older than 50 years and UV-exposure (26). MCCs can appear anywhere on the body, but most often presents in UV-

exposed areas of the skin, most commonly in the head and neck area and second most commonly in the limbs (2, 17, 18, 20, 26).

2.5 Diagnosis

The diagnosis is made based on histopathological findings and the presence of neuroendocrine markers in a tumour biopsy (5). Histologically, MCC cells present as round blue cells in haematoxylin-eosin staining, with hyperchromatic nuclei, very little cytoplasm, and a high mitotic rate. The MCC cells resemble those of other malignancies, such as small cell lung cancer cells, carcinoid tumours and malignant lymphoma, which is why neuroendocrine immunological markers are used in the pathological identification of MCC. MCCs commonly stain positive for CK20 (cytokeratin 20) and NSE (neuron-specific enolase), and stain negative for TTF1 (thyroid transcription factor 1) and LCA (leukocyte common antigen) commonly found in small cell lung carcinoma and lymphomas respectively. (27, 28, 31)

Based on tumour size and spreading at time of diagnosis it is possible to give a stage classification to the disease. This can be done by using the American Joint Committee on Cancer (AJCC) 2018 staging chart (Appendix 1). Most patients present with a stage I or II disease at time of diagnosis. (2, 5, 17, 24)

2.6 Treatment

The recommended treatment for primary MCC is a histologically controlled 1-2 cm margin excision of the primary lesion, sentinel lymph node biopsy (SLNB) or complete lymph node dissection (CLND) at the time of the surgery depending on clinical lymph node status, with adjuvant radiotherapy to the primary location and/or local lymph node beds. In metastatic MCC chemotherapy was the only additional treatment aside from radiation therapy available until 2016, with mostly platinum-based therapeutics being in use. (31, 32) Although the responses to chemotherapy in metastatic MCC have been fairly good, the responses tend to be short lived (31). Due to the immunogenic character of MCC, with immunosuppression presenting as a large risk factor, immunotherapies have been of interest as a possible treatment approach for some time, and since 2016 new immune checkpoint inhibitors have become available as a treatment for metastatic MCC.

The anti-PD-L1-immunotherapy avelumab has been proven to be effective in treatment of chemotherapy-refractory metastatic MCC, or as a first-line treatment of patients with MCC with distant metastases. (39, 40) Avelumab is a human anti-PD-L1 IgG1 monoclonal antibody. It targets PD-L1 and inhibits the PD-L1/PD-1 interactions which leads to inhibition of the inactivation of the T cell. (39, 40)

MCC is a radiosensitive tumour (34, 37). Several studies have found that radiation therapy as an adjuvant treatment to MCC improves both locoregional control and patient over-all and disease-free survival, regardless of excision margin status (34-36). However, radiation therapy is not given to all MCC patients, often because long radiotherapy treatments are strenuous and the patients are usually elderly and may be suffering from other diseases simultaneously. It is possible that hypo-fractionated or single fraction radiation therapy could be beneficial for patients who are not eligible for long radiotherapy regimens. Single fraction radiation therapy has also been shown to be effective in palliative treatment of metastatic MCC. (33) It has also been presented that radiotherapy effects are achieved at least partially through immune system response, and that radiotherapy does not only have an effect on the tumour areas receiving radiotherapy, but that it through these immunological processes can have a beneficial effect on malignant growth in other areas as well (38, 42).

2.7. MCC survival and disease progression

Five-year relative survival of MCC patients varies between around 40 and 70 percent in most literature (5, 16, 19, 24). The survival is generally stage-dependant, with lower survival percentages within higher tumour stages. Agelli et al. showed that the 5-year relative survival for patients with distant metastases at time of diagnosis was only 25%, 59% for patients with regional MCC spreading, and 75% for patients with local disease at time of diagnosis in a study presented in 2003 regarding MCC patients in the US. (6) Kukko et al. presented similar findings in a Finnish study published in 2011, with 5-year relative survival at 69% with stage I disease, 67% with stage II disease and 17% with stage III disease. (24) Recurrence usually occurs within 3 years from primary diagnosis. Metastases most commonly occur in regional lymph nodes, and have also been found in distant lymph nodes, skin, liver, lungs, brain, adrenal glands and bones. (5)

3 Material and methods

3.1 Patient cohort

The patient cohort was compiled by collecting data of all Merkel Cell Carcinomas that had been entered in to the Helsinki University Hospital (HUH) pathology database QPAT during the time period between 1.1.2010 and 31.12.2018. A total of 156 MCC samples from 52 patients were found in the database. 4 out of these 52 patients had received their treatment elsewhere than at HUH, and were thus excluded from the study. Out of the remaining 48 patients one had received neoadjuvant radiotherapy and thus differed significantly from the remaining patient cohort and was therefore excluded from the study.

3.2 Collected data

Of the remaining 47 patients the following information was collected from the patient filing system URANUS: sex, age at time of MCC diagnosis, occurrence of rheumatological or haematological diseases in the patient, previous immunosuppressive or biological drug treatment, previous chemotherapy, detection of metastases at time of diagnosis, whether sentinel lymph node biopsies (SLNB) or complete lymph node dissections (CLND) were done, final surgery date, what adjuvant treatment the patients received for the MCC, radiation therapy start date, radiotherapy dose, length of follow-up period, whether local relapse, lymph node progression or systemic metastases occurred, and whether the patient had died, the time of death and if it was as a consequence of the MCC. The cut-off date was 28th of April 2019.

From the HUH laboratory database Weblab the following data was collected: date of pathological Merkel Cell Carcinoma diagnosis, size of the primary tumour, whether there was lympho-vascular invasion present. In the cases where SLNB had been performed data was collected regarding whether lymph node metastases were diagnosed, and data was also collected regarding if later local relapse, lymph node metastases or systemic metastases were diagnosed by a pathologist until the cut-off date 28.4.2019.

3.3 Statistical analysis

The statistical analysis was outsourced and done using the software NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

In the survival analyses and the relapse analyses logistical regressions were calculated for the following independent variables: radiotherapy to the primary tumour site, radiotherapy to the lymph nodes, tumour location in the head and neck, tumour location in the upper limb, tumour stage at time of diagnosis, patient age at time of diagnosis and tumour size at time of diagnosis. The variables with a p-value < 0.10 were included in a multi variable analysis. Kaplan-Meier analyses were made regarding the complete over-all and disease-specific survival, as well as for the over-all and disease-specific survival of the patients receiving adjuvant radiation therapy compared to those who did not receive radiation therapy. Cox regressions were calculated regarding the effect of delays between diagnosis and treatment on survival, regarding over all survival and regarding survival in the group receiving adjuvant radiation therapy compared to the group not receiving radiation therapy.

All tumours in this study were given a stage classification in accordance with the recommendation by the AJCC (Appendix 1). The staging is based on tumour size, lymph node status and distant metastasising at the time of diagnosis. The lymph node and distant metastases status is categorized as negative or positive either by a clinical exam or by a sample exam by a pathologist.

The patient survival was studied both regarding overall survival and disease specific survival, overall survival indicating that death due to any cause is considered, whereas disease-specific survival only includes patients who died of MCC in the deceased cohort.

4 Results

4.1 Patient and tumour characteristic

The patient and tumour characteristics at baseline are presented in Table 2. 47 patients were included in this study, out of which the majority, 28 patients, were female and 19 were male. The median age at the time of diagnosis was 79 years, ranging from 47 to 102 years. The mean age was 78.7 years. The mean primary tumour size was 20.3 mm, ranging from 3 to 100 mm. Most primary tumours appeared in the head and neck area, with 31 primary tumours in this area. Out of the remaining primary tumours 11 were located in the upper limbs, 3 in the lower limbs, and one on the trunk. There was also one case with no primary tumour.

Characteristic	Value
Patient number (n)	47
Median age at time of diagnosis	79 years (47 - 102)
Gender balance:	
Women	28 (59.6%)
Men	19 (40.4%)
Median tumour size	20.3 mm
Location of primary tumour:	
Head and neck	31 (66.0%)
Upper extremity	11 (23.4%)
Lower extremity	3 (6.4%)
Trunk	1 (2.1%)
Unknown primary tumour	1 (2.1%)

Table 2. Baseline patient and tumour characteristics

In this study the tumours were retrospectively given stage classification as recommended by the AJCC (Appendix 1), presented in Table 3. 24 patients had a stage I clinical or pathological disease at time of diagnosis, 9 patients had a stage IIA clinical or pathological disease, 1 patient had a stage IIB clinical disease, and 12 patients had a stage III clinical or pathological disease at time of diagnosis. Only 1 patient had a stage IV clinical disease at the time of diagnosis. In Table 3 there is also included the number of patients in each stage group receiving adjuvant radiation therapy and the share of the patients of the group receiving radiation therapy stated in percentages as well.

Tumour stage	Number of patients		Adjuvant RT
	N = 47	%	N = 28
I Clinical	10	21.3	6
I Pathological	14	29.8	10
IIA Clinical	4	8.5	2
IIA Pathological	5	10.6	4
IIB Clinical	1	2.1	0
IIB Pathological	0	0.0	0
III Clinical	4	8.5	0
IIIA Pathological	3	6.4	2
IIIB Pathological	5	10.6	4
IV Clinical	1	2.1	0
IV Pathological	0	0.0	0

Table 3. Tumour stage at time of diagnosis for all patients and number of patients in each stage group receiving adjuvant radiation therapy.

4.2 Treatments and treatment delays

The treatments given to the patients are presented in Table 4. Out of the 47 patients in this study 43 had a re-excision after the primary diagnostic excision, 35 of these with the margin status defined as sufficient by a pathologist. The remaining 8 patients who had a surgical re-excision either had positive resection margins (2 patients) or insufficient margins (6 patients). 4 patients did not have a surgical re-excision of the primary tumour after the primary biopsy.

22 patients had a sentinel lymph node biopsy. 4 patients had a positive sentinel lymph node biopsy (SLNB) and went on to have a complete lymph node dissection (CLND) but only one of these patients had a positive result in the CLND as well. Two patients had a CLND without a prior SLNB, and in both cases with a positive finding.

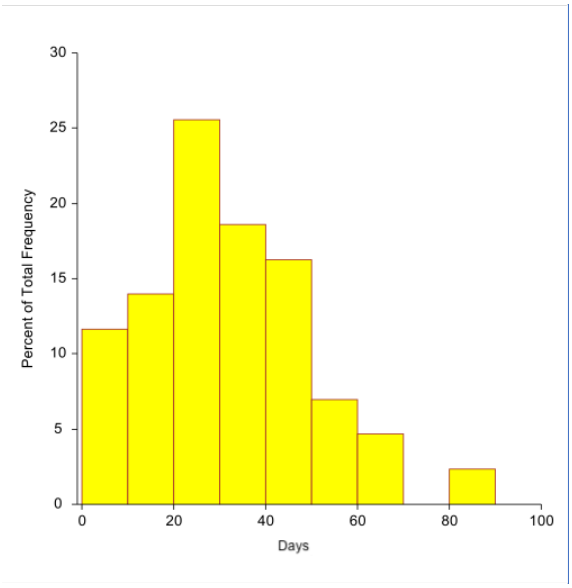
28 patients received adjuvant radiation therapy (RT). 27 patients received adjuvant RT to the primary tumour site. 15 out of these patients received radiation therapy only to the primary tumour site and 12 patients received radiation therapy both to the primary tumour location and to the proximal lymph node beds. One patient received radiation therapy only to the nearby lymph node area. 89.6% of the patients receiving adjuvant RT received

a hyper fractionated RT regimen, with a total radiation dose between 45 and 66 Gray (Gy) divided into 2 or 2.5 Gy doses. The most common primary tumour area RT dose was 50 Gy divided into 2 Gy doses, given to 9 out of the 26 patients receiving RT to the primary tumour location. The second most common dose was 60 Gy divided into 2 Gy doses, given to 7 patients. 3 patients received 56 Gy divided into 2 Gy doses.
Only one patient received chemotherapy, in this case a combination of carboplatine and etoposide. One patient was treated with the immunotherapeutic drug avelumab.

Treatment	**Number of patients**	
	N = 47	**% of all**
No surgical re-excision	4	8.5
Surgical re-excision	43	91.5
Re-excision with negative margins	35	74.5
Re-excision with insufficient margins	6	12.8
Re-excision with positive margins	2	4.3
SLNB	22	46.8
CLND	6	12.8
Any adjuvant RT	28	59.6
Only primary location RT	15	31.9
Primary location + lymph node area RT	12	25.5
Only lymph node area RT	1	2.1
45-66 Gy / 2-2.5 Gy	25	53.2
Chemotherapy	1	2.1
Immunological therapy	1	2.1

Table 4. Patient treatments. RT = radiation therapy.

The mean delay between histological diagnosis from the tumour biopsy to the definitive wide-margin re-excision was 32.4 days, ranging from 1 to 83 days. 50% of the patients had their re-excision within 30 days from the time of diagnosis (Figure 1).
The mean delay between definitive re-excision and first RT dose was 99.1 days, ranging from 49 to 292 days. 50% of the patients received their first dose of RT within 83.5 days from their definitive surgery (Figure 2). The main reasons for the delays in RT initiation were hospital resource factors and wound healing problems. Two patients had a significantly longer delay until RT start (283 and 292 days).



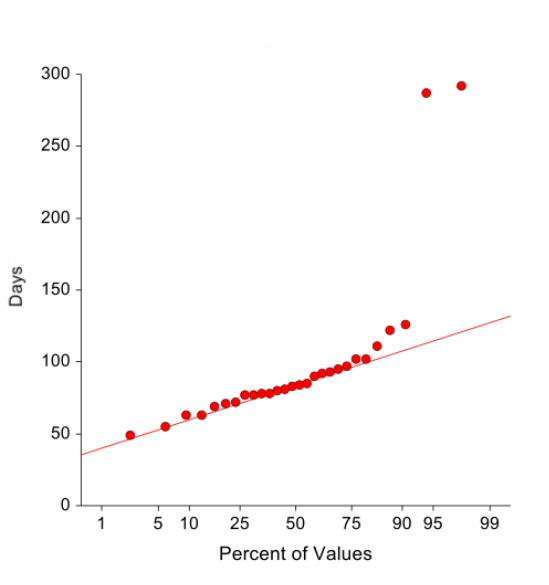


Figure 1. Frequency of delays between diagnosis and re-excision. *Figure 2. Distribution of delays between re-excision and RT start.*

The time between diagnosis and re-excision and re-excision and adjuvant radiotherapy was analysed through cox-regressions regarding the effect of the delay on patient over-all and disease-specific survival, however no significant p-values were achieved (Table 5).

		DSS			OS		
Variable	Mean (days)	Risk Ratio	P-value	Pseudo R^2	Risk Ratio	P-value	Pseudo R^2
Re-excision delay	32.4	1.0006	0.976	0.0001	1.0048	0.755	0.0075
RT delay	99.1	1.0062	0.254	0.2454	1.0042	0.379	0.0992

Table 5. Re-excision and RT delay effect on disease-specific (DSS) and over-all (OS) survival.

4.3 Over-all and disease-specific survival

Out of the 47 patients in this study, 19 had died at the cut-off date of 28th of April 2019. The mean over-all survival time from time of histological diagnosis for all patients was 887 days, ranging from 20 to 2987 days.

The survival time from time of diagnosis for the deceased patients ranged from 20 to 1822 days. Of the 28 patients still alive at cut-off the survival time at cut-off varied from 122 to 2987 days from diagnosis. This makes for an over-all survival of 59.6%. Kaplan-Meier analyses were made regarding the over-all and disease-specific survival of the patients. The calculated linear over-all survival at 1065 days or approximately three years was 55.4

% (38.7% - 72.1%). (Figure 3) The Kaplan-Meier over-all survival plot is illustrated in Figure 3, and the over-all hazard function plot is shown in Figure 4. These figures illustrate that the highest risk of death of any cause occurs within approximately the first two years from the MCC diagnosis.

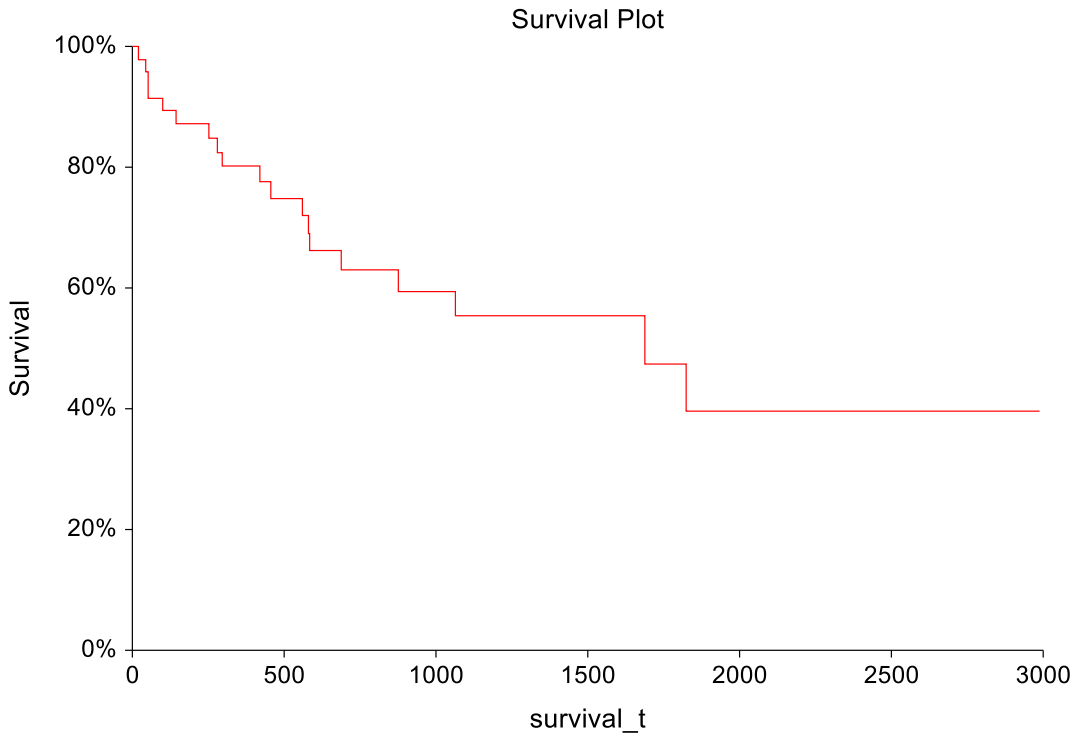


Figure 3. Kaplan-Meier plot for over-all survival for all patients. Survival time in days since time of diagnosis are shown on the x-axis, survival in percentages on the y-axis.

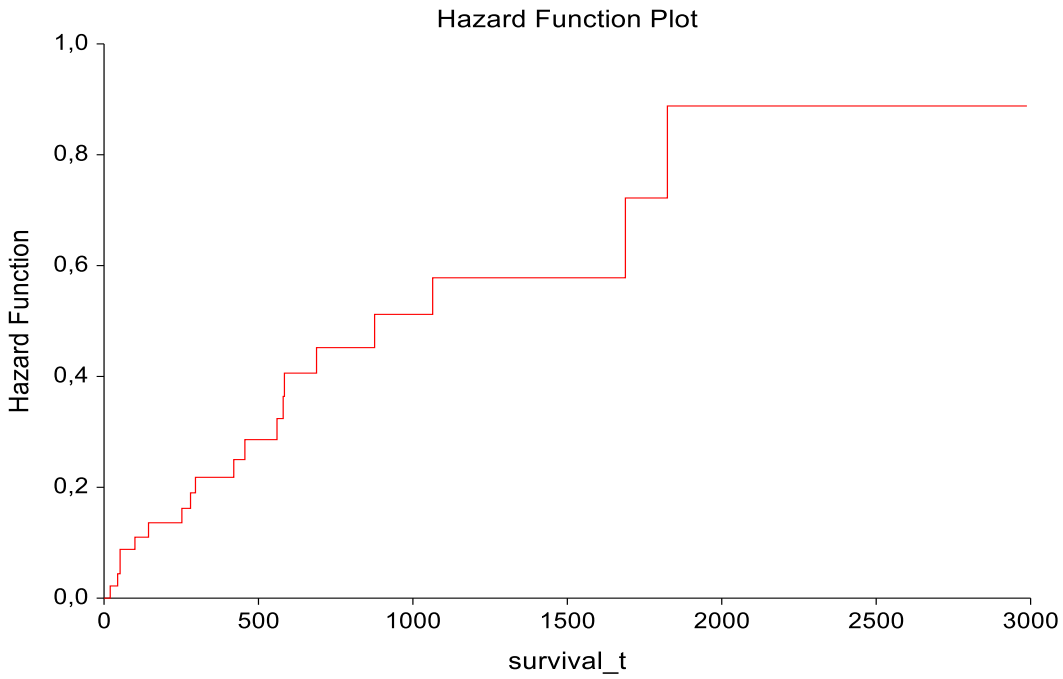


Figure 4. Hazard function plot for over-all survival for all patients. Survival time in days since time of diagnosis is shown on the x-axis, the hazard function on the y-axis.

Out of the patients that had died at the cut-off time on 28th of April 2019, 13 had died due to MCC, whereas 6 had died due to other causes. This gives a disease-specific survival of 72.3% at cut-off for all patients, not regarding time since diagnosis. Out of the people who died as a consequence of MCC during the follow-up time, the person who survived the longest lived for 1065 days. The calculated disease-specific survival at 1065 days or approximately 3 years from diagnosis was 64.7% (47.9 – 81.5%), which is illustrated in the survival plot in Figure 5. Of the people who were alive at cut-off time the person who has survived the longest had been alive for 2987 days at the cut-off date. From the Hazard function analysis (Figure 6) it can be seen that the highest disease-specific mortality risk is within the first year from diagnosis.

Out of the 47 patients in this study, a minimum of two years had passed from the time of diagnosis to the cut-off date of this study for 35 of the patients. Out of these patients 15 had passed away within two years of follow up, giving a real world 2-year over-all survival of 57.1%. 11 out of the 35 patients died of MCC, and 4 patients died of other causes, the 2-year disease specific survival being 68.6% for this patient cohort.

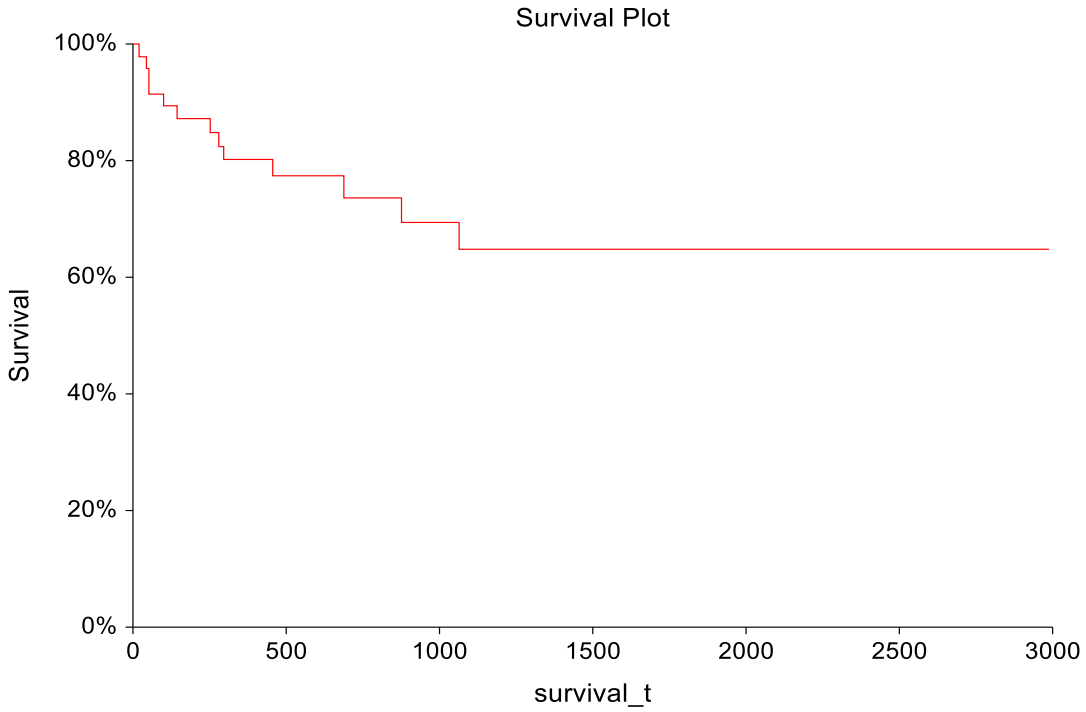


Figure 5. Disease-specific survival plot. The survival time in days from time of diagnosis is plotted on the x-axis, the survival percentages on the y-axis.

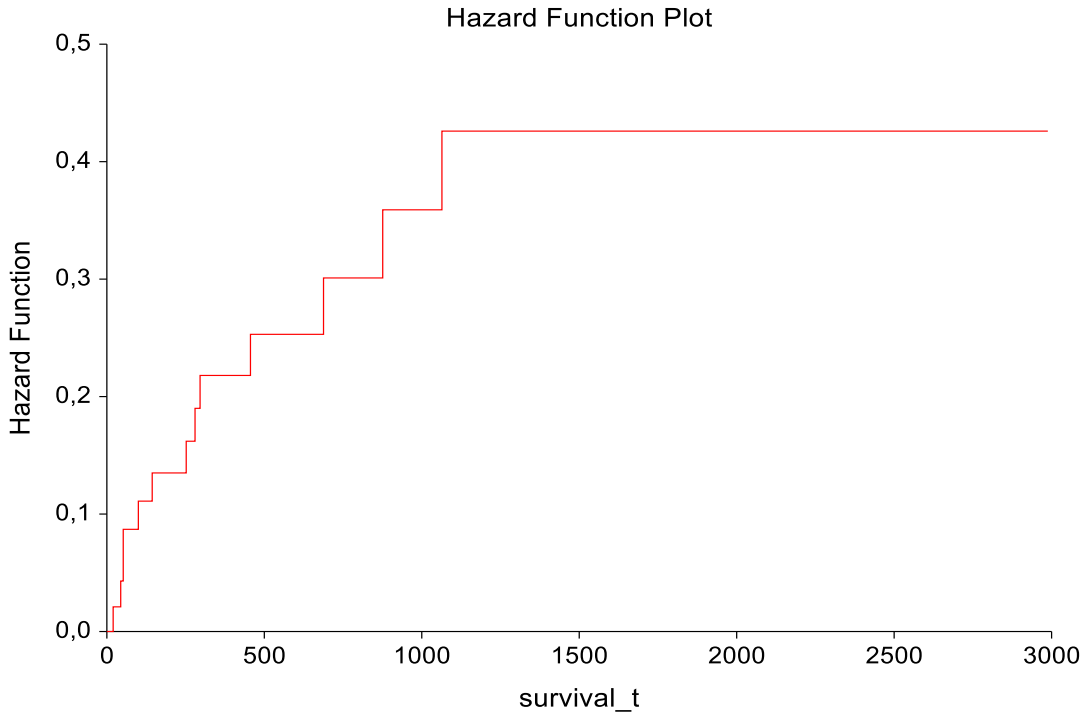


Figure 6. Hazard function plot for disease specific survival. The survival time in days since diagnoss is plotted on the x-axis, the hazard function on the y-axis.

4.4 Disease progression

Out of the 47 patients included in this study, reliable information regarding disease progression during the study period could be found for 45 patients. 22 patients suffered either local disease progression, nodal progression, systemic metastases or a combination of these during the follow-up period. 15 patients out of the 45 patients (33.3%) suffered a local disease relapse during the time period, 18 patients (40.0%) had nodal progression during the time period, and 14 patients (31.1%) were diagnosed with a systemic metastasis during the follow up time. 10 of the patients with systemic metastasis were also among the patients with recorded local relapse and nodal progression.

	Effect on nodal progression			**Effect on systemic metastases**		
Variable	**Odds ratio (OR)**	**95% CI**	**Wald P-value**	**Odds ratio (OR)**	**95% CI**	**Wald P-value**
Tumour size	1.11	1.02-1.19	0.011	-	-	-
Tumour stage	-	-	-	1.72	1.15-2.59	0.009
Adjuvant RT	0.19	0.04-0.83	0.028	0.20	0.04-0.95	0.043

Table 6. Growing tumour size, tumour stage and adjuvant radiation therapy (RT) effect on nodal progression and metastases.

Regression analyses on the effect of independent variables on disease progression were made, however no independent variable reached statistical significance regarding local disease progression. Regarding nodal progression the effect of the independent variables ‘radiotherapy of the primary tumour area’ and ‘tumour size’ reached statistical significance, and the analysis shows that the likelihood for nodal progression increases marginally with increasing primary tumour size, whereas the likelihood for nodal progression decreases with adjuvant radiotherapy to the primary tumour area. (Table 6) For systemic metastases, the variables ‘adjuvant radiotherapy of the primary tumour location’ and ‘tumour stage’ were significant regarding patient outcome. Higher tumour stage correlated with a higher likelihood of metastases, whereas adjuvant radiotherapy to the primary tumour correlated with at lower likelihood of metastases (Table 6).

4.5 Baseline characteristics and treatment effects on survival

For the survival analyses regression equations and p-values for single variables were calculated through Cox regressions. The variables with $p < 0.10$ were included in a multi

variable analysis. The variables included were 'adjuvant RT to the primary tumour location', 'adjuvant RT to the proximal lymph nodes', 'tumour location in the head and neck area', 'tumour location in the upper limb', 'tumour size', 'tumour stage' and 'patient age at time of diagnosis'. Out of these variables 'adjuvant RT to the primary tumour location', 'tumour size', 'tumour stage' and 'patient age at time of diagnosis' remained significant in a multivariable analysis for over-all survival, and thus seem to be solitary risk factors. As seen in Table 7, a bigger tumour size, higher patient age and higher tumour stage are all independently associated with a lower over-all survival, whereas receiving adjuvant RT is associated with a higher survival. For the disease-specific survival, the variables 'RT to the primary tumour location', 'tumour size' and 'tumour stage' remained significant in the multi-variable analysis. The findings are presented in Table 7. Once again, a higher risk ratio is associated with bigger tumour size and higher tumour stage, whereas a lower risk ratio is associated with receiving RT.

		Effect on OS				Effect on DSS			
Variable	**Mean**	**Risk ratio (95% CI)**	**P-value**	**Pseudo R^2**	**χ^2**	**Risk ratio (95% CI)**	**P-value**	**Pseudo R^2**	**χ^2**
Tumour size	20.28	1.09 (1.03 – 1.15)	0.002	0.402	15.1	1.08 (1.02 – 1.14)	0.008	0.410	10.1
Patient age	78.96	1.06 (1.01 – 1.12)	0.025	0.252	5.4	-	-	-	
Tumour stage	2.28†	1.49 (1.02 – 2.18)	0.041	0.217	4.1	1.41 (1.01 – 1.97)	0.046	0.285	3.8
RT	0.54*	0.19 (0.06 – 0.64)	0.007	0.325	8.2	0.12 (0.03 – 0.50)	0.004	0.460	10.7

Table 7. Tumour size, patient age, tumour stage and RT effect on over-all survival (OS) and disease-specific survival (DSS).
*RT = adjuvant radiotherapy to primary tumour. * 0 = no RT, 1 = RT. † Tumour stage I = 1, IIA = 2, IIB = 3, III = 4, IIIA = 5, IIIB = 6, IV = 7.*

4.5 Adjuvant radiation therapy effect on survival

The effect of adjuvant radiation therapy on survival compared to no radiation therapy was analysed further. All patients receiving RT either to the primary location or to associated lymph node beds were included in the RT-group. The groups were compared with several different tests, the Logrank test gave a χ^2 value of 4.253, and a p-value of 0.039.

The Kaplan-Meier analysis of over-all survival (OS) comparing the two groups is presented in Figure 11. The survival at three-years from diagnosis for patients receiving radiotherapy was 66.5% (45.4 – 87.6%, 95% CI), whereas the three-year survival for patients who did not receive radiotherapy was 40.3% (15.5 – 65.1%, 95% CI). The survival was constantly higher among patients receiving adjuvant radiotherapy.

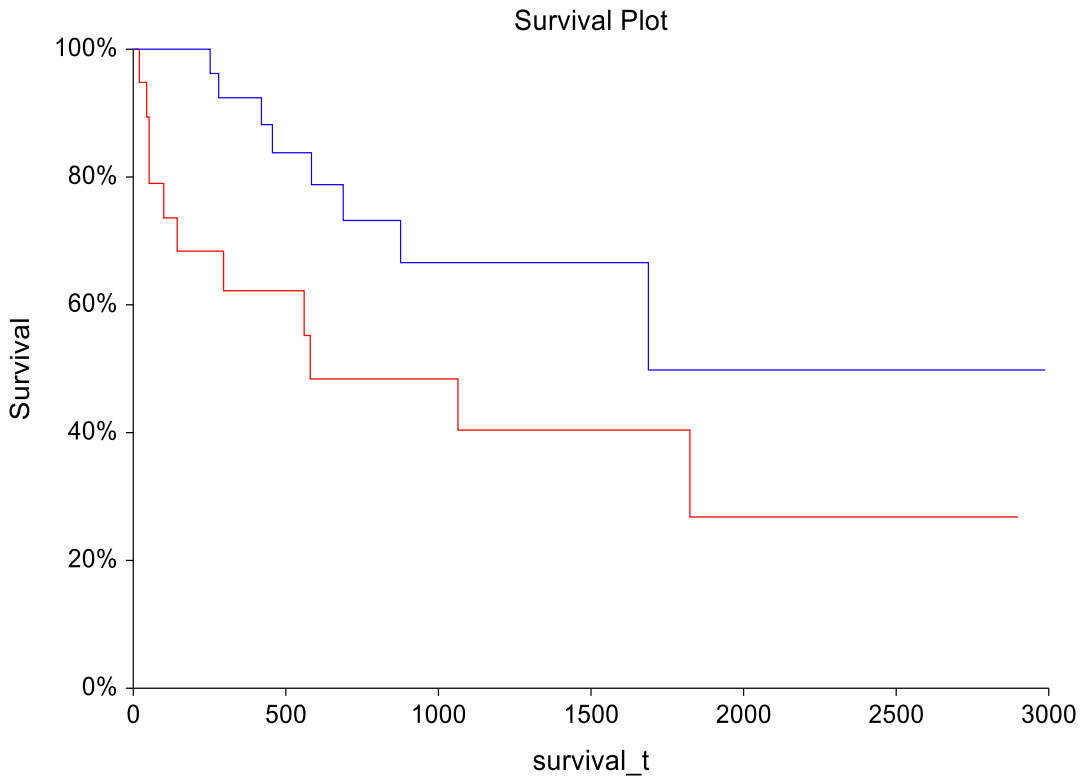


Figure 11. Survival plot comparing OS in patients receiving adjuvant RT (blue) to patients who did not receive adjuvant RT (red). Survival time in days since diagnosis are plotted on the x-axis, survival in percentages on the y-axis.

The Cox-Mantel hazard ratio for no RT compared to RT was 2.51. Hazard functions over survival time were calculated for both the RT and the no RT groups and are presented in Figure 12. The figure shows that the risk for death or disease progression is constantly higher in the group that did not receive adjuvant radiotherapy.

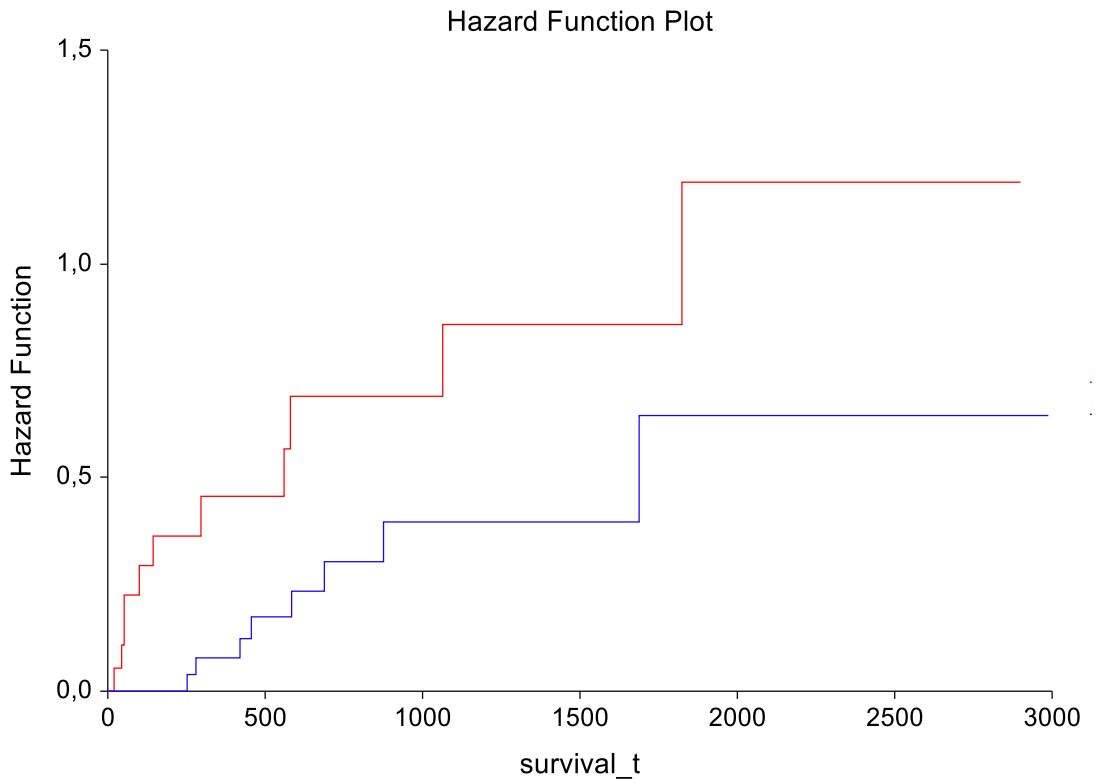


Figure 12. Over-all hazard function plot comparing patients who received adjuvant RT (blue) to patients who did not receive RT (red). Survival time in days since diagnosis are plotted on the x-axis, the hazard function is plotted on the y-axis.

The disease-specific survival (DSS) and hazard was also compared between the group that received adjuvant RT and the group that did not receive adjuvant RT. The groups were also compared with a Logrank test, providing a χ^2 of 4.048 and a p-value of 0.044. The survival plot (Figure 13) shows a disease-specific survival advantage for the patient group receiving adjuvant RT. The three-year disease-specific survival for the group that did not receive RT was 51.8% (25.6 – 78.1%, 95% C.I.), and the three-year survival for the RT-group was 74.0% (53.3 – 94.8%, 95% C.I.).

The disease-specific Cox-Mantel Hazard Ratio between the group who did not receive adjuvant RT and the group who received adjuvant RT was 2.97. Figure 14 illustrates the Hazard Functions over survival time for the RT-group and the no-RT-group, and it shows that the risk for disease progression or death is constantly higher in the no-RT-group.

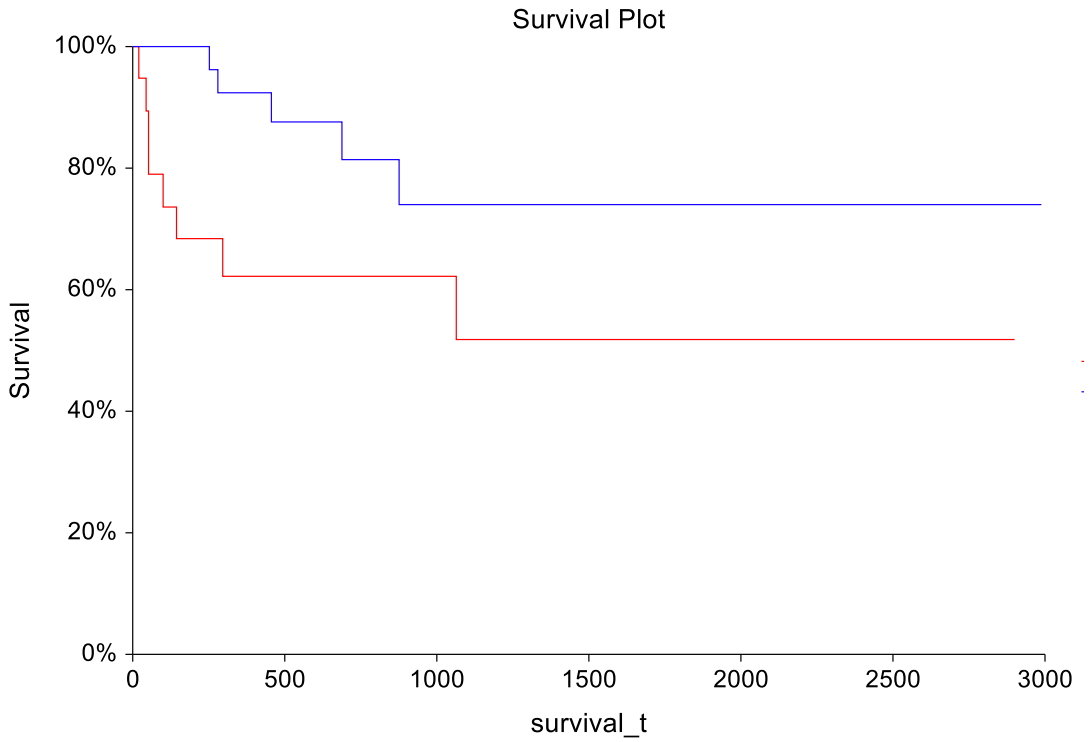


Figure 13. Survival plot comparing DSS in patients receiving RT (blue) to patients who did not receive RT (red). Survival time in days since diagnosis are plotted on the x-axis, survival in percentages on the y-axis.

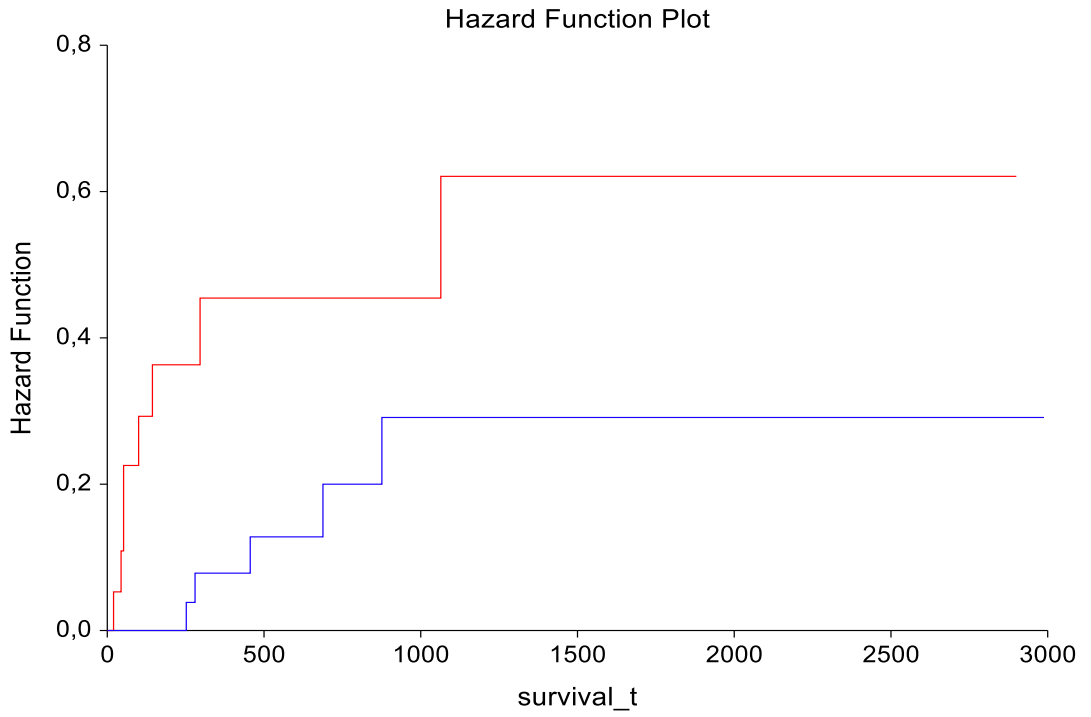


Figure 14. Disease-specific hazard function plot comparing patients who received RT (blue) to patients who did not receive RT (red). Survival time in days since diagnosis are plotted on the x-axis, the hazard function is plotted on the y-axis.

5 Discussion

In this study MCC patients at Helsinki University Hospital diagnosed in the time period between 1.1.2010 and 31.12.2018 were analysed regarding their baseline characteristics, MCC treatments, disease recurrence and survival. The findings of this study suggest that there is a survival advantage for patients with small tumour size and low tumour stage at time of diagnosis, for patients of younger age, as well as a statistically significant survival advantage for patients receiving adjuvant radiation therapy.

The main restriction for this study, as for many MCC studies, is the small patient cohort. This is mainly as a result of the rarity of MCC. The patient cohort was also collected during a number of years, which gives large variety in the follow-up time, since the earliest diagnoses included in this study were made in 2010 and the latest in 2018. The patient with the most recent time of diagnosis was only followed up for 122 days before the cut-off date, and thus the relapse occurrence and survival times regarding these more recent cases is not completely comparable to those who have had their MCC diagnosis for a longer time.

An advantage for this study is the consistent documentation of the patient findings and characteristics, making them very easily comparable. The reliability of the data collected is generally good, with a consistency in the recordings. Still, some data, mainly regarding treatment and disease progression, had not been recorded conclusively for all patients, which might have affected the outcome.

The baseline characteristics of the patients were mainly in line with findings from previous studies, with a median patient age of 79 years at time of diagnosis, mostly stage I-II diseases being recorded, and most tumours occurring in the head and neck area and the limbs (2, 15-18, 24, 25). However, whereas in most MCC studies there are more male than female patients, in this study the majority of patients were women (60:40). This is a common distinction for MCC patients in Finland, and similar findings have been recorded in previous Finnish studies. (2, 15, 18, 20, 24) Data collected regarding some patient characteristics, mainly regarding co-morbidities and previous immunosuppressive

treatments, were not included in the statistical analyses due to the insecurity of the coverage of the information available, and thus not included in the study.

The small patient cohort provided some challenges in analysing independent variable effect on disease progression and survival. Only a few independent variables remained statistically significant in the statistical analyses, and thus the impact on disease progression and survival of some characteristics could not be determined in this study. However, some variables remained significant in a majority of the calculations, and thus this study managed to provide significant data regarding the effects of adjuvant radiation therapy, tumour size and stage and patient age effect on disease progression and patient mortality. The beneficial survival effects of small tumour size, low stage and low age have been shown in various earlier studies, and the beneficial effects of adjuvant radiation therapy has been presented in some previous studies as well. (2, 19, 24, 34-36, 41)

The varying follow-up times for the patients provided some difficulties in calculating survival times, and only 35 patients (74.4%) had been followed up for a minimum of two years at the cut of date, giving a real world two-year disease specific survival of 68.6% for this patient group. The survival analyses giving calculated three-year survival data showed that MCC survival at HUH has not significantly improved during the last decade. The calculated over-all three-year survival of the patients in this study was 59.6%, with a disease-specific three-year survival of 72.3%. The difference between the disease specific survival and over-all survival is concordant with the fact that MCC is a cancer of the elderly, and patients are lost during follow-up to other causes than MCC.

As shown in the survival plots, the mortality among the patient group who did not receive adjuvant RT is high during the first year from diagnosis, whereas there are no mortalities during the first year within the group receiving adjuvant RT. This suggests that the patients who received RT were healthier to begin with, and that perhaps that some of the patients in the no adjuvant RT group died before RT could have been administered. A more reliable way of analysing the survival would have been to start the survival follow-up for example 100 days from the time of diagnosis. The baseline characteristics of the patients such as age, co-morbidities and MCC disease characteristics could not be taken into consideration in the survival analysis since the patient cohort was so small, so the

possibility that a selection bias could explain the survival advantage among the group receiving adjuvant RT should be taken into consideration.

Even though the recommended primary treatment combination for MCC is histologically controlled wide-margin resection of the primary tumour, SLNB and adjuvant radiotherapy, not nearly all the patients in this study received this treatment combination (32). Although almost all patients (43 out of 47) had a re-excision, only 24 out of 47 patients had a SLNB or CLND or both, and only 28 patients received adjuvant radiation therapy to the primary tumour, nearby lymph nodes or both. The reasons for this probably vary. In rare cancers such as MCC proper treatment guidelines might not be established or known to all clinicians, but at HUH hospitals the MCC treatment guidelines are well established and therefore a lack of proper guidelines is not likely to be the explanation for the varying treatment schemes. The fact that MCC patients often are elderly might play a bigger part, since additional treatments might be strenuous and thus not considered for patients who are frailer. It has been suggested that hypo-fractionated or even single-fraction radiotherapy regimens could be beneficial at least as a palliative treatment for patients that are not eligible for long radiotherapy treatments (33). These shorter RT regimens are currently mainly used as palliative MCC treatments at HUH, but in the future they might be implemented more into the primary adjuvant treatment plans if the benefits are proved in future studies.

Similar to this study, a fairly recently published MCC overview in Clinical Oncology described several studies showing clear advantages on local control and survival for stage I and II MCC patients receiving both surgery and adjuvant radiotherapy to the primary tumour. Radical radiotherapy alone is generally not preferable compared to radical surgery, even though these findings might not be conclusive since patient characteristics have not taken into consideration in the existing studies. (43) There is a possibility of a selection bias being present in this study as well as in other studies with similar findings regarding RT benefits, because as stated previously adjuvant RT is more commonly given to healthier and younger individuals. Although the findings in this study suggest that both over-all and disease-specific survival improves when patients are treated with adjuvant radiotherapy, the results might have been reached through a selection bias. Since the survival analyses do not take into consideration any patient and tumour characteristics, it may be that the patients treated with adjuvant RT are younger, have a lower tumour stage

or are generally healthier than the patients who did not receive RT. There is clearly a need for more conclusive trials regarding the benefits of adjuvant radiation therapy in the treatment of both local and metastatic MCC.

Out of the 47 patients in this study, only one patient was treated with chemotherapy for metastatic MCC, even though 14 patients were diagnosed with systemic metastases during the follow-up period, and a few more with either local relapses or nodal progression. Even though clinically significant response rates to chemotherapy as a treatment for metastatic MCC is fairly good, 20-61% across various studies, the responses have a tendency to be short-lived. (5, 31)

One patient in the cohort was treated for metastatic MCC with the immunotherapy avelumab. The benefits of avelumab were first presented in 2016 with findings from clinical trials. (39) Avelumab had only been available at HUH for a short time when the data for this study was collected, which probably explains why only one patient received the immunotherapy. In the future it is quite possible that more patients will be treated with avelumab or other immunotherapies since studies on the effect of treatments with PD-L1-inhibitors in MCC have presented very promising results. (39, 40)

The mean delay between diagnosis and surgical re-excision was 32.4 days, with 50% of patients having their re-excision within 30 days from time of diagnosis. The mean delay between re-excision and radiation therapy initiation was 99.1 days. There were two outliers shifting this curve slightly, but still the earliest RT initiation was on 49 days from the re-excision, and only 50% of patients begun their RT treatment within 83.5 days from re-excision. The delay from re-excision to RT initiation was fairly long, but no statistically significant effect of longer intervention delays on survival could be shown, as was also the case for the effect of the delay between diagnosis and surgical re-excision. The small patient cohort might explain the lack of statistically significant results, and larger studies could possibly give valuable additional insight regarding this.

The results from this study show that Merkel Cell Carcinoma is still a very aggressive and lethal cancer. The treatments given still vary largely between patients, and the national and European guidelines are not always followed in patient treatment. A statistically significant survival advantage could be seen in the patient group receiving

adjuvant radiation therapy, however it can not be ruled out that this advantage is due to a selection bias. In concordance with previous studies this study suggests that a smaller primary tumour and low tumour stage correlate with a higher patient survival. It should still be stated that a possible improvement in survival could be achieved by treating patients with adjuvant radiation therapy, and the possible advantage of giving shorter hypo-fractionated or single-fraction radiotherapy to frailer patients is something that should be studied further in the future.

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Appendix

Stage	Primary Tumour	Lymph node	Metastasis
0	In situ (within epidermis only)	No regional lymph node metastasis	No distant metastasis
I Clinical	≤ 2 cm maximum tumour dimension	Nodes negative by clinical exam (no pathological exam performed)	No distant metastasis
I Pathological	≤ 2 cm maximum tumour dimension	Nodes negative by pathological exam	No distant metastasis
IIA Clinical	> 2 cm tumour dimension	Nodes negative by clinical exam (no pathological exam performed)	No distant metastasis
IIA Pathological	> 2 cm tumour dimension	Nodes negative by pathological exam	No distant metastasis
IIB Clinical	Primary tumour invades bone, muscle, fascia or cartilage	Nodes negative by clinical exam (no pathological exam performed)	No distant metastasis
IIB Pathological	Primary tumour invades bone, muscle, fascia or cartilage	Nodes negative by pathological exam	No distant metastasis
III Clinical	Any size / depth tumour	Nodes positive by clinical exam (no pathological exam performed)	No distant metastasis
IIIA Pathological	Any size / depth tumour	Nodes positive by pathological exam only (nodal diseade not apparent on clinical exam)	No distant metastasis
	Not detected (“unknown primary”)	Nodes positive by clinical exam, and confirmed via pathological exam	No distant metastasis
IIIB Pathological	Any size / depth tumour	Nodes positive by clinical exam, and confirmed via pathological exam OR in-transit metastasis	No distant metastasis
IV Clincal	Any	+/- regional nodal involvement	Distant metastasis detected via clinical exam
IV Pathological	Any	+/- regional nodal involvement	Distant metastasis confirmed via pathological exam

Table 2. AJCC MCC-classification