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Basic and clinical features of cutaneous squamous cell carcinoma in organ transplant recipients

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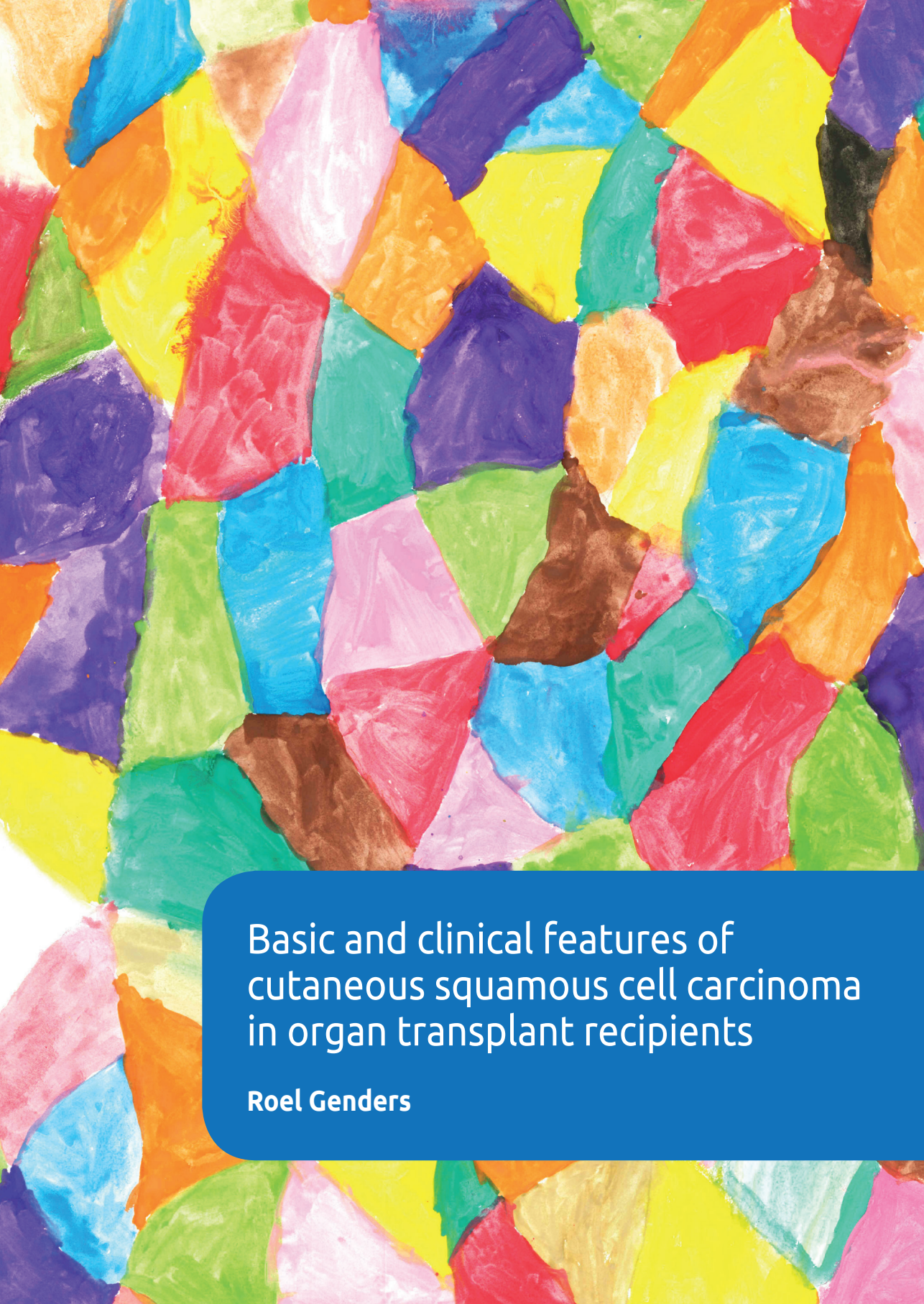


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Roel Genders

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CHAPTER 1

General introduction

Cutaneous squamous cell carcinoma in the general population

Cutaneous squamous cell carcinoma (cSCC) is a skin cancer originating from keratinocytes. It is the second most frequent form of keratinocyte carcinoma (KC) after basal cell carcinoma (BCC) and contributes to approximately 20% of all KC.[1,2] SCC is a common malignancy. In the Netherlands a significant increase was reported of the European Standardised Rates (ESR) from 22.2–35.4 per 100,000 inhabitants for males and from 7.9 to 20.5 for females between 1989 and 2008. In 2017, approximately 14700 individuals developed a cSCC in the Netherlands. (https://www.volksgezondheidenzorg.info/search/site/huidkanker).[3] The number of newly diagnosed patients may exceed 11,000 by 2020.[4,5] The incidence of cSCC varies globally, with a higher incidence in whites living closer to the equator. A systematic review of 19 studies examining incidence trends of cSCC in white populations showed geographic variation with the highest incidence rates in the United Kingdom (31.7 per 100,000 person-years) and Switzerland (28.9 per 100,000 person-years) and the lowest in Croatia (8.9 per 100,000 person-years).[6] In Australia, these numbers are reported to be 1,035 for men and 472 for women per 100,000 person years, respectively.[7,8] With a lifetime incidence of cSCC between 7 and 14%, cSCC is considered a major health problem for the Caucasian population at high costs.[9] The mortality from cSCC is likely to be underreported and in the USA may approach mortality rates in melanoma.[10] In the Netherlands, however the death rate among patients with cSCC is approximately 6 to 8 times lower than the death rate of melanoma.[3]

Cutaneous squamous cell carcinoma in organ transplant recipients

Organ transplant recipients (OTR) are lifelong immunosuppressed and experience a high burden of actinic keratoses and cSCC.[11,12] The incidence of cSCC in OTR is 60–250 times increased compared to the immunocompetent population.[12-15] In OTR, cSCC are more frequently observed than BCC, with a cSCC/BCC ratio of 5:1 compared with the general population where a cSCC/BCC ratio of 1:4 is found.[16] Twenty to 75% of solid organ transplant recipients develop at least 1 cSCC within 20 years after transplantation.[17,18] After a first invasive cSCC, within 3 years, multiple subsequent cSCC will develop in 60 to 80%.[19,20]

Because of this burden of numerous cSCC (Figure 1), the management of skin cancer in OTR requires a dedicated transplant dermatology clinic with easy access to



Figure 1. Illustrative organ transplant recipient with multiple cSCC.

dermatologic care and more frequent surveillance for high-risk patients. In the Leiden University Medical Center, there is a specialized clinic for skin cancer screening and treatment of OTR for more than 25 years. Intervention with regular skin screening examinations may lessen morbidity associated with advanced skin cancer and improve overall quality of life post transplantation.[21]

Risk factors for cutaneous squamous cell carcinoma

The pathogenesis of cSCC is multifactorial and includes synergistic interplay between the carcinogenic effects of ultraviolet (UV) radiation, human papillomavirus infection, host genetic susceptibility factors and compromised immune surveillance (Figure 2). Other risk factors for cSCC are male gender, older age, smoking, exposure to ionizing radiation and exposure to arsenic. There is also a higher risk for developing cSCC in scars, burn scars and chronic ulcers or inflammation.[22-24]

Ultraviolet radiation

UV radiation, particularly among fair-skinned persons, is the primary risk factor for cSCC development.[25] Cumulative or occupational sun exposure during lifetime is related to developing cSCC.[23] UV radiation appears to have dual effects in mutagenesis and immune suppression as well.[26]

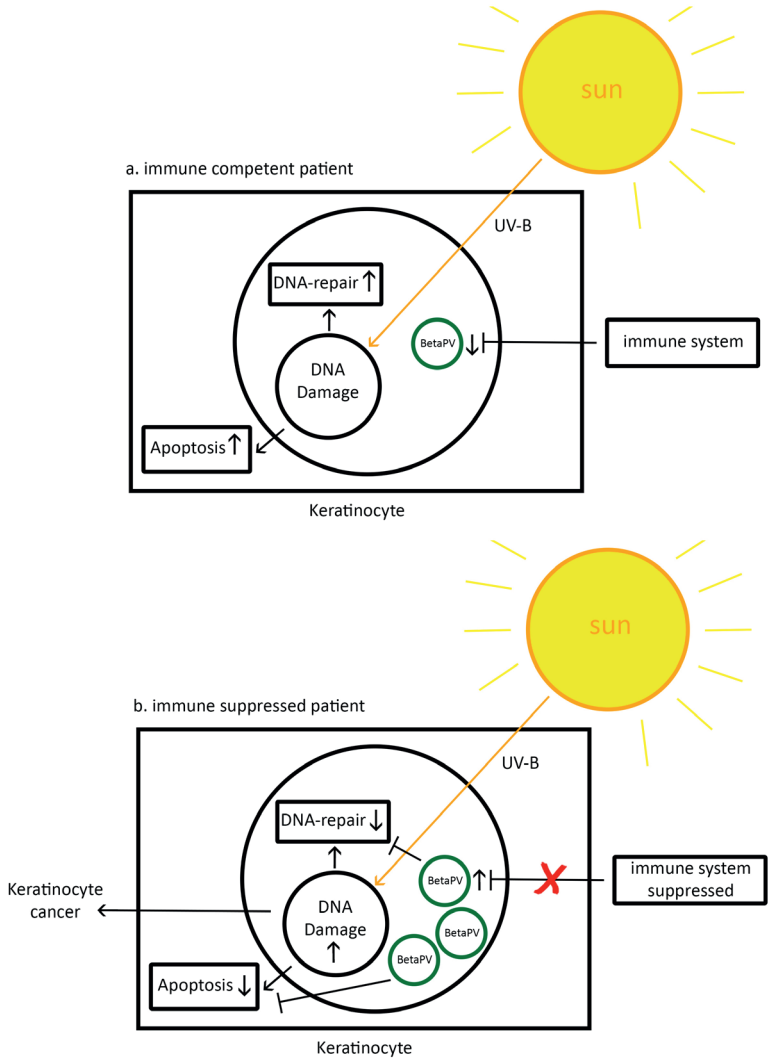


Figure 2. Proposed mechanism for Beta-PV infection and keratinocyte carcinoma development. In immunocompetent individuals (a), Beta-PV infections are suppressed by the immune system. In this case, only low levels of Beta-PV protein will be present to interact with host cell proteins involved in DNA damage repair and apoptosis, and genotoxic damage imposed by UV radiation will be managed adequately. In the presence of immunosuppression, however (b), productive Beta-PV infection occurs and sufficient amounts of Beta-PV protein, particularly E6, are expressed, inhibiting DNA damage repair mechanisms and apoptosis. As a result, genomic instability develops in the infected keratinocytes, which may lead ultimately to the development of keratinocyte carcinoma. Adapted from J Pathol. [26]

The molecular events taking place in cSCC carcinogenesis are most likely heterogenic, and current data argue for a multi-step process frequently involving UV-induced p53 mutations.[27] UV radiation is a prevailing factor implicated in the etiology of actinic keratoses and cSCC as evidenced by the high frequency of UV-related mutations in both the p53 and p16 tumor suppressor genes.[28,29] Besides UV radiation, immunosuppressive treatment and human papillomavirus (HPV) also are considered risk factors for the enhanced cutaneous carcinogenesis.[30-34]

Immunosuppression

Immunosuppression is an important risk factor for cSCC. Patients with hematologic malignancies, HIV or chronic diseases on immunosuppressive drugs are to a certain extent immunosuppressed and carry a higher risk for cSCC.[36-38]

In OTR, the long-term immunosuppressive therapy is an important risk factor for the development of cSCC. Higher risk is associated with older age at transplantation, longer time after transplantation and more sun exposure before the transplantation.[39] Available data suggest that cSCC burden is correlated to the level of immunosuppression and is highest among heart and lung TR, intermediate in kidney TRs and lowest in liver TRs reflecting differences in level of immunosuppression required to prevent immunological graft-rejection.[40] The increased incidence of KC and inverted BCC/cSCC ratio in immunosuppressed compared with immunocompetent individuals suggests a central role of the immune system in the genesis of cSCC.[41]

Immunosuppressive drugs (e.g. azathioprine, calcineurin inhibitors) are linked to aberrant production of cytokines that promote tumor growth, angiogenesis and metastasis.[42] Immunosuppressive drugs have a potential pro-oncogenic action in cells or by facilitating tumor cell escape from immunosurveillance.[26,43]

Azathioprine is an antimetabolite and purine analog, inhibiting purine and DNA synthesis. It is associated with an increased risk of cSCC in OTR.[16,44,45] Mycophenolate mofetil (MMF) is an acid prolog and also anti metabolite, inhibiting purine and DNA synthesis. It was introduced as an immunosuppressant after kidney transplantation in 1995 and has nowadays replaced azathioprine in many centers. Its use seems to be associated with a lower risk of cSCC in OTR.[46-48]

Cyclosporin and tacrolimus are both calcineurin inhibitors, inhibiting phosphatase activity of calcineurin. First cyclosporine and from mid-nineties tacrolimus are used in OTR. The research as to whether use of a calcineurin inhibitor would increase the risk of skin cancer in OTR, and if tacrolimus would have a lower risk than cyclosporin is conflicting.[44,45,49,50]

Traditionally varying doses of azathioprine and cyclosporin, combined with corticosteroids were used. Nowadays this has largely been replaced in more recent recipients by the modern MMF with tacrolimus.[45]
The prolonged immunosuppression leading to the development of numerous actinic keratoses and cSCC also makes a role for a viral pathogenesis very likely.

Human papillomavirus

The most common malignancies in immunosuppressed patients are those due to oncogenic viruses.[41] In the skin, these include Kaposi sarcoma (human herpes virus 8) and Merkel cell carcinoma (Merkel cell polyomavirus).[51-54] Cutaneous human papillomavirus (HPV) of the Beta genus (Beta-PV) has been most closely linked with cSCC.[35,51,55-57]
It is not known how many and which of the more than 200 cHPVs are responsible for the increased risk of cSCC.[35,55-64] HPV DNA of various genotypes has been detected in KC.[65] Some studies report a higher HPV prevalence in lesions from immunosuppressed patients.[66,67] There is evidence suggesting that HPV infection is involved in cSCC development both in immunocompetent patients and in OTR.[11,35,51,55,63,64,68-81] This will be described in more detail in chapter 2.
Recent animal and human studies show that Beta-PV infection in combination with UV exposure significantly accelerates the development of cSCC.[58,59,61] It has been shown that Beta-PV infection facilitates the accumulation of UV-induced DNA mutations by different mechanisms, which in turn leads to cellular transformation and malignant transformation by UV radiation.[35,55-57,82]
Beta-PVs are found in the skin and hair follicles of more than 80% of healthy people. Colonization occurs from early childhood onwards. Increased viral load, number of infecting HPV genotypes and Beta-PV-seropositivity have all been associated with cSCC.[35,51,55,58,59,61,83]
Most studies investigating the association between serological responses to HPV and cSCC are cross-sectional or case-control studies. Those studies were carried out in the immunocompetent population and showed an association between detection of Beta-PV antibodies and cSCC.[72,73,81,84-86] Cohort studies investigating the association between serological responses to HPV and later development of a first KC were not carried out in OTR. Therefore, we designed a retrospective follow-up study to establish if there is a relationship between the presence of serological responses to HPV, in particular Beta-PV, around transplantation and the development of KC (cSCC and BCC) in the years after the organ transplantation (chapter 3).

Role of p16

The mechanisms involved in Beta-PV mediated skin carcinogenesis are fundamentally different from those occurring in the malignant transformation driven by the alpha-PV genotypes (HPV16, 18).[56] In cervical squamous epithelium high risk HPV oncogene expression is accompanied by strong nuclear and/or cytoplasmic diffuse overexpression of the cellular cyclin-dependent kinase inhibitor p16INK4a.[87,88] Immunohistochemistry for p16 is established as a surrogate marker of HPV in oropharyngeal cancer.[89]

p16INK4a (p16) is a tumor suppressor protein encoded by the INK4a (also called MTSI, CDK4I, or CDKN2) gene on chromosome 9p21 and acts by inhibiting D-type cycle independent kinases 4 and 6 involved in the regulation of the retinoblastoma gene (Rb) activity. The formation of the p16CDK4/6 complex allows Rb to continue cell cycle arrest, whereas the failure of complex formation allows cell division to continue.[90-93] Functional or structural loss of p16, could lead to cell cycle propagation of potential genetically damaged cells and subsequent risk of tumor development. In skin neoplasia the relation between cSCC and HPV is not as clear and there is no significant association between p16 protein expression and the presence of HPV.[35,94-96] There is evidence that UV radiation and immune suppression, both of which are associated with increased risk of cSCC of the head and neck, can also lead to p16 up-regulation, unrelated to HPV.[97] Nearly 32% of cSCC, particularly poorly differentiated, show p16 expression.[89] P16 protein expression in human keratinocytes is induced by UVB irradiation at low and high doses, possibly at a posttranscriptional level.[98] Actinic keratosis and cSCC have been shown to have different expression of p16 by immunohistochemistry.[99]

Previous studies using staining for p16 in KC have shown discrepant results and reports on KC in OTR are few.[100,101] We aimed to investigate p16 immunostaining patterns in a spectrum of premalignant and invasive keratinocyte lesions from OTR (chapter 4). Distinguishing between benign keratinocyte neoplasia and malignancies is not an uncommon histologic diagnostic dilemma. The main goal was to find out if differences in p16 immunostaining might be useful to differentiate between different keratinocyte lesions (Figure 3).

Clinical characteristics of cSCC

cSCC can develop in the clinical spectrum of actinic keratosis and Bowen's disease, which are pre-cursor lesions and has several clinical presentations (Figures 2-5).

The classical presentation of a cSCC is that of a skin-colored to erythematous tumor with central erosion or hyperkeratosis with well-defined borders. However, sometimes a cSCC resembles an actinic keratosis (pre-cursor lesion) as a flat erythematous hyperkeratotic lesion with ill-defined borders. It can also present as an ulceration with erythematous borders.



Figure 3. Actinic keratoses. Severely actinically damaged skin, with multiple erythematous hyperkeratotic lesions on the scalp and on the lower extremity. The lesion encircled on the scalp are squamous cell carcinomas.

The clinical presentation often reflects the histopathological differentiation grade.[102,103] This is graded on a 4-tier system developed by Broders.[104] In this classification, the grade reflects the ratio of differentiated cells to undifferentiated cells. Grade 1 is well differentiated (keratinized), grade 2 is moderately differentiated, grade 3 is poorly differentiated and grade 4 is undifferentiated (unkeratinized).
Most cSCC are between 0.5-1.5cm in diameter at time of presentation. The majority of cSCC arise in sun-exposed skin, like the scalp, ears, lips, back of the hands and lower legs. The surrounding skin often shows evident sun damage.[105]
Lesions can be painful. It has been found that tenderness was significantly associated with cSCC versus actinic keratosis.[106] OTR with cSCC usually have numerous keratotic skin lesions, such as actinic keratoses, common warts, seborrheic warts and hyperkeratotic papillomas and they may mimic invasive cSCC hampering timely diagnosis making.[11] Pain as a clinical symptom has been proposed to be a useful factor in differentiating cSCC from other lesions.[107] Especially in patients with severely sun damaged skin and numerous keratotic lesions, pain can be helpful in identifying a cSCC in a field of numerous suspicious lesions, which is often the case in OTR.[108]
Validated patient-reported warning signals as pain are useful to facilitate rapid screening of large numbers of keratotic skin lesions in OTR to identify invasive SCC for early diagnosis and treatment. We therefore performed a multi-center study with the aim to identify clinically meaningful independent patient reported signs and symptoms predicting the presence of cSCC in OTR (chapter 5).

Metastasis

cSCC has the potential to metastasize, usually to the regional lymph nodes. The overall incidence rate of cSCC metastasis is variable, ranging from 0.1-9.9%, with a tendency to a higher incidence in tertiary hospitals.[109-117] The risk of developing metastases of low-risk cSCC is 0-5% in the general population. Large tumors, recurrent tumors, deep infiltration, location on the lip or ear, poor differentiation grade and perineural and lymphovascular invasion increase the risk for metastasis up to 45%.[1,110,115,118,119] A 13% risk of metastatic disease in immunocompromised patients has been described, twice the risk in patients without immunosuppression.[110]
Because of the large numbers of cSCC in individual patients, OTR present more often with aggressive cSCC (thicker tumors, poorly differentiated and infiltrative), with a higher predilection for metastasis.[120] Broadly, it is stated in literature that cSCC in

OTR frequently seems to exhibit a more aggressive behavior, irrespective of the size of the tumor, with more frequent metastases and worse outcome compared to immunocompetent patients.[121-124]

It has been reported that about 5 to 23 % of all patients with metastatic cSCC are immuno-compromised and in these studies investigating metastatic cSCC, immunosuppression is often reported as a risk factor.[111,125,126] However, the definition of an immunosuppressed patient is not clear-cut. OTR are subject to lifelong immunosuppressive therapy and therefore ideal to study the influence of immunosuppression on cSCC behavior. Especially in OTR, numbers and conclusions are based on small studies, so there is limited evidence regarding cSCC metastasis risk in OTR compared to the general cSCC population. We performed a systematic review of the available literature to investigate whether the risk for cSCC metastasis is increased for OTR compared to immunocompetent patients (chapter 6).



Figure 4.
Different clinical presentations of Bowen's disease. It is characteristically presented as a well circumscribed erythematous plaque with light scaling and or hyperkeratosis. Sometimes pigmentation is present.

High risk factors for metastasis

The challenge in daily practice is to identify high risk cSCC and to detect a metastasis as early as possible, in both low and high risk populations.[127-129]

A staging system can help to accomplish this task. Three different systems are currently in use; the International Union Against Cancer (UICC), the American Joint Committee on Cancer (AJCC) classification and the Brigham and Women's Hospital (BWH) tumor staging system.[130-132] The AJCC staging system is the most commonly used and is subject to regular modifications. Most recently the 8th edition of the American Joint Committee on Cancer was published and showed some changes with the 7th edition regarding high risk features to upgrade the T-stage.[131,133]



Figure 5. Different presentations of cutaneous squamous cell carcinoma. It often presents as a skin-colored to erythematous tumor with central erosion or hyperkeratosis with well-to-moderate-defined borders. It can also present as an ulceration with erythematous borders.

These staging systems are based upon defined series of major and minor criteria, which contain both clinical and histological features. High risk features that are used for tumor staging are tumor size, depth of invasion, location on head and neck, differentiation grade and perineural invasion.[109]
A higher risk is related to increase in size, as this is also an important feature of the TNM classification.[131,133]
Histologic differentiation grade is correlated with prognosis. Well differentiated cSCC have a better prognosis then poorly differentiated cSCC.[109,110,128,134,135]
Also other histological features are correlated with worse prognosis, namely depth of invasion, perineural invasion and lymphovascular invasion.[102,103]
Location on the central face, ear and lip are high risk criteria, as well as rapid growth, recurrent tumor, prior radiation therapy and immunocompromised status.[1,25,105]
However, in the most recent AJCC classification system only size, perineural invasion and depth of invasion > 6mm or infiltrating beyond subcutis are incorporated as high risk features. On the other hand, risk factors as poor differentiation grade and location on the head and neck area, especially lip and ear, are still considered as high risk factors for cSCC to take into account as a prognostic factor for clinical practice. This also accounts for immunosuppression, although immunosuppressed status is not included in the staging systems.[113,136-139] OTR are an ideal population to analyze the influence of immunosuppression on metastatic behavior. We aimed to identify the risk factors and to calculate the risk of cSCC metastasis in OTR and immunocompetent population in our institution over a 10-year period by a retrospective cohort study (chapter 7).

Aim and outline of the thesis

This thesis consists of several aspects of cSCC in OTR. The aim of the studies presented in this thesis is three fold. First, in chapter 2 and 3, we give an overview of the role of HPV in cSCC and study HPV as a prognostic factor in the etiology of cSCC. Subsequently we sought to identify p16 as a possible histopathological marker for cSCC in chapter 4 and the clinical implication of pain to differentiate cSCC from other keratinocyte lesions in chapter 5. Third we endeavored to achieve metastasis risk, and subsequently risk factors, of cSCC in OTR compared to immunocompetent patients in chapter 6 and 7.

Chapter 2 gives an overview of HPV and the epidemiology of HPV Infection related to skin cancer, with the emphasis on HPV as a risk factor for cSCC in OTR.

Chapter 3 describes a retrospective follow-up study to establish whether there is a relationship between the presence of serological responses to HPV, in particular Beta-PV, between 1 year before and 1 year after transplantation and the development of KC (SCC and BCC) in the years after the organ transplantation in 445 OTR (of whom 60 developed cSCC) during maximum 22-year follow-up period.

Chapter 4 investigates p16 immunostaining patterns in order to differentiate between different types of keratinocyte neoplasia in 59 actinic keratosis, 51 Bowen's disease , 63 cSCC and 16 benign keratotic lesions from 31 OTR patients and 25 control eczema and psoriasis patients.

Chapter 5 defines pain as a clinically meaningful patient-reported warning signal predicting the presence of cSCC in a prospective, multi-center cohort of 812 consecutively biopsied skin lesions from 410 OTR performed in 10 centers dedicated to surveillance of OTR with skin problems in Europe and the United States.

Chapter 6 compares the cSCC metastasis risk in OTR and the general population in a systematic review of the literature, up to January 2018.

Chapter 7 studies in a stratified cohort study, during a 10-year period, in OTR and non-OTR patients if there is a difference in metastasis risk for cSCC and whether their risk factors differ.

References

1. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. NEnglJMed. 2001;344(13): 975-83.
2. Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. Lancet. 2010;375(9715): 673-85.
3. https://www.volksgezondheidenzorg.info/search/site/huidkanker.
4. Hollestein LM, de Vries E, Aarts MJ, Schroten C, Nijsten TE. Burden of disease caused by keratinocyte cancer has increased in The Netherlands since 1989. J Am Acad Dermatol. 2014;71(5):896-903.
5. Hollestein LM, de VE, Nijsten T. Trends of cutaneous squamous cell carcinoma in the Netherlands: increased incidence rates, but stable relative survival and mortality 1989-2008. EurJCancer. 2012;48(13):2046-53.
6. Xiang F, Lucas R, Hales S, Neale R. Incidence of Nonmelanoma Skin Cancer in Relation to Ambient UV Radiation in White Populations, 1978-2012: Empirical Relationships. JAMA Dermatol. 2014.
7. Green A, Battistutta D, Hart V, Leslie D, Weedon D. Skin cancer in a subtropical Australian population: incidence and lack of association with occupation. The Nambour Study Group. Am J Epidemiol. 1996;144(11): 1034-40.
8. Hannuksela-Svahn A, Pukkala E, Karvonen J. Basal cell skin carcinoma and other nonmelanoma skin cancers in Finland from 1956 through 1995. Arch Dermatol. 1999;135(7):781-6.
9. Yoong C, De'Ambrosis B. Cutaneous invasive squamous cell carcinoma: 10-year experience and recommendations for follow up. AustralasJDermatol. 2009;50(4):261-5.
10. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: Estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. JAmAcadDermatol. 2013.
11. Bouwes Bavinck JN, Euvrard S, Naldi L, Nindl I, Proby CM, Neale R,... Harwood CA. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in The Netherlands, United Kingdom, Germany, France, and Italy. JInvest Dermatol. 2007;127(7):1647-56.
12. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in The Netherlands. Transplantation. 1990;49(3):506-9.
13. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. NEnglJMed. 2003;348(17):1681-91.
14. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. BrJDermatol. 2006;154(3):498-504.
15. Tessari G, Naldi L, Boschiero L, Nacchia F, Fior F, Forni A,... Girolomoni G. Incidence and clinical predictors of a subsequent nonmelanoma skin cancer in solid organ transplant recipients with a first nonmelanoma skin cancer: a multicenter cohort study. ArchDermatol. 2010;146(3):294-9.
16. Wisgerhof HC, van der Boog PJ, de Fijter JW, Wolterbeek R, Haasnoot GW, Claas FH,... Bouwes Bavinck JN. Increased risk of squamous-cell carcinoma in simultaneous pancreas kidney transplant recipients compared with kidney transplant recipients. JInvest Dermatol. 2009;129(12): 2886-94.
17. Harwood CA, Mesher D, McGregor JM, Mitchell L, Leedham-Green M, Raftery M,... Proby CM. A surveillance model for skin cancer in organ transplant recipients: a 22-year prospective study in an ethnically diverse population. AmJTransplant. 2013;13(1):119-29.
18. Krynitz B, Edgren G, Lindelof B, Baecklund E, Brattstrom C, Wilczek H, Smedby KE. Risk of skin cancer and other malignancies in kidney, liver, heart and lung transplant recipients 1970 to 2008--a Swedish population-based study. International journal of cancer. 2013;132(6):1429-38.
19. Collett D, Mumford L, Banner NR, Neuberger J, Watson C. Comparison of the incidence of malignancy in recipients of different types of organ: a UK Registry audit. Am J Transplant. 2010;10(8):1889-96.

20. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part I. Epidemiology of skin cancer in solid organ transplant recipients. JAmAcadDermatol. 2011; 65(2):253-61.
21. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part II. Management of skin cancer in solid organ transplant recipients. JAmAcadDermatol. 2011;65(2):263-79.
22. London NJ, Farmery SM, Will EJ, Davison AM, Lodge JP. Risk of neoplasia in renal transplant patients. Lancet. 1995;346(8972):403-6.
23. Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: Incidence, risk factors, diagnosis, and staging. J Am Acad Dermatol. 2018;78(2):237-47.
24. Ramsay HM, Fryer AA, Reece S, Smith AG, Harden PN. Clinical risk factors associated with nonmelanoma skin cancer in renal transplant recipients. Am J Kidney Dis. 2000;36(1):167-76.
25. Nehal KS, Bichakjian CK. Update on Keratinocyte Carcinomas. N Engl J Med. 2018; 379(4):363-74.
26. Rangwala S, Tsai KY. Roles of the immune system in skin cancer. Br J Dermatol. 2011; 165(5):953-65.
27. Boukamp P. Non-melanoma skin cancer: What drives tumor development and progression? Carcinogenesis. 2005;26(10): 1657-67.
28. McGregor JM, Berkhout RJ, Rozycka M, ter Schegget J, Bouwes Bavinck JN, Brooks L, Crook T. p53 mutations implicate sunlight in post-transplant skin cancer irrespective of human papillomavirus status. Oncogene. 1997;15(14):1737-40.
29. Soufir N, Daya-Grosjean L, de La Salmoniere P, Moles JP, Dubertret L, Sarasin A, Basset-Seguin N. Association between INK4a-ARF and p53 mutations in skin carcinomas of xeroderma pigmentosum patients. J Natl Cancer Inst. 2000;92(22):1841-7.
30. Kubo Y, Urano Y, Matsumoto K, Ahsan K, Arase S. Mutations of the INK4a locus in squamous cell carcinomas of human skin. Biochem Biophys Res Commun. 1997;232(1): 38-41.
31. Soufir N, Moles JP, Vilmer C, Moch C, Verola O, Rivet J,... Basset-Seguin N. P16 UV mutations in human skin epithelial tumors. Oncogene. 1999;18(39):5477-81.
32. de Jong-Tieben LM, Berkhout RJ, Smits HL, Bouwes Bavinck JN, Vermeer BJ, van der Woude FJ, ter Schegget J. High frequency of detection of epidermodysplasia verruciformis-associated human papillomavirus DNA in biopsies from malignant and premalignant skin lesions from renal transplant recipients. The Journal of investigative dermatology. 1995;105(3):367-71.
33. Euvrard S, Kanitakis J, Pouteil-Noble C, Dureau G, Touraine JL, Faure M,... Thivolet J. Comparative epidemiologic study of premalignant and malignant epithelial cutaneous lesions developing after kidney and heart transplantation. JAmAcadDermatol. 1995;33(2 Pt 1):222-9.
34. Weinstein T, Korzets A, Chagnac A, Ori Y, Herman M, Zevin D,... Gafter U. Effect of immunosuppressive therapy on DNA repair and cancer incidence in renal transplant recipients. Transplant Proc. 2000;32(4):694-5.
35. Quint KD, Genders RE, de Koning MN, Borgogna C, Gariglio M, Bouwes Bavinck JN,... Feltkamp MC. Human Beta-papillomavirus infection and keratinocyte carcinomas. J Pathol. 2015;235(2):342-54.
36. Falchi L, Vitale C, Keating MJ, Lerner S, Wang X, Elhor Gbito KY,... Ferrajoli A. Incidence and prognostic impact of other cancers in a population of long-term survivors of chronic lymphocytic leukemia. Ann Oncol. 2016;27(6):1100-6.
37. Jennings L, Schmults CD. Management of high-risk cutaneous squamous cell carcinoma. Journal of Clinical and Aesthetic Dermatology. 2010;3(4):39-48.
38. Silverberg MJ, Leyden W, Warton EM, Quesenberry CP, Jr., Engels EA, Asgari MM. HIV infection status, immunodeficiency, and the incidence of non-melanoma skin cancer. J Natl Cancer Inst. 2013;105(5):350-60.
39. Madeleine MM, Patel NS, Plasmeijer EI, Engels EA, Bouwes Bavinck JN, Toland AE,...

the Keratinocyte Carcinoma Consortium Immunosuppression Working G. Epidemiology of keratinocyte carcinomas after organ transplantation. Br J Dermatol. 2017; 177(5):1208-16.
40. Garrett GL, Blanc PD, Boscardin J, Lloyd AA, Ahmed RL, Anthony T,... Arron ST. Incidence of and Risk Factors for Skin Cancer in Organ Transplant Recipients in the United States. JAMA Dermatol. 2017;153(3):296-303.
41. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370(9581):59-67.
42. Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation. 2004;77(12):1777-82.
43. Gutierrez-Dalmau A, Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients - A systematic review. Drugs. 2007;67(8):1167-98.
44. Ingvar A, Smedby KE, Lindelof B, Fernberg P, Bellocco R, Tufveson G,... Adami J. Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma. NephrolDialTransplant. 2010;25(8): 2764-71.
45. Jiyad Z, Olsen CM, Burke MT, Isbel NM, Green AC. Azathioprine and Risk of Skin Cancer in Organ Transplant Recipients: Systematic Review and Meta-Analysis. Am J Transplant. 2016;16(12):3490-503.
46. Coghill AE, Johnson LG, Berg D, Resler AJ, Leca N, Madeleine MM. Immunosuppressive Medications and Squamous Cell Skin Carcinoma: Nested Case-Control Study Within the Skin Cancer after Organ Transplant (SCOT) Cohort. AmJTransplant. 2016;16(2):565-73.
47. Molina BD, Leiro MG, Pulpon LA, Mirabet S, Yanez JF, Bonet LA,... Muniz J. Incidence and risk factors for nonmelanoma skin cancer after heart transplantation. Transplant Proc. 2010;42(8):3001-5.
48. O'Neill JO, Edwards LB, Taylor DO. Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2006;25(10):1186-91.
49. Fuchs U, Klein S, Zittermann A, Ensminger SM, Schulz U, Gummert JF. Incidence of malignant neoplasia after heart transplantation--a comparison between cyclosporine a and tacrolimus. Ann Transplant. 2014;19: 300-4.
50. Navarro MD, Lopez-Andreu M, Rodriguez-Benot A, Aguera ML, Del Castillo D, Aljama P. Cancer incidence and survival in kidney transplant patients. Transplant Proc. 2008;40(9):2936-40.
51. Arron ST, Jennings L, Nindl I, Rosl F, Bouwes Bavinck JN, Seckin D,... Skin Care in Organ Transplant Patients E. Viral oncogenesis and its role in nonmelanoma skin cancer. Br J Dermatol. 2011;164(6):1201-13.
52. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science. 1994;266(5192):1865-9.
53. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008;319(5866):1096-100.
54. zur Hausen H. Oncogenic DNA viruses. Oncogene. 2001;20(54):7820-3.
55. Howley PM, Pfister HJ. Beta genus papillomaviruses and skin cancer. Virology. 2015;479-480:290-6.
56. Viarisio D, Gissmann L, Tommasino M. Human papillomaviruses and carcinogenesis: well-established and novel models. Curr Opin Virol. 2017;26:56-62.
57. Tommasino M. The biology of beta human papillomaviruses. Virus Res. 2017;231:128-38.
58. Viarisio D, Muller-Decker K, Accardi R, Robitaille A, Durst M, Beer K,... Tommasino M. Beta HPV38 oncoproteins act with a hit-and-run mechanism in ultraviolet radiation-induced skin carcinogenesis in mice. PLoS Pathog. 2018;14(1):e1006783.
59. Hasche D, Stephan S, Braspenning-Wesch I, Mikulec J, Niebler M, Grone HJ,... Vinzon SE. The interplay of UV and cutaneous papillo-

mavirus infection in skin cancer development. PLoS Pathog. 2017;13(11):e1006723.
60. Harwood CA, Toland AE, Proby CM, Euvrard S, Hofbauer GFL, Tommasino M,... KeraCon C. The pathogenesis of cutaneous squamous cell carcinoma in organ transplant recipients. Br J Dermatol. 2017;177(5): 1217-24.
61. Bouwes Bavinck JN, Feltkamp MCW, Green AC, Fiocco M, Euvrard S, Harwood CA,... group E-H-U-C. Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study. Am J Transplant. 2018;18(5): 1220-30.
62. Vinzon SE, Rosl F. HPV vaccination for prevention of skin cancer. Hum Vaccin Immunother. 2015;11(2):353-7.
63. Genders RE, Mazlom H, Michel A, Plasmeijer EI, Quint KD, Pawlita M,... Bouwes Bavinck JN. The presence of betapapillomavirus antibodies around transplantation predicts the development of keratinocyte carcinoma in organ transplant recipients: a cohort study. JInvest Dermatol. 2015;135(5):1275-82.
64. Hasche D, Vinzon SE, Rosl F. Cutaneous Papillomaviruses and Non-melanoma Skin Cancer: Causal Agents or Innocent Bystanders? Front Microbiol. 2018;9:874.
65. de Villiers EM, Lavergne D, McLaren K, Benton EC. Prevailing papillomavirus types in non-melanoma carcinomas of the skin in renal allograft recipients. International journal of cancer. 1997;73(3):356-61.
66. Harwood CA, Surentheran T, McGregor JM, Spink PJ, Leigh IM, Breuer J, Proby CM. Human papillomavirus infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. J Med Virol. 2000;61(3):289-97.
67. Stockfleth E, Nindl I, Sterry W, Ulrich C, Schmook T, Meyer T. Human papillomaviruses in transplant-associated skin cancers. Dermatol Surg. 2004;30(4 Pt 2):604-9.
68. Borgogna C, Lanfredini S, Peretti A, De Andrea M, Zavattaro E, Colombo E,... Gariglio M. Improved detection reveals active beta-papillomavirus infection in skin lesions from kidney transplant recipients. Mod Pathol. 2014;27(8):1101-15.
69. Bouwes Bavinck JN, Neale RE, Abeni D, Euvrard S, Green AC, Harwood CA,... Feltkamp MC. Multicenter study of the association between betapapillomavirus infection and cutaneous squamous cell carcinoma. Cancer Res. 2010;70(23):9777-86.
70. Chahoud J, Semaan A, Chen Y, Cao M, Rieber AG, Rady P, Tyring SK. Association Between beta-Genus Human Papillomavirus and Cutaneous Squamous Cell Carcinoma in Immunocompetent Individuals-A Meta-analysis. JAMA Dermatol. 2016;152(12): 1354-64.
71. Chockalingam R, Downing C, Tyring SK. Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients. J Clin Med. 2015;4(6):1229-39.
72. Karagas MR, Nelson HH, Sehr P, Waterboer T, Stukel TA, Andrew A,... Pawlita M. Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. JNatlCancer Inst. 2006;98(6):389-95.
73. Karagas MR, Waterboer T, Li Z, Nelson HH, Michael KM, Bouwes Bavinck JN Pawlita M. Genus beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. BMJ. 2010;341:c2986.
74. Neale RE, Weissenborn S, Abeni D, Bouwes Bavinck JN, Euvrard S, Feltkamp MC,... Pfister H. Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. Cancer EpidemiolBiomarkers Prev. 2013;22(4):719-27.
75. Proby CM, Harwood CA, Neale RE, Green AC, Euvrard S, Naldi L,... Bouwes Bavinck JN. A case-control study of betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. AmJTransplant. 2011;11(7):1498-508.
76. Weissenborn S, Neale RE, Waterboer T, Abeni D, Bouwes Bavinck JN, Green AC,... group E-H-U-C. Beta-papillomavirus DNA loads in hair follicles of immunocompetent people and organ transplant recipients. Med Microbiol Immunol. 2012;201(2): 117-25.
77. Bouwes Bavinck JN, Feltkamp MC. Milk of

human kindness?--HAMLET, human papillomavirus, and warts. NEnglJMed. 2004;350(26):2639-42.
78. Feltkamp MC, de Koning MN, Bouwes Bavinck JN, Ter Schegget J. Betapapillomaviruses: innocent bystanders or causes of skin cancer. J Clin Virol. 2008;43(4):353-60.
79. Plasmeijer EI, Neale RE, Buettner PG, de Koning MN, Ter Schegget J, Quint WG,... Feltkamp MC. Betapapillomavirus infection profiles in tissue sets from cutaneous squamous cell-carcinoma patients. International journal of cancer. 2010;126(11):2614-21.
80. Tessari G, Girolomoni G. Nonmelanoma skin cancer in solid organ transplant recipients: update on epidemiology, risk factors, and management. DermatolSurg. 2012; 38(10):1622-30.
81. Feltkamp MC, Broer R, di Summa FM, Struijk L, van der Meijden E, Verlaan BP,... Bouwes Bavinck JN. Seroreactivity to epidermodysplasia verruciformis-related human papillomavirus types is associated with nonmelanoma skin cancer. Cancer Res. 2003;63(10):2695-700.
82. Tommasino M. The human papillomavirus family and its role in carcinogenesis. Semin Cancer Biol. 2014;26:13-21.
83. de Koning MN, Struijk L, Bouwes Bavinck JN, Kleter B, ter Schegget J, Quint WG, Feltkamp MC. Betapapillomaviruses frequently persist in the skin of healthy individuals. The Journal of general virology. 2007;88(Pt 5):1489-95.
84. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? DermatolSurg. 2013;39(1 Pt 1):1-23.
85. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G. A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology. 2013;445(1-2):224-31.
86. Waterboer T, Abeni D, Sampogna F, Rother A, Masini C, Sehr P,... Pawlita M. Serological association of beta and gamma human papillomaviruses with squamous cell carcinoma of the skin. BrJDermatol. 2008;159(2): 457-9.
87. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U,... von Knebel DM. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. IntJCancer. 2001;92(2): 276-84.
88. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol. 1998;153(6): 1741-8.
89. Satgunaseelan L, Chia N, Suh H, Virk S, Ashford B, Lum T,... Gupta R. p16 expression in cutaneous squamous cell carcinoma of the head and neck is not associated with integration of high risk HPV DNA or prognosis. Pathology. 2017;49(5):494-8.
90. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature. 1993;366(6456):704-7.
91. Tam SW, Shay JW, Pagano M. Differential expression and cell cycle regulation of the cyclin-dependent kinase 4 inhibitor p16Ink4. Cancer Res. 1994;54(22):5816-20.
92. Russo AA, Tong L, Lee JO, Jeffrey PD, Pavletich NP. Structural basis for inhibition of the cyclin-dependent kinase Cdk6 by the tumour suppressor p16INK4a. Nature. 1998;395(6699):237-43.
93. Sherr CJ. Cancer cell cycles. Science. 1996;274(5293):1672-7.
94. Kusters-Vandevelde HV, de Koning MN, Melchers WJ, Quint WG, de Wilde PC, de Jong EM,... Blokx WA. Expression of p14ARF, p16INK4a and p53 in relation to HPV in (pre-)malignant squamous skin tumours. JCell MolMed. 2009;13(8B):2148-57.
95. Murao K, Yoshioka R, Kubo Y. Human papillomavirus infection in Bowen disease: negative p53 expression, not p16(INK4a) overexpression, is correlated with human papillomavirus-associated Bowen disease. JDermatol. 2014;41(10):878-84.
96. Svajdler M, Jr., Mezencev R, Kaspirkova J, Kacerovska D, Kazakov DV, Ondic O, Michal M. Human Papillomavirus Infection and p16 Expression in Extragenital/Extraungual

Bowen Disease in Immunocompromised Patients. AmJDermatopathol. 2016.
97. Pavey S, Conroy S, Russell T, Gabrielli B. Ultraviolet radiation induces p16CDKN2A expression in human skin. Cancer Res. 1999;59(17):4185-9.
98. Chazal M, Marionnet C, Michel L, Mollier K, Dazard JE, Della V, V,... Basset-Seguin N. P16(INK4A) is implicated in both the immediate and adaptative response of human keratinocytes to UVB irradiation. Oncogene. 2002;21(17):2652-61.
99. Hodges A, Smoller BR. Immunohistochemical comparison of p16 expression in actinic keratoses and squamous cell carcinomas of the skin. ModPathol. 2002;15(11): 1121-5.
100. Blokx WA, de Jong EM, de Wilde PC, Bulten J, Link MM, Ruiter DJ, van de Kerkhof PC. P16 and p53 expression in (pre)malignant epidermal tumors of renal transplant recipients and immunocompetent individuals. ModPathol. 2003;16(9):869-78.
101. Conscience I, Jovenin N, Coissard C, Lorenzato M, Durlach A, Grange F,... Bernard P. P16 is overexpressed in cutaneous carcinomas located on sun-exposed areas. EurJDermatol. 2006;16(5):518-22.
102. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification--part two. JCutanPathol. 2006;33(4):261-79.
103. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part one. JCutanPathol. 2006;33(3):191-206.
104. Broders AC. Squamous-Cell Epithelioma of the Skin: A Study of 256 Cases. Ann Surg. 1921;73(2):141-60.
105. Rudolph R, Zelac DE. Squamous cell carcinoma of the skin. PlastReconstrSurg. 2004;114(6):82e-94e.
106. Cook BA, Gravely AA, Nelson DB, Warshaw EM. Is tenderness a reliable predictor for differentiating squamous cell carcinomas from actinic keratoses? J Am Acad Dermatol. 2011;65(1):211-2.
107. Mills KC, Kwatra SG, Feneran AN, Pearce DJ, Williford PM, D'Agostino RB, Yosipovitch G. Itch and pain in nonmelanoma skin cancer: pain as an important feature of cutaneous squamous cell carcinoma. Arch Dermatol. 2012;148(12):1422-3.
108. Kwatra SG, Mills KC, Zeitany A, Pearce DJ, Williford PM, D'Agostino RB, Jr., Yosipovitch G. Pain and nonmelanoma skin cancer in transplant patients. J Am Acad Dermatol. 2012;67(6):1387-8.
109. Farasat S, Yu SS, Neel VA, Nehal KS, Lardaro T, Mihm MC,... Liegeois NJ. A new American Joint Committee on Cancer staging system for cutaneous squamous cell carcinoma: creation and rationale for inclusion of tumor (T) characteristics. JAmAcadDermatol. 2011;64(6):1051-9.
110. Rowe DE, Carroll RJ, Day CL, Jr. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. JAmAcadDermatol. 1992;26(6):976-90.
111. Veness MJ, Palme CE, Morgan GJ. High-risk cutaneous squamous cell carcinoma of the head and neck: results from 266 treated patients with metastatic lymph node disease. Cancer. 2006;106(11):2389-96.
112. Weinberg AS, Ogle CA, Shim EK. Metastatic cutaneous squamous cell carcinoma: an update. DermatolSurg. 2007;33(8):885-99.
113. Brantsch KD, Meisner C, Schonfisch B, Trilling B, Wehner-Caroli J, Rocken M, Breuninger H. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. Lancet Oncol. 2008;9(8):713-20.
114. Brougham ND, Dennett ER, Cameron R, Tan ST. The incidence of metastasis from cutaneous squamous cell carcinoma and the impact of its risk factors. JSurgOncol. 2012;106(7):811-5.
115. Chuang TY, Popescu NA, Su WP, Chute CG. Squamous cell carcinoma. A population-based incidence study in Rochester, Minn. ArchDermatol. 1990;126(2):185-8.
116. Dinehart SM, Pollack SV. Metastases from squamous cell carcinoma of the skin and lip. An analysis of twenty-seven cases. JAmAcadDermatol. 1989;21(2 Pt 1):241-8.

117. Tavin E, Persky M. Metastatic cutaneous squamous cell carcinoma of the head and neck region. Laryngoscope. 1996;106(2 Pt 1):156-8.
118. Czarnecki D, Staples M, Mar A, Giles G, Meehan C. Metastases from squamous cell carcinoma of the skin in southern Australia. Dermatology. 1994;189(1):52-4.
119. Breuninger H, Schaumburg-Lever G, Holzschuh J, Horny HP. Desmoplastic squamous cell carcinoma of skin and vermilion surface: a highly malignant subtype of skin cancer. Cancer. 1997;79(5):915-9.
120. Smith KJ, Hamza S, Skelton H. Histologic features in primary cutaneous squamous cell carcinomas in immunocompromised patients focusing on organ transplant patients. DermatolSurg. 2004;30(4 Pt 2): 634-41.
121. Carucci JA. Squamous cell carcinoma in organ transplant recipients: approach to management. Skin therapy letter. 2004;9(4):5-7.
122. Veness MJ, Quinn DI, Ong CS, Keogh AM, Macdonald PS, Cooper SG, Morgan GW. Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience. Cancer. 1999;85(8):1758-64.
123. Carucci JA, Martinez JC, Zeitouni NC, Christenson L, Coldiron B, Zweibel S, Otley CC. In-transit metastasis from primary cutaneous squamous cell carcinoma in organ transplant recipients and nonimmunosuppressed patients: clinical characteristics, management, and outcome in a series of 21 patients. DermatolSurg. 2004;30(4 Pt 2):651-5.
124. Martinez JC, Otley CC, Stasko T, Euvrard S, Brown C, Schanbacher CF, Weaver AL. Defining the clinical course of metastatic skin cancer in organ transplant recipients: a multicenter collaborative study. ArchDermatol. 2003;139(3):301-6.
125. Dinehart SM, Chu DZ, Maners AW, Pollack SV. Immunosuppression in patients with metastatic squamous cell carcinoma from the skin. JDermatolSurgOncol. 1990;16(3): 271-4.
126. Khurana VG, Mentis DH, O'Brien CJ, Hurst TL, Stevens GN, Packham NA. Parotid and neck metastases from cutaneous squamous cell carcinoma of the head and neck. AmJSurg. 1995;170(5):446-50.
127. Veness MJ. Defining patients with high-risk cutaneous squamous cell carcinoma. AustralasJDermatol. 2006;47(1):28-33.
128. Cherpelis BS, Marcusen C, Lang PG. Prognostic factors for metastasis in squamous cell carcinoma of the skin. DermatolSurg. 2002;28(3):268-73.
129. Martorell-Calatayud A, Sanmartin JO, Cruz MJ, Guillen BC. Cutaneous squamous cell carcinoma: defining the high-risk variant. Actas Dermosifiliogr. 2013;104(5):367-79.
130. In: Sobin L, Gospodarowicz M, Wittekind C, editors. UICC International Union Against Cancer TNM Classification of Malignant Tumors. 7th ed. West Sussex, United Kingdom: Wiley-Blackwell; 2009.
131. Califano JA LW, Nehal KS, et al. Cutaneous Squamous Cell Carcinoma of the Head and Neck. In: Amin MB ES, Greene F, et al, eds., editor. AJCC Cancer Staging Manual. 8th ed. ed. New York, NY: Springer; 2017. p. 171-81.
132. Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF, Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. JClinOncol. 2014;32(4):327-34.
133. Karia PS, Morgan FC, Califano JA, Schmults CD. Comparison of Tumor Classifications for Cutaneous Squamous Cell Carcinoma of the Head and Neck in the 7th vs 8th Edition of the AJCC Cancer Staging Manual. JAMA Dermatol. 2018;154(2):175-81.
134. Clayman GL, Lee JJ, Holsinger FC, Zhou X, Duvic M, El-Naggar AK,... Lippman SM. Mortality risk from squamous cell skin cancer. JClinOncol. 2005;23(4):759-65.
135. Mullen JT, Feng L, Xing Y, Mansfield PF, Gershenwald JE, Lee JE,... Cormier JN. Invasive squamous cell carcinoma of the skin: defining a high-risk group. AnnSurgOncol. 2006;13(7):902-9.
136. Haisma MS, Plaat BE, Bijl HP, Roodenburg JL, Diercks GF, Romeijn TR, Terra JB. Multivariate analysis of potential risk factors for

lymph node metastasis in patients with cutaneous squamous cell carcinoma of the head and neck. J Am Acad Dermatol. 2016;75(4):722-30.
137. Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. JAMA Dermatol. 2013;149(5): 541-7.
138. Thompson AK, Kelley BF, Prokop LJ, Murad MH, Baum CL. Risk Factors for Cutaneous Squamous Cell Carcinoma Recurrence, Metastasis, and Disease-Specific Death: A Systematic Review and Meta-analysis. JAMA Dermatol. 2016;152(4):419-28.
139. http://www.nvdv.nl/wp-content/uploads/2014/08/Herziening-richtlijn-Plaveiselcelcarcinoom-van-de-huid-2018_Definitieve-versie-2.pdf.



CHAPTER 2

Update on our understanding of HPV as a risk factor for cutaneous squamous cell carcinoma in organ transplant recipients

Advances in Transplant Dermatology: Clinical and Practical Implications.
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Abstract

Keratinocyte carcinomas are by far the most common malignancies seen in organ transplant recipients (OTR). Life-long immunosuppressive therapy is a major risk factor for developing squamous cell carcinoma (SCC) in OTR. In the years after transplantation, OTR develop numerous warts and wart-like lesions followed by the development of SCC. This resembles the clinical picture of epidermodysplasia verruciformis patients in which human papillomavirus (HPV) infections were associated with skin cancer. HPV can be divided into genera and cause several distinct benign and (pre-) malignant diseases.

There is evidence linking Beta-PV infection with the development of SCC in OTR. However, the role of Beta-PV in cutaneous squamous cell carcinoma carcinogenesis is still enigmatic. Unlike the carcinogenic Alpha-PV types, Beta-PV is not integrated in the human cellular DNA and is not necessary for the maintenance of the malignant phenotype of SCC.

The current view is that the carcinogenic effect of Beta-PV in OTR is subtle and probably exerted early in carcinogenesis.

Introduction

Organ transplant recipients, skin cancer and immunosuppressive therapies

Keratinocyte carcinomas are by far the most common malignancies seen in organ transplant recipients (OTR). The incidence of squamous cell carcinoma (SCC) is 60-250 times increased compared to the immunocompetent population, and for basal cell carcinoma (BCC) this is 10-40 times.[1-4]

Life-long immunosuppressive therapy is the most important risk factor for developing SCC in OTR. Other important risk factors include cumulative sun exposure, smoking and fair skin type with susceptibility to sunburn, which are risk factors similar to the immunocompetent population.[5]

Long term immunosuppressive therapy predisposes to the development of skin cancer and this is related to the type, duration and intensity of the immunosuppressive therapy. Azathioprine increases the photosensitivity of human skin to UVA radiation and when exposed to UVA radiation the active metabolite, methyl-thioinosine monophosphate (MeTIMP), which is incorporated into cellular DNA, generates reactive mutagenic oxygen species.[6,7] The carcinogenic effect of calcineurin inhibitors (cyclosporine and tacrolimus) is linked to aberrant production of cytokines that promote tumor growth, metastasis and angiogenesis.[8]

Immunosuppressive therapy with mammalian target of rapamycin (mTOR) inhibitors is possibly associated with a reduced risk of cutaneous SCC by antitumor and anti angiogenic properties, but seems only effective when the number of SCC is still low, and during the first year after conversion to mTOR inhibitor.[9-11]

Human papillomavirus infection

Human papillomaviruses (HPV) cause several distinct benign and (pre-) malignant diseases. HPV can be divided into Alpha, Beta, Gamma, Mu and Nu genera. Well known associations with benign lesions are with common skin warts (verruca vulgaris) and genital warts (condyloma accuminata). The most prevalent HPV types associated with common warts are the Alpha-PV types 2, 27 and 57 and the Gamma-PV type 4.[12-14] The majority of genital warts are caused by the mucosal Alpha-PV types 6 and 11, but other mucosal HPV types of the Alpha genera are also detected in genital warts.

The range of infections, precancers and malignancies associated with HPV continues to grow. The International Agency on Cancer Research IARC has classified mucosal

types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as carcinogenic types (class 1), mainly causing cervical cancer; type 68 as probably carcinogenic (Class 2A); and types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85 and 97 as possibly carcinogenic (Class 2B). HPV6 and HPV11 were not classifiable as to its carcinogenicity to humans, and the remaining mucosal HPV types were not taken into consideration by IARC (Class 3).[15]
The first time that HPV infection was linked with skin cancer was in patients with epidermodysplasia verruciformis (EV). EV is a rare autosomal recessive disease, initially described by Lewandowsky and Lutz in 1922, that has been proposed as a model for Beta-PV-mediated skin carcinogenesis.[16] EV patients have an increased susceptibility to widespread Beta-PV infections of the skin and develop pityriasis versicolor-like lesions and flat warts.[17,18] Skin cancers develop in one-third of the patients, mainly on sun-exposed sites in young to middle aged adult patients.[19]
In the years after transplantation, OTR develop numerous warts and wart-like lesions followed by the development of SCC (Figure 1,2), which resembles the clinical picture of EV patients. The association between wart-like lesions and SCC in OTR is an argument that HPV infection may play a role in SCC carcinogenesis.[20,21] This chapter will further focus on Beta-PV infection as a possible risk factor for cutaneous SCC carcinogenesis in OTR. In the following paragraphs laboratory and epidemiological evidence linking HPV infection with the development of SCC will be discussed. The HPV genome and taxonomy, replication and influence on cell cycle, and Beta-PV detection methods will be covered as well.



Figure 1.
Multiple wart-like lesions on dorsum of the hands

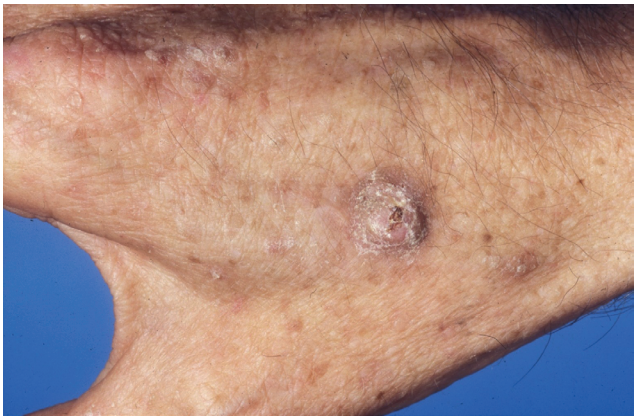


Figure 2.
Squamous cell carcinoma on dorsum of the hand

Human papillomaviruses

HPV genome and taxonomy

Papillomaviruses (PV) are non-enveloped circular double-stranded DNA viruses belonging to the family *Papillomaviridae*. The genome is approximately 8 kb and slightly varies in size between types. More than 150 types have been identified today and the number is still increasing (Figure 3). The genome is subdivided in an early (E) coding region, a late (L) coding region, and a long control region (LCR). The early region generally encodes for six non-structural viral regulatory proteins (E1, E2, E4, E5, E6 and E7 in most PV types and an additional E8 in some PV types) involved in several functions including transformation, transcription and viral adaptation to different cellular milieus.[22] The late region encodes for two structural proteins, namely L1 and L2.[23] Whereas all PV appear to have an E1, E2, L1 and L2 open reading frame (ORF) the other ORFs are not consistently present in every PV.[13] Beta-PV have E1, E2, E4, E6, E7, L1 and L2 ORFs, but lack E5.

The L1 ORF encoding for the major capsid protein L1 is relatively well conserved between HPV types. The L1 protein is the basis for currently registered prophylactic vaccines against HPV types 6, 11, 16 and 18 infections that cause genital warts and cervical cancer. The current papillomavirus classification system is based on DNA sequence homology of the L1 ORF and comprises a division in genus, species, type, subtype and variants. The phylogenetic tree is shown in Figure 3.[24]

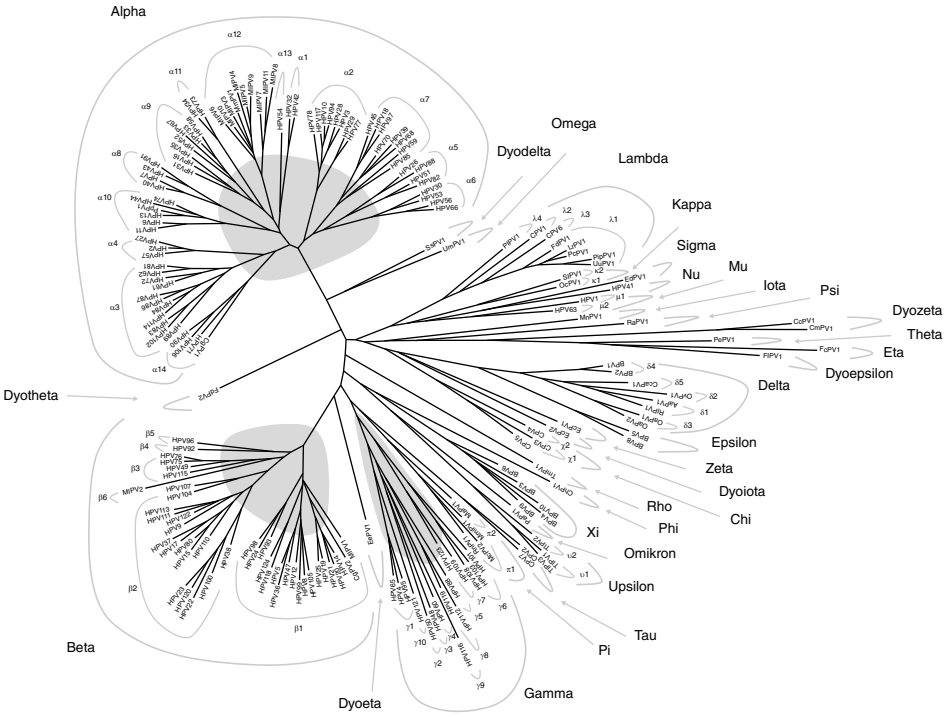


Figure 3. Phylogenetic Tree

As stated before, HPV can be divided into Alpha, Beta, Gamma, Mu and Nu genera. Species of the same genera share at least 60% homology of the L1 ORF. A new type is defined as one in which the complete nucleotide sequence ORF of the L1 gene differs by more than 10% from the most closely related known PV type. Table 1 shows all the HPV types according to the genus, based on the Papillomavirus Episteme database (http://pave. niaid. nih. gov/#home). The Beta, Gamma, Mu and Nu genera are cutaneous types. The Alpha genus contains all the mucosal types but also some cutaneous types (HPV2, 3, 10, 27, 28, 29, 57, 77, 94) and mucocutaneous types (HPV7, 40, 43, 91).[24]

HPV life cycle

HPV infection occurs when the virus enters the basal layer of the epithelium, supposedly achieved by small abrasions of the epithelium. However, the body-wide

Table 1. HPV types sorted by genus

genus	HPV type
Alpha	2,3,6,7,10,11,13,16,18,26,27,28,29,30,31,32,33,34,35,39,40,42,43,44,45,51,52,53, 54,55,56,57,58,59,61,62,66,67,68,69,70,71,72,73,74,77,78,81,82,83,84,85,86,87 ,89,90,91,94,97,102,106,114,117,125,160
Beta	5,8,9,12,14,15,17,19,20,21,22,23,24,25,36,37,38,47,49,75,76,80,92,93,96,98,99, 100,104,105,107, 110,111,113,115,118,120,122,124,143,145,150,151,159
Gamma	4,48,50,60,65,88,95,101,103,108,109,112,116,119,121,123,126,127,128,129, 130,131,132,133,134,135,136,137,138,139,140,141,142,144,146,147,148,149, 153,154,155,156,161,162,163,164,165,166,169,170
Mu	1,63
Nu	41

Source: http://pave. niaid. nih. gov/#home

distribution of especially Beta-PV infections suggests a direct route of infection, bypassing the requirement of epithelial injury or abrasion.[25-29] Life cycle studies have been mainly performed for the HPV types causing cervical cancer but the life cycle of several HPV types with a cutaneous tropism appears to be similar.[30]

E6 and E7 are mainly expressed in the (supra) basal layer and the granular layer of the epithelium and are associated with PV genome maintenance and cellular proliferation. E1 and E2 are involved in viral DNA replication and the regulation of E6 and E7 transcription. E4 of some cutaneous HPV types appears to be expressed throughout the epithelium except in the basal cell layer and presumably promotes viral DNA replication.[31,32] There is some variability in viral life cycle between PV types. However, the order of expression of viral genes throughout the differentiating epithelia is similar, although the localisation of E4 and L1 expression is variable and starts either in the lower part of the epithelium (HPV1 and 2) or in the upper part (HPV63 and 65).[33]

Beta-PV detection methods

There are different methods for Beta-PV detection, based on measurement of viral protein, viral DNA or serum antibody responses. Tissue specimens can be collected via several sampling methods like skin swabs, plucked hairs, biopsies and blood.[34] Several PCR-based methods targeting the HPV L1, E1 or E7 ORF have been developed to detect Beta-PV types in skin biopsies, plucked hairs and skin swabs.[25,26,35-45]

These PCR methods can be divided into I) type-specific PCRs, using HPV type-specific primers for the detection of a single HPV type, II) multiplex type-specific PCR

methods, in which multiple type-specific primer sets are combined in a single PCR reaction, III) broad-spectrum PCR methods using consensus primers, which permit simultaneous amplification of multiple types, and IV) quantitative PCR methods that allow viral DNA quantification.

Multiplex type-specific PCR and broad-spectrum PCR based methods amplify multiple HPV types in a single reaction. Subsequent genotyping in the case of amplification systems that target multiple HPV types is generally performed by sequence analysis, either directly or preceded by cloning of the amplimer. Sequencing, however, does not easily permit the identification of HPV types present in frequently occurring multiple infections.[39,46] Therefore, the more recent developed PCRs, are followed by reverse hybridisation technologies or APEX for the simultaneous identification of multiples HPV types.[39,41,45,47]

With virus-like particle (VLP) enzyme-linked immunoassay (ELISA) or multiplex technology (Luminex), antibodies against Beta-PV viral proteins can be detected, to determine a person's Beta-PV serological status. Serologic responses are usually measured against the major capsid protein L1 and the non-structural protein E6 using HPV virus-like particle (VLP) or GST-HPV fusion proteins in ELISA or with multiplex serology using GST-L1 fusion proteins, respectively.[48-50] The latter method (Luminex) is based on fluorescent bead technology that allows simultaneous detection of antibodies against up to 100 different in situ affinity-purified recombinant HPV proteins.[51] However, not all HPV infections induce an antibody response to HPV, and cross-reactive antibodies between different HPV types may be produced.[52] Nevertheless, serologic antibody responses provide a useful epidemiologic tool to explore HPV infections in certain populations.

Detection of papillomavirus proteins and DNA in paraffin-embedded tissue sections is possible by combining in situ hybridization and fluorescence detection methods,(FISH=Fluorescent DNA in situ hybridization).[53] Also laser capture microdissection can be used to specifically test a particular part of an histological specimen for HPV presence and viral load.[54]

Beta-PV replication, cell transformation and carcinogenesis

After infection, Beta-PV will start to replicate in the epidermal keratinocytes. As for mucosal HPV, keratinocyte differentiation is probably also crucial for Beta-PV to fulfill their life cycle.[55,56] It is expected that in the basal epidermal layers a low level of replication of Beta-PV DNA is maintained to ensure viral episome distribution

among daughter cells, and especially the early (E) genes will be expressed.[57]
Transformation of human cells has been studied in great detail for the HPV types causing cervical cancer (e. g. HPV16 and HPV18). In general, transformation requires long-term and deregulated expression of viral oncogenes E6 and E7. This process is facilitated by integration of the viral episome into the cellular DNA, disturbing the E2 ORF, thereby causing a lack of control of E6 and E7 expression.[32] In general, Beta-PV are found episomal, also in actinic keratoses and SCC, although (integrated) HPV has been occasionally isolated from an SCC metastatic lesion in an OTR.[43,58]
Since papillomaviruses depend on (unscheduled) host cell DNA replication in order to produce progeny, the virus tends to keep its host cell in a replicative state (S-phase) as long as possible. This is particularly relevant as terminal keratinocyte differentiation is a dead-end road resembling programmed cell death (apoptosis).[59,60] For high-risk mucosal HPV types it is known that the E7 early gene product drives cells into S-phase by binding and degrading the tumor suppressor protein pRb.[61] For HPV38, a Beta-PV type that has been associated with SCC in some studies, this has been shown as well, but it does not seem to be a mechanism exploited by all Beta-PV types. For the high-risk mucosal HPVs, such as HPV16 and HPV18, this mechanism acts through ubiquitination and subsequent degradation of the tumor suppressors p53 and pRb. Presumably, persistent infections are needed to accumulate sufficient mutations in the host cellular genome to generate a malignant cell. The proliferative phenotype of these malignant cells remains dependent on E6/E7 expression.[62]
HPV38 E7 was shown to bind and degrade the tumor suppressor pRb similar as HPV16 E7[63,64]. Furthermore, HPV38 E6 and E7 are sufficient to deregulate the cell cycle and senescence programs in primary human keratinocytes, thereby increasing the lifespan of human skin keratinocytes.[63,65,66] HPV38 E6, possibly in combination with E7 induces telomerase activity, which plays a key role in transformation of human keratinocytes.[67]
Another mechanism behind the lack of cell cycle arrest in Beta-PV expressing cells might be the upregulation of delta-Np73 as a result of p53 accumulation.[68,69] This upregulation prevents p53 to induce the transcription of genes involved in apoptosis and growth suppression, altering the regulation of cell cycle checkpoints that are normally activated by UV radiation.[69]
Several studies investigating the effect of Beta-PV E6 and E7 in organotypic (raft) cultures showed that Beta-PV could delay cell differentiation and disturb keratinocyte outgrowth.[66,70-72]

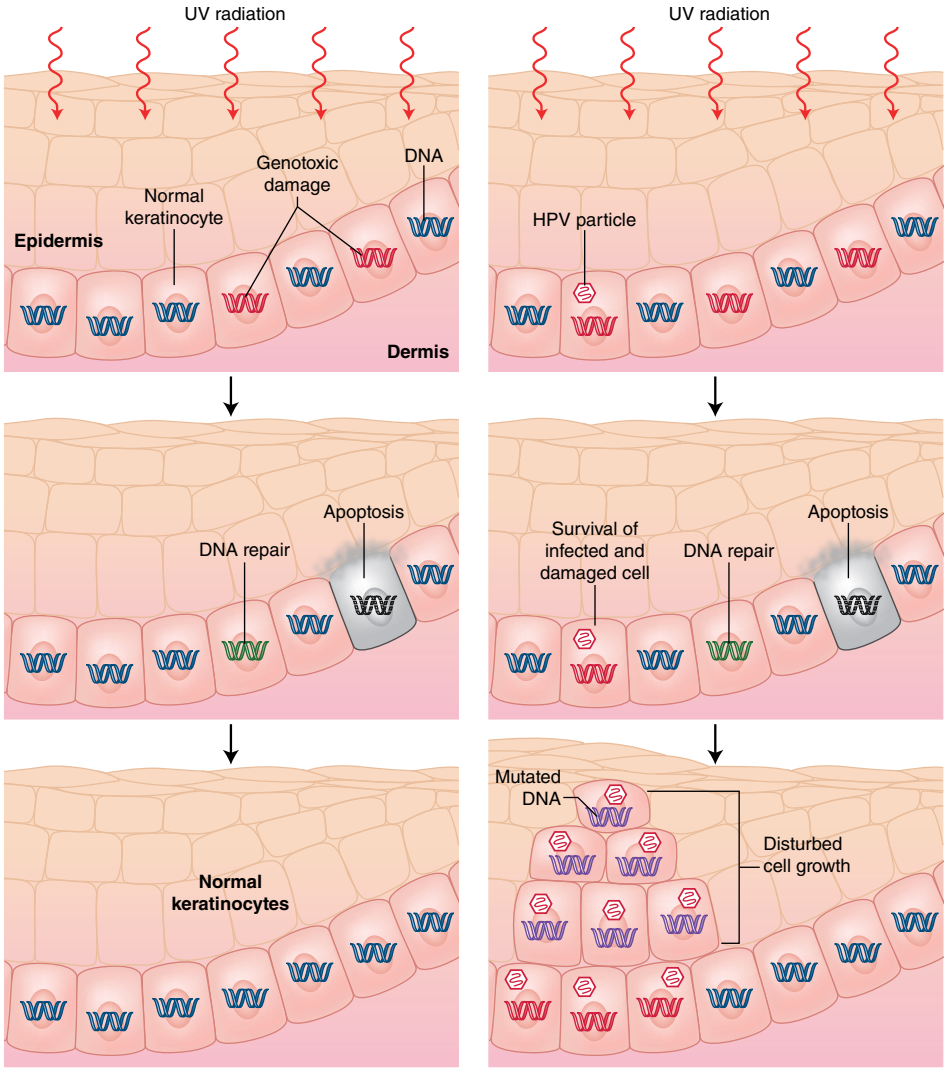


Figure 4. Ultraviolet radiation causes genotoxic damage in the keratinocytes in the basal layer of the epidermis. Panels on the left illustrate DNA repair in the cells or dying due to apoptosis. The panels on the right show the situation when a keratinocyte is infected with HPV. The infected keratinocyte has low ability for DNA repair and is less sensitive to apoptosis. The DNA mutated cell can proliferate and multiply and lead to dysplastic keratinocytes. Currently it is believed that not every mutated cell carries a virus episome. Permission for reprint accepted by NEJM (rights JNBB).

As the vast majority of SCC occurs on sun-exposed sites, it is generally believed that Beta-PV, if involved in skin cancer development, somehow cooperates with UV-radiation in transforming cutaneous epithelial keratinocytes. Beta-PV could confer its possible carcinogenic effect via inhibition of DNA repair and apoptosis in UV-damaged cells which is illustrated in Figure 4.[73]

HPV5 E6 appeared to exert this effect via the degradation of Bak, a protein involved in induction of apoptosis.[74-76] The E6 of HPV8, 20, 22, 38, 76, 92 and 96 can protect UV-treated keratinocytes from apoptosis.[77,78] The oncoproteins E6 and E7 from Beta-PV38 significantly contribute to SCC development in the skin rendering keratinocytes more susceptible to UV-induced carcinogenesis in mice.[79,80] Interference with UV-induced DNA repair and apoptosis by Beta-PV might occur, and epidemiological studies seem suggestive of a joint effect of UV-radiation and Beta-PV infection as well.[50,73,81]

Still other studies have shown that the E6 proteins of HPV5 and HPV8 inhibit the transforming growth factor Beta (TGF-Beta) signaling pathway by the degradation of the SMAD3 transcription factor.[82] TGF-Beta-triggered pathways lead to the synthesis of inhibitors (p16, p17, p21, and p27) of the cyclin-dependent kinases that play a crucial role in the cell cycle. It can be postulated that specific degradation of SMAD3 could negatively regulate inhibitors of the cell cycle and favor cell transition from the G1 to the S phase, allowing viral DNA vegetative replication and, as a side effect, cell transformation.

Additionally, there is evidence that the association of HPV5 and HPV8 E6 proteins with MAML1 inhibits Notch signaling and that Notch signaling plays a role in both virus–host perturbations and tumor genesis.[83]

In HPV positive skin cancers, not all tumor cells contain HPV DNA and the viral load is higher in actinic keratoses.[84] If Beta-PV are involved in the development of skin cancer, they probably play a role in tumor initiation and progression, not in maintenance of the malignant phenotype, through a “hit-and-run” mechanism of viral carcinogenesis.[79]

Epidemiology of HPV infection and skin cancer

HPV carriage in humans

HPV DNA can frequently be detected in skin swabs and plucked hairs both in newborns, children and adults.[29,34,50,85-89] Family members may share some of the same HPV types.[29,90] The prevalence of HPV DNA ranges between 42 and 87%, and 84 and

91%, respectively, using skin swab and plucked hair sampling, and varies also between geographical regions and ethnicities.[25,29,46,90-95]
Viral DNA is persistent in/on the skin.[86,89,95] Increasing age, sun exposure, sunburn, skin type and a medical history of skin cancer are factors which are associated with an increased prevalence of HPV DNA.[25,46,91,96,97] Duration of immunosuppression and immunosuppression itself in OTR is also associated with an increased prevalence of HPV DNA in plucked hair samples compared with the immunocompetent population.[46]
Seropositivity for one or more HPV types is approximately 60% in the general population (most frequently for HPV8 followed by HPV15, 17, 38 and 49), but can be higher in certain subpopulations.[97-99] Serological responses to HPV are also more common in OTR and vary between 80 and 90% to at least one HPV type. Between 45 and 56% of these positive seroresponses are against Beta-PV types. Most commonly detected Beta-PV types are HPV8, 15, 17, 38 and 49.[25,100,101]

Presence of HPV DNA in skin cancer and precursor lesions

Several studies have investigated the prevalence of HPV DNA in SCC and precursor lesions in both immunocompetent individuals and OTR. HPV carriage was nicely summarized by Aldabagh et al.[34] The percentages of HPV DNA positivity vary between immunocompetent individuals and OTR and depend on the detection methods used. The prevalences of HPV DNA in SCC in the immunocompetent population range between 26 and 69%. HPV DNA can also be found in benign lesions, actinic keratosis and normal and sun-exposed skin from both patients with and without SCC.[84,94,102-105] Frequently, Beta-PV types, including HPV5 and 8, are detected in higher rates in actinic keratoses compared to SCC.[106] Viral load of HPV infection was found to be significantly higher in actinic keratoses compared to SCC.[84] In a small study Beta-PV DNA was found to be more often present in perilesional skin than in SCC and mirror site healthy skin, but there are also studies reporting that Beta-PV species 2 is more likely to be identified in SCC than in adjacent healthy skin.[102,103,107].
Numerous studies were carried out in OTR to assess the presence of HPV DNA in keratinocyte carcinomas. De Villiers et al found HPV DNA positivity in 91% of SCC and in situ SCC in 25 OTR, the most prevalent types being among the Beta-PV types.[38] Berkhout et al found similar frequencies and distribution of Beta-PV types in hyperkeratotic papillomas, actinic keratosis and SCC, but lower in BCC, benign lesions and normal skin.[108]

Some studies have compared the prevalence of HPV DNA in SCC and precursor lesions of immunocompetent individuals with OTR. In a German study, HPV DNA was detected more frequently in SCC of OTR (75%) than in immunocompetent patients (47%).[109] Similar HPV prevalences were found in cutaneous warts (91% vs. 94%), pre-malignant skin tumors (38% vs. 36%) and normal skin specimens (17% vs. 16%) for both patient populations. HPV types 5 and 8 were found more frequently in SCC and only in the SCC of the OTR.[109] In a Dutch group of OTR with and without skin cancer, the prevalence of Beta-PV DNA in benign keratotic skin lesions was equally high, around 50%.[108-110] A higher prevalence of Beta-PV DNA was found in lesions from sun-exposed sites with a history of skin cancer.[110] In a Scottish study no difference was detected between lesions from immunocompetent individuals and OTR with HPV15, 24 and 38 as the most frequently detected types. In this study multiple infections were more common in tumors from immunocompetent individuals (70%) compared with those from OTR (26%).[111]

Association between HPV DNA and skin cancer

Case-control studies investigating the association between the presence of Beta-PV DNA and SCC are summarized in Table 2. Most studies were performed with immunocompetent patients and show a statistically significant association between the presence of Beta-PV DNA and SCC or its precursor actinic keratoses, or a non-significant trend in the same direction (Table 2). However, in a large international case-control study, the presence of Beta-PV DNA in eyebrow hairs was only significantly associated with an increased risk of SCC in the Netherlands, but not in Italy and Australia.[87] Overall the Beta-PV DNA positivity was more than 90% for all participants.[87]So far, only one large study was performed with European OTR showing a 2.4 times increased risk of SCC in OTR with Beta-PV DNA in eyebrow hairs.[116]

High viral load of Beta-PV DNA in eyebrow hairs was reported to be associated with increased risk of cutaneous SCC in immunocompetent Australian patients and in OTR, with total load seemingly more important than the load of any specific type.[117] Exposure to individual cutaneous HPV types and the risk of SCC was recently summarized.[118] Many Beta-PV types are associated with an increased risk of SCC, but no specific type stands out. HPV9, 24, 36, 76 and 92 showed the strongest association with SCC.[118]

Table 2. Epidemiological studies summarizing the association between Beta-PV DNA and SCC development

Author (ref)	Study type	Population / country	Infection marker	Method	HPV-types	Cases	Controls	Adjusted odds ratio (95% CI)	Comments
Boxman [36]	Nested case-control/	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	64 KC* 51 BCC 25 SCC	64 51 25	0. 8 (0. 3-1. 8) 0. 6 (0. 2-1. 5) 2. 0 (0. 5-8. 0)	*BCC/SCC/intra-epithelial carcinoma/ KC undefined
Boxman [112]	Cross-sectional	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	276 AK	231	3. 4 (1. 8-6. 5) (M) 1. 0 (0. 6-1. 8) (F)	Significant association between Beta-PV and AK only in men
Struijk [113]	Case-control	IC / the Netherlands	DNA in eyebrow hairs	Type-specific PCR	2, 5, 8, 15, 16, 20, 24, 38	155 SCC	371	1. 7 (1. 1-2. 7)	Association between Beta-PV and SCC with increasing age and male sex
Harwood [114]	Case-control	IC / U.K.	DNA in normal skin biopsies	Degenerate/ Nested PCR	Beta	10 KC*	29	6. 4 (1. 8-22. 9)	*BCC/SCC
Struijk [49]	Case-control	IC / Australia	DNA in eyebrow hairs	Type-specific PCR	5, 8, 15, 20, 24, 38	126 AK 64 SCC	57	1. 6 (0. 8-3. 0) 0. 9 (0. 4-2. 0)	
McBride [115]	Prospective	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	71 1-10 AK 41 > 10 AK	179	1. 8 (0. 7-4. 4)	Association with having more than 10 AK. Significant associations with age over 60 years, fair skin color, high sun exposure
Bouwes Bavinck [87]	Case control	IC / Australia (AU), Italy (I), the Netherlands (NL)	DNA in eyebrow hairs	PCR-reverse hybridization assay	Beta	689 SCC	845	2. 8 (1. 3-5-8) NL 1. 7 (0. 79-3. 6) I 0. 91 (0. 53-1. 6) AU	
Proby [116]	Case-control	IS / France, Italy, the Netherlands, U.K.	DNA in eyebrow hairs	PCR-reverse hybridization assay	Beta	210 SCC	394	2. 4 (1-5. 4)	For HPV5, 9, 24 and 36

IC;immunocompetent, IS;immunosuppressed/transplant patients, KC; keratinocyte cancer, AK; actinic keratosis, BCC; basal cell carcinoma, SCC; squamous cell carcinoma

Association between HPV serology and skin cancer

Most studies investigating the association between serologic antibody responses to HPV and SCC were also carried out in the immunocompetent population and show a statistically significant association between the presence of Beta-PV antibodies and SCC, or a non-significant trend in the same direction (Table 2 and 3).

The odds ratios are increasing when there are more positive serological responses against increasing numbers of Beta-PV types.[48,50,121,124,125]The association with SCC is exclusively found for Beta-PV types, particularly HPV5, 8, 9, 15, 17, 20, 24, 36, 38, 49, 75, 76 and 92, but not for Alpha, Gamma, Mu or Nu HPV types.[118]

In an international case-control study a positive antibody response against 4 or more Beta-PV types was associated with a doubled risk for SCC in Australia and the Netherlands.[87] A population-based cohort study in Australia, however, revealed no associations between the presence of any Beta-PV antibodies and the occurrence of SCC. However, among people who were less than 50 years old in 1992, the presence of Beta-PV antibodies was associated with a two-fold increased risk of SCC.[126] In the United Kingdom in a prospective pilot study among 39 SCC cases and 80 controls also no statistically significant differences were found in the seroprevalence of antibodies against any of 38 HPV types.[128] HPV seropositivity was strongly associated with the risk of developing a second SCC after 5 years for both Beta and Gamma HPV types.[127]

With respect to the presence of Beta-PV DNA in (pre-)malignant and benign skin tumors, no correlation was found with serum antibodies to the same HPV type measured. However, seropositivity to any Beta-PV type was significantly more common among patients positive for HPV DNA of any HPV type and seroprevalences were higher for SCC patients compared to BCC patients.[85]

Viral transcriptional activity and skin cancer

Viral transcriptional activity was investigated in SCC from both immunocompetent individuals and OTR. Only a portion of SCC was positive for cutaneous HPV using in situ hybridization (122), but similarly low transcriptional activity in warts in OTR was observed.[129] The biological activity of HPV types found in tumor tissues was assessed by examinating HPV E6/E7 RNA expression for cutaneous HPV types. HPV DNA was detected in 25 of 31 tissue samples. E6/E7 transcripts of HPV8, 9 and 15 were found in low copy numbers in one SCC and three AKs, but not in normal skin or verrucae vulgaris.[130]

Table 3 Epidemiological studies summarizing the association between Beta-PV seroprevalence and SCC development

Author (ref)	Study type	Population / country	Infection marker	Method	HPV-types	Cases	Controls	Adjusted odds ratio (95% CI)	Comments
Steger [119]	Case-control	IC / Germany	L1 serology	Western blot	8	11	445	10. 7 (2. 5-63. 2)	
Stark [120]	Case-control	IC / Germany	L1 serology	ELISA	8	14 SCC	210	30. 3 (7. 4-142. 5)	
Bouwes Bavinck [121]	Case-control	IC / the Netherlands	L1 serology	ELISA	8	13 SCC	82	3. 1 (0. 7-13. 3)	
Feltkamp [48]	Case-control	IC / the Netherlands	L1 serology	ELISA	5, 8, 15, 20, 24, 38	540 SCC	333	1. 4 (0. 8-2. 5)	
Masini [122]	Case-control	IC / Italy	L1 serology	ELISA	8, 15, 23, 36	46 SCC	84	3. 2 (1. 3-7. 9) (HPV8) 0. 4 (0. 2-0. 9) (HPV15) 1. 0 (0. 3-3. 3) HPV23) 2. 8 (0. 8-10. 0) (HPV36)	
Karagas [50]	Case-control	IC / U.S.A.	L1 serology	multiplex	Beta	252 SCC	461	1. 5 (1. 1-2. 1)	
Struijk [49]	Case-control	IC / Australia	L1/E6 serology	ELISA	5, 8, 15, 16, 20, 24, 38	126 AK 64 SCC	57	2. 3 (0. 9-4. 9)(L1) 0. 6 (0. 3-1. 3) (E6) 3. 9 (1. 4-10. 7) (L1) 0. 5 (0. 2-1. 1) (E6)	Associations between Beta-PV L1 and E6 serology and AK/SCC
Casabonne [123]	Nested case-control	IC / U.K.	L1 serology	multiplex	Beta	39 SCC	80	0. 5 (0. 1-1. 7)* 1. 0 (0. 4-2. 5) **	Association between 1* or 2+** Beta-PV type(s) and SCC
Karagas [124]	Case-control	IC / U.S.A.	L1 serology	multiplex	Beta	663 SCC	805	1. 0 (0. 7-1. 3)* 1. 4 (1. 0-2. 0)** 1. 5 (1. 0-2. 2)*** 1. 7 (1. 1-2. 6)****	Association between 1* or 2-3**, 4-8*** or >8**** Beta-PV type(s) and SCC
Proby [116]	Case-control	IS / France, Italy, the Netherlands, U.K.	L1 serology	multiplex	Beta	210 SCC	394	1. 6 (1. 1-2. 5)	Association for concordant detection of both antibodies and DNA for at least one Beta-PV type

Waterboer [125]	Case-control	IC / Italy	L1 serology	multiplex	Beta / Gamma	43 SCC	77	2. 8 (1. 1-7. 1)* 2. 6 (1. 0-6. 5)** 3. 0 (1. 2-7. 9)*** 3. 4 (1. 2-9. 5)****	*HPV15 **HPV17 ***HPV38 ****HPV50
Bouwes Bavinck [87]	Case control	IC / Australia (AU), Italy (I), the Netherlands (NL)	L1 serology	multiplex	Beta	689 SCC	845	>= 4 types postive 2. 0 (1. 2-3. 4) * 2. 2 (1. 4-3. 3) ** 1. 6 (0. 94-2. 7) ***	 *NL **AU ***I fair skinned
Plasmeijer [126]	Prospective	IC / Australia	L1 serology	multiplex	Beta	150 SCC	1161	1. 0 (0. 7-1. 4)* 2. 1 (1. 0-4. 6)**	*HR for being positive for at least 1 Beta-PV type **HR in subset of people <50y
Paradisi [127]	Case-control	IC / Italy	L1 serology	multiplex	Beta / Gamma	16 SCC	48	4. 25 (1. 22-15. 58)* 8. 08 (1. 05-62. 42)** 3. 01 (1. 01-8. 97)*** 3. 46 (1. 08-11. 5)****	*HR HPV24 **HR HPV15 ***HR HPV95 ****HR HPV60

IC;immunocompetent, IS;immunosuppressed/transplant patients, KC; keratinocyte cancer, AK; actinic keratosis, BCC; basal cell carcinoma, SCC; squamous cell carcinoma

Transcriptome sequencing, performed on 31 SCC, failed to identify HPV expression in any of the skin tumors.[131]

Human papillomavirus and basal cell carcinoma

A recent systematic review showed no association between Beta-PV DNA or Beta-PV serological responses and BCC.[118] In a subgroup of patients with Beta-PV DNA positive BCC, however, an association between a serological response against Beta-PV and BCC could be observed.[132] Some Beta-PV types could up-regulate the p16INK4a and Akt/P13K pathway and might play a role in the carcinogenesis of BCC.[133] The evidence that HPV is involved in BCC carcinogenesis is clearly much weaker than the possible involvement of HPV in cutaneous SCC carcinogenesis.

Conclusions

The role of Beta-PV in cutaneous SCC carcinogenesis is still enigmatic and clearly different from the role of mucosal Alpha-PV types in cervical cancer. Despite experimental evidence and a plausible hypothesis of a biological mechanism supporting a carcinogenic role of Beta-PV in SCC carcinogenesis, the epidemiological studies are still difficult to interpret. The viral load and the plurality of Beta-PV types are an important obstacle to identify the responsible HPV types in epidemiological studies. Several case-control studies observed an association between Beta-PV antibodies in the serum and/or Beta-PV DNA in plucked eyebrow hairs and development of cutaneous SCC, but so far no specific high risk Beta-PV types could be identified. Possibly much larger studies will be needed to identify the responsible HPV types in between the massive number of bystander HPV types. It is also not clear what the implications are in daily practice for Beta-PV infections in OTR.

Since Beta-PV is not integrated in the human cellular DNA and is not necessary for the maintenance of the malignant phenotype of cutaneous SCC, the carcinogenic effect, if present, is subtle and probably exerted early in carcinogenesis. This fits with observations that premalignant lesions such as actinic keratoses often contain a higher load of Beta-PV compared to SCC. By impairing DNA repair and UV-induced apoptosis, these persistent skin viruses may, after a long period, lead to cellular instability and sequentially cause field changes, actinic keratoses and finally

cutaneous SCC. Whether different Beta-PV types have different effects on cellular mechanisms and a combination of these HPV types may further increase the risk of cutaneous SCC is unknown. Further research is clearly needed to unravel the secrets of Beta-PV in SCC carcinogenesis.

References

1. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. NEnglJMed. 2003;348(17):1681-91.
2. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in the Netherlands. Transplantation. 1990;49(3):506-9.
3. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. BrJDermatol. 2006;154(3):498-504.
4. Tessari G, Naldi L, Boschiero L, Nacchia F, Fior F, Forni A,... Girolomoni G. Incidence and clinical predictors of a subsequent nonmelanoma skin cancer in solid organ transplant recipients with a first nonmelanoma skin cancer: a multicenter cohort study. ArchDermatol. 2010;146(3):294-9.
5. Terhorst D, Drecoll U, Stockfleth E, Ulrich C. Organ transplant recipients and skin cancer: assessment of risk factors with focus on sun exposure. BrJDermatol. 2009;161 Suppl 3:85-9.
6. O'Donovan P, Perrett CM, Zhang X, Montaner B, Xu YZ, Harwood CA,... Karran P. Azathioprine and UVA light generate mutagenic oxidative DNA damage. Science. 2005;309(5742):1871-4.
7. Perrett CM, Walker SL, O'Donovan P, Warwick J, Harwood CA, Karran P, McGregor JM. Azathioprine treatment photosensitizes human skin to ultraviolet A radiation. BrJDermatol. 2008;159(1):198-204.
8. Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation. 2004;77(12):1777-82.
9. Euvrard S, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I,... Dantal J. Sirolimus and secondary skin-cancer prevention in kidney transplantation. NEnglJMed. 2012;367(4):329-39.
10. JM H-vdA, Harden PN, Hoitsma AJ, Proby CM, Wolterbeek R, Bouwes Bavinck JN, de Fijter JW. Two-year randomized controlled prospective trial converting treatment of stable renal transplant recipients with cutaneous invasive squamous cell carcinomas to sirolimus. JClinOncol. 2013;31(10):1317-23.
11. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. Transplantation. 2005;80(7):883-9.
12. Bruggink SC, de Koning MN, Gussekloo J, Egberts PF, Ter SJ, Feltkamp MC,... Eekhof JA. Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. JClinVirol. 2012;55(3):250-5.
13. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur HH. Classification of papillomaviruses. Virology. 2004;324(1):17-27.
14. Jablonska S, Orth G, Obalek S, Croissant O. Cutaneous warts. Clinical, histologic, and virologic correlations. ClinDermatol. 1985;3(4):71-82.
15. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El GF,... Cogliano V. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009;10(4):321-2.
16. Jablonska S, Dabrowski J, Jakubowicz K. Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. Cancer Res. 1972;32(3):583-9.
17. Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rzesa G. Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. ProcNatlAcadSciUSA. 1978;75(3):1537-41.
18. Orth G, Jablonska S, Jarzabek-Chorzelska M, Obalek S, Rzesa G, Favre M, Croissant O. Characteristics of the lesions and risk of malignant conversion associated with the type of human papillomavirus involved in epidermodysplasia verruciformis. Cancer Res. 1979;39(3):1074-82.
19. Lutzner MA. Epidermodysplasia verruciformis. An autosomal recessive disease characterized by viral warts and skin cancer. A model for viral oncogenesis. BullCancer. 1978;65(2):169-82.
20. Human papillomaviruses. IARC Monogr EvalCarcinogRisks Hum. 2007;90:1-636.
21. Bouwes Bavinck JN, Euvrard S, Naldi L, Nindl I, Proby CM, Neale R,... Harwood CA. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in the Netherlands, United Kingdom, Germany, France, and Italy. JInvest Dermatol.

2007;127(7):1647-56.
22. Burk RD, Chen Z, Van DK. Human papillomaviruses: genetic basis of carcinogenicity. Public Health Genomics. 2009;12(5-6):281-90.
23. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci. 2006;11:2286-302.
24. Bernard HU, Burk RD, Chen Z, Van DK, zur HH, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9.
25. Antonsson A, Forslund O, Ekberg H, Sterner G, Hansson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensalic nature of these viruses. JVirol. 2000;74(24):11636-41.
26. Boxman IL, Berkhout RJ, Mulder LH, Wolkers MC, Bouwes Bavinck JN, Vermeer BJ, Ter SJ. Detection of human papillomavirus DNA in plucked hairs from renal transplant recipients and healthy volunteers. JInvest Dermatol. 1997;108(5):712-5.
27. Feltkamp MC, de Koning MN, Bouwes Bavinck JN, Ter SJ. Betapapillomaviruses: innocent bystanders or causes of skin cancer. JClinVirol. 2008;43(4):353-60.
28. Kohler A, Forschner T, Meyer T, Ulrich C, Gottschling M, Stockfleth E, Nindl I. Multifocal distribution of cutaneous human papillomavirus types in hairs from different skin areas. BrJDermatol. 2007;156(5):1078-80.
29. Weissenborn SJ, de Koning MN, Wieland U, Quint WG, Pfister HJ. Intrafamilial transmission and family-specific spectra of cutaneous Betapapillomaviruses. JVirol. 2009;83(2):811-6.
30. Middleton K, Peh W, Southern S, Griffin H, Sotlar K, Nakahara T,... Doorbar J. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. JVirol. 2003;77(19):10186-201.
31. Peh WL, Middleton K, Christensen N, Nicholls P, Egawa K, Sotlar K,... Doorbar J. Life cycle heterogeneity in animal models of human papillomavirus-associated disease. JVirol. 2002;76(20):10401-16.
32. Doorbar J. Papillomavirus life cycle organization and biomarker selection. DisMarkers. 2007;23(4):297-313.
33. Egawa K, Iftner A, Doorbar J, Honda Y, Iftner T. Synthesis of viral DNA and late capsid protein L1 in parabasal spinous cell layers of naturally occurring benign warts infected with human papillomavirus type 1. Virology. 2000;268(2):281-93.
34. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? DermatolSurg. 2013;39(1 Pt 1):1-23.
35. Berkhout RJ, Tieben LM, Smits HL, Bouwes Bavinck JN, Vermeer BJ, Ter SJ. Nested PCR approach for detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients. JClinMicrobiol. 1995;33(3):690-5.
36. Boxman IL, Russell A, Mulder LH, Bouwes Bavinck JN, Schegget JT, Green A. Case-control study in a subtropical Australian population to assess the relation between non-melanoma skin cancer and epidermodysplasia verruciformis human papillomavirus DNA in plucked eyebrow hairs. The Nambour Skin Cancer Prevention Study Group. IntJCancer. 2000;86(1):118-21.
37. de Jong-Tieben LM, Berkhout RJ, Smits HL, Bouwes Bavinck JN, Vermeer BJ, van der Woude FJ, Ter SJ. High frequency of detection of epidermodysplasia verruciformis-associated human papillomavirus DNA in biopsies from malignant and premalignant skin lesions from renal transplant recipients. JInvest Dermatol. 1995;105(3):367-71.
38. de Villiers EM, Lavergne D, McLaren K, Benton EC. Prevailing papillomavirus types in non-melanoma carcinomas of the skin in renal allograft recipients. IntJCancer. 1997;73(3):356-61.
39. de KM, Quint W, Struijk L, Kleter B, Wanningen P, van Doorn LJ,... Ter SJ. Evaluation of a novel highly sensitive, broad-spectrum PCR-reverse hybridization assay for detection and identification of Beta-papillomavirus DNA. JClinMicrobiol. 2006;44(5):1792-800.
40. Forslund O, Ly H, Higgins G. Improved detection of cutaneous human papillomavirus DNA by single tube nested 'hanging droplet' PCR. JVirolMethods. 2003;110(2):129-36.
41. Gheit T, Billoud G, de Koning MN, Gemig-

nani F, Forslund O, Sylla BS,... Tommasino M. Development of a sensitive and specific multiplex PCR method combined with DNA microarray primer extension to detect Betapapillomavirus types. JClinMicrobiol. 2007;45(8):2537-44.
42. Harwood CA, Spink PJ, Surentheran T, Leigh IM, Hawke JL, Proby CM,... McGregor JM. Detection of human papillomavirus DNA in PUVA-associated non-melanoma skin cancers. JInvest Dermatol. 1998;111(1):123-7.
43. Nindl I, Koehler A, Meyer T, Forschner T, Meijer CJ, Snijders PJ,... Stockfleth E. Detection of human papillomavirus DNA in primary squamous cell carcinoma and metastases. BrJDermatol. 2006;154(4):797-9.
44. Weissenborn SJ, Wieland U, Junk M, Pfister H. Quantification of Beta-human papillomavirus DNA by real-time PCR. NatProtoc. 2010;5(1):1-13.
45. Michael KM, Forslund O, Bacevskij O, Waterboer T, Bravo IG, Pawlita M, Schmitt M. Bead-based multiplex genotyping of 58 cutaneous human papillomavirus types. JClinMicrobiol. 2011;49(10):3560-7.
46. de Koning MN, Weissenborn SJ, Abeni D, Bouwes Bavinck JN, Euvrard S, Green AC,... Feltkamp MC. Prevalence and associated factors of Betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma. JGenVirol. 2009;90(Pt 7):1611-21.
47. Nindl I, Kohler A, Gottschling M, Forschner T, Lehmann M, Meijer CJ,... Stockfleth E. Extension of the typing in a general-primer-PCR reverse-line-blotting system to detect all 25 cutaneous Beta human papillomaviruses. JVirolMethods. 2007;146(1-2):1-4.
48. Feltkamp MC, Broer R, di Summa FM, Struijk L, van der Meijden E, Verlaan BP,... Bouwes Bavinck JN. Seroreactivity to epidermodysplasia verruciformis-related human papillomavirus types is associated with nonmelanoma skin cancer. Cancer Res. 2003;63(10):2695-700.
49. Struijk L, Hall L, van der Meijden E, Wanningen P, Bouwes Bavinck JN, Neale R,... Feltkamp MC. Markers of cutaneous human papillomavirus infection in individuals with tumor-free skin, actinic keratoses, and squamous cell carcinoma. Cancer EpidemiolBiomarkers Prev. 2006;15(3):529-35.
50. Karagas MR, Nelson HH, Sehr P, Waterboer T, Stukel TA, Andrew A,... Pawlita M. Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. JNatlCancer Inst. 2006;98(6):389-95.
51. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO,... Pawlita M. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. ClinChem. 2005;51(10):1845-53.
52. Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. JNatlCancer Inst-Monogr. 2003(31):80-8.
53. Peh WL, Doorbar J. Detection of papillomavirus proteins and DNA in paraffin-embedded tissue sections. Methods MolMed. 2005;119:49-59.
54. Chew K, Rooney PH, Cruickshank ME, Murray GI. Laser capture microdissection and PCR for analysis of human papilloma virus infection. Methods MolBiol. 2005;293:295-300.
55. Doorbar J. The papillomavirus life cycle. JClinVirol. 2005;32 Suppl 1:S7-15.
56. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. MicrobiolMolBiolRev. 2004;68(2):362-72.
57. Haller K, Stubenrauch F, Pfister H. Differentiation-dependent transcription of the epidermodysplasia verruciformis-associated human papillomavirus type 5 in benign lesions. Virology. 1995;214(1):245-55.
58. Ostrow RS, Bender M, Niimura M, Seki T, Kawashima M, Pass F, Faras AJ. Human papillomavirus DNA in cutaneous primary and metastasized squamous cell carcinomas from patients with epidermodysplasia verruciformis. ProcNatlAcadSciUSA. 1982;79(5):1634-8.
59. Lippens S, Denecker G, Ovaere P, Vandenabeele P, Declercq W. Death penalty for keratinocytes: apoptosis versus cornification. Cell DeathDiffer. 2005;12 Suppl 2:1497-508.
60. Blanpain C, Fuchs E. Epidermal stem cells of the skin. AnnuRevCell DevBiol. 2006;22:339-73.
61. Munger K, Basile JR, Duensing S, Eichten A, Gonzalez SL, Grace M, Zacny VL. Biological activities and molecular targets of the human papillomavirus E7 oncoprotein.

Oncogene. 2001;20(54):7888-98.
62. zur Hausen H. Immortalization of human cells and their malignant conversion by high risk human papillomavirus genotypes. SeminCancer Biol. 1999;9(6):405-11.
63. Caldeira S, Zehbe I, Accardi R, Malanchi I, Dong W, Giarre M,... Tommasino M. The E6 and E7 proteins of the cutaneous human papillomavirus type 38 display transforming properties. JVirol. 2003;77(3):2195-206.
64. Dong WL, Caldeira S, Sehr P, Pawlita M, Tommasino M. Determination of the binding affinity of different human papillomavirus E7 proteins for the tumour suppressor pRb by a plate-binding assay. JVirol Methods. 2001;98(1):91-8.
65. Bedard KM, Underbrink MP, Howie HL, Galloway DA. The E6 oncoproteins from human Betapapillomaviruses differentially activate telomerase through an E6AP-dependent mechanism and prolong the lifespan of primary keratinocytes. JVirol. 2008;82(8):3894-902.
66. Cordano P, Gillan V, Bratlie S, Bouvard V, Banks L, Tommasino M, Campo MS. The E6E7 oncoproteins of cutaneous human papillomavirus type 38 interfere with the interferon pathway. Virology. 2008;377(2):408-18.
67. Gabet AS, Accardi R, Bellopede A, Popp S, Boukamp P, Sylla BS,... Tommasino M. Impairment of the telomere/telomerase system and genomic instability are associated with keratinocyte immortalization induced by the skin human papillomavirus type 38. FASEB J. 2008;22(2):622-32.
68. Accardi R, Dong W, Smet A, Cui R, Hautefeuille A, Gabet AS,... Tommasino M. Skin human papillomavirus type 38 alters p53 functions by accumulation of deltaNp73. EMBO Rep. 2006;7(3):334-40.
69. Dong W, Arpin C, Accardi R, Gissmann L, Sylla BS, Marvel J, Tommasino M. Loss of p53 or p73 in human papillomavirus type 38 E6 and E7 transgenic mice partially restores the UV-activated cell cycle checkpoints. Oncogene. 2008;27(20):2923-8.
70. Akgul B, Garcia-Escudero R, Ghali L, Pfister HJ, Fuchs PG, Navsaria H, Storey A. The E7 protein of cutaneous human papillomavirus type 8 causes invasion of human keratinocytes into the dermis in organotypic cultures of skin. Cancer Res. 2005;65(6): 2216-23.
71. Boxman IL, Mulder LH, Noya F, de W, V, Gibbs S, Broker TR,... Ter SJ. Transduction of the E6 and E7 genes of epidermodysplasia-verruciformis-associated human papillomaviruses alters human keratinocyte growth and differentiation in organotypic cultures. JInvest Dermatol. 2001;117(6): 1397-404.
72. Kazem S, van der Meijden E, Struijk L, de Gruijl FR, Feltkamp MC. Human papillomavirus 8 E6 disrupts terminal skin differentiation and prevents pro-Caspase-14 cleavage. Virus Res. 2012;163(2):609-16.
73. Bouwes Bavinck JN, Feltkamp MC. Milk of human kindness?--HAMLET, human papillomavirus, and warts. NEnglJMed. 2004;350(26):2639-42.
74. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev. 2000;14(23):3065-73.
75. Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. Oncogene. 2000;19(4):592-8.
76. Leverrier S, Bergamaschi D, Ghali L, Ola A, Warnes G, Akgul B,... Storey A. Role of HPV E6 proteins in preventing UVB-induced release of pro-apoptotic factors from the mitochondria. Apoptosis. 2007;12(3):549-60.
77. Struijk L, van der Meijden E, Kazem S, Ter SJ, de Gruijl FR, Steenbergen RD, Feltkamp MC. Specific Betapapillomaviruses associated with squamous cell carcinoma of the skin inhibit UVB-induced apoptosis of primary human keratinocytes. JGenVirol. 2008;89(Pt 9):2303-14.
78. Underbrink MP, Howie HL, Bedard KM, Koop JI, Galloway DA. E6 proteins from multiple human Betapapillomavirus types degrade Bak and protect keratinocytes from apoptosis after UVB irradiation. JVirol. 2008;82(21):10408-17.
79. Pfister H. Chapter 8: Human papillomavirus and skin cancer. JNatlCancer InstMonogr. 2003(31):52-6.
80. Viarisio D, Mueller-Decker K, Kloz U, Aengeneyndt B, Kopp-Schneider A, Grone HJ,... Tommasino M. E6 and E7 from Beta-PV38 cooperate with ultraviolet light in the de-

velopment of actinic keratosis-like lesions and squamous cell carcinoma in mice. PLoSPathog. 2011;7(7):e1002125.
81. Hall L, Struijk L, Neale RE, Feltkamp MC. Re: Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. JNatlCancer Inst. 2006;98(19):1425-6.
82. Mendoza JA, Jacob Y, Cassonnet P, Favre M. Human papillomavirus type 5 E6 oncoprotein represses the transforming growth factor Beta signaling pathway by binding to SMAD3. JVirol. 2006;80(24):12420-4.
83. Rozenblatt-Rosen O, Deo RC, Padi M, Adelmant G, Calderwood MA, Rolland T,... Vidal M. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. Nature. 2012; 487(7408):491-5.
84. Weissenborn SJ, Nindl I, Purdie K, Harwood C, Proby C, Breuer J,... Wieland U. Human papillomavirus-DNA loads in actinic keratoses exceed those in non-melanoma skin cancers. JInvest Dermatol. 2005;125(1):93-7.
85. Andersson K, Waterboer T, Kirnbauer R, Slupetzky K, Iftner T, de Villiers EM,... Dillner J. Seroreactivity to cutaneous human papillomaviruses among patients with nonmelanoma skin cancer or benign skin lesions. Cancer EpidemiolBiomarkers Prev. 2008;17(1):189-95.
86. Antonsson A, Karanfilovska S, Lindqvist PG, Hansson BG. General acquisition of human papillomavirus infections of skin occurs in early infancy. JClinMicrobiol. 2003;41(6):2509-14.
87. Bouwes Bavinck JN, Neale RE, Abeni D, Euvrard S, Green AC, Harwood CA,... Feltkamp MC. Multicenter study of the association between Betapapillomavirus infection and cutaneous squamous cell carcinoma. Cancer Res. 2010;70(23):9777-86.
88. Boxman IL, Mulder LH, Russell A, Bouwes Bavinck JN, Green A, ter Schegget J. Human papillomavirus type 5 is commonly present in immunosuppressed and immunocompetent individuals. BrJDermatol. 1999;141(2):246-9.
89. de Koning MN, Struijk L, Bouwes Bavinck JN, Kleter B, Ter SJ, Quint WG, Feltkamp MC. Betapapillomaviruses frequently persist in the skin of healthy individuals. JGenVirol. 2007;88(Pt 5):1489-95.
90. Hsu JY, Chen AC, Keleher A, McMillan NA, Antonsson A. Shared and persistent asymptomatic cutaneous human papillomavirus infections in healthy skin. JMedVirol. 2009;81(8):1444-9.
91. Alotaibi L, Provost N, Gagnon S, Franco EL, Coutlee F. Diversity of cutaneous human papillomavirus types in individuals with and without skin lesion. JClinVirol. 2006;36(2):133-40.
92. Antonsson A, Erfurt C, Hazard K, Holmgren V, Simon M, Kataoka A,... Hansson BG. Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents. JGenVirol. 2003;84(Pt 7):1881-6.
93. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. JGenVirol. 1999;80 (Pt 9):2437-43.
94. Forslund O, Ly H, Reid C, Higgins G. A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis. BrJDermatol. 2003;149(1):64-73.
95. Hazard K, Karlsson A, Andersson K, Ekberg H, Dillner J, Forslund O. Cutaneous human papillomaviruses persist on healthy skin. JInvest Dermatol. 2007;127(1):116-9.
96. Chen AC, McMillan NA, Antonsson A. Human papillomavirus type spectrum in normal skin of individuals with or without a history of frequent sun exposure. JGenVirol. 2008;89(Pt 11):2891-7.
97. Iannacone MR, Michael KM, Giuliano AR, Waterboer T, Pawlita M, Rollison DE. Risk factors for cutaneous human papillomavirus seroreactivity among patients undergoing skin cancer screening in Florida. JInfectDis. 2010;201(5):760-9.
98. Waterboer T, Neale R, Michael KM, Sehr P, de Koning MN, Weissenborn SJ,... Pawlita M. Antibody responses to 26 skin human papillomavirus types in the Netherlands, Italy and Australia. JGenVirol. 2009;90(Pt 8):1986-98.
99. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H,... Pawlita M. Seroprevalence of 34 human papillomavirus types in the German general population. PLoSPat-

hog. 2008;4(6):e1000091.
100. Antonsson A, Waterboer T, Bouwes Bavinck JN, Abeni D, de KM, Euvrard S,... Neale RE. Longitudinal study of seroprevalence and serostability of 34 human papillomavirus types in European organ transplant recipients. Virology. 2013;436(1):91-9.
101. Casabonne D, Waterboer T, Michael KM, Pawlita M, Lally A, Mitchell L,... Harwood C. The sero-epidemiology of human papillomavirus among Caucasian transplant recipients in the UK. InfectAgentCancer. 2009;4:13.
102. Asgari MM, Kiviat NB, Critchlow CW, Stern JE, Argenyi ZB, Raugi GJ,... de Villiers EM. Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. JInvest Dermatol. 2008;128(6):1409-17.
103. Forslund O, Iftner T, Andersson K, Lindelof B, Hradil E, Nordin P,... de Villiers EM. Cutaneous human papillomaviruses found in sun-exposed skin: Beta-papillomavirus species 2 predominates in squamous cell carcinoma. JInfectDis. 2007;196(6):876-83.
104. Meyer T, Arndt R, Christophers E, Nindl I, Stockfleth E. Importance of human papillomaviruses for the development of skin cancer. Cancer DetectPrev. 2001;25(6):533-47.
105. Patel AS, Karagas MR, Perry AE, Nelson HH. Exposure profiles and human papillomavirus infection in skin cancer: an analysis of 25 genus Beta-types in a population-based study. JInvest Dermatol. 2008;128(12):2888-93.
106. Pfister H, Fuchs PG, Majewski S, Jablonska S, Pniewska I, Malejczyk M. High prevalence of epidermodysplasia verruciformis-associated human papillomavirus DNA in actinic keratoses of the immunocompetent population. ArchDermatolRes. 2003;295(7):273-9.
107. Plasmeijer EI, Neale RE, Buettner PG, de Koning MN, ter Schegget J, Quint WG,... Feltkamp MC. Betapapillomavirus infection profiles in tissue sets from cutaneous squamous cell-carcinoma patients. IntJCancer. 2010;126(11):2614-21.
108. Berkhout RJ, Bouwes Bavinck JN, Ter SJ. Persistence of human papillomavirus DNA in benign and (pre)malignant skin lesions from renal transplant recipients. JClinMicrobiol. 2000;38(6):2087-96.
109. Meyer T, Arndt R, Nindl I, Ulrich C, Christophers E, Stockfleth E. Association of human papillomavirus infections with cutaneous tumors in immunosuppressed patients. TransplInt. 2003;16(3):146-53.
110. de Jong-Tieben LM, Berkhout RJ, ter Schegget J, Vermeer BJ, de Fijter JW, Bruijn JA,... Bouwes Bavinck JN. The prevalence of human papillomavirus DNA in benign keratotic skin lesions of renal transplant recipients with and without a history of skin cancer is equally high: a clinical study to assess risk factors for keratotic skin lesions and skin cancer. Transplantation. 2000;69(1):44-9.
111. Mackintosh LJ, de Koning MN, Quint WG, Ter SJ, Morgan IM, Herd RM, Campo MS. Presence of Beta human papillomaviruses in nonmelanoma skin cancer from organ transplant recipients and immunocompetent patients in the West of Scotland. BrJDermatol. 2009;161(1):56-62.
112. Boxman IL, Russell A, Mulder LH, Bouwes Bavinck JN, Ter SJ, Green A. Association between epidermodysplasia verruciformis-associated human papillomavirus DNA in plucked eyebrow hair and solar keratoses. JInvest Dermatol. 2001;117(5):1108-12.
113. Struijk L, Bouwes Bavinck JN, Wanningen P, van der Meijden E, Westendorp RG, ter Schegget J, Feltkamp MC. Presence of human papillomavirus DNA in plucked eyebrow hairs is associated with a history of cutaneous squamous cell carcinoma. JInvest Dermatol. 2003;121(6):1531-5.
114. Harwood CA, Surentheran T, Sasieni P, Proby CM, Bordea C, Leigh IM,... McGregor JM. Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. BrJDermatol. 2004;150(5):949-57.
115. McBride P, Neale R, Pandeya N, Green A. Sun-related factors, Betapapillomavirus, and actinic keratoses: a prospective study. ArchDermatol. 2007;143(7):862-8.
116. Proby CM, Harwood CA, Neale RE, Green AC, Euvrard S, Naldi L,... Bouwes Bavinck JN. A case-control study of Betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. AmJTransplant. 2011;11(7):1498-508.

117. Neale RE, Weissenborn S, Abeni D, Bouwes Bavinck JN, Euvrard S, Feltkamp MC,... Pfister H. Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. Cancer EpidemiolBiomarkers Prev. 2013;22(4):719-27.
118. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G. A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology. 2013;445(1-2):224-31.
119. Steger G, Olszewsky M, Stockfleth E, Pfister H. Prevalence of antibodies to human papillomavirus type 8 in human sera. JVirol. 1990;64(9):4399-406.
120. Stark S, Petridis AK, Ghim SJ, Jenson AB, Bouwes Bavinck JN, Gross G,... Pfister H. Prevalence of antibodies against virus-like particles of Epidermodysplasia verruciformis-associated HPV8 in patients at risk of skin cancer. JInvest Dermatol. 1998;111(4):696-701.
121. Bouwes Bavinck JN, Stark S, Petridis AK, Marugg ME, ter Schegget J, Westendorp RG,... Pfister H. The presence of antibodies against virus-like particles of epidermodysplasia verruciformis-associated humanpapillomavirus type 8 in patients with actinic keratoses. BrJDermatol. 2000;142(1):103-9.
122. Masini C, Fuchs PG, Gabrielli F, Stark S, Sera F, Ploner M,... Abeni D. Evidence for the association of human papillomavirus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. ArchDermatol. 2003;139(7):890-4.
123. Casabonne D, Waterboer T, Michael KM, Pawlita M, Mitchell L, Newton R,... Proby C. The seroprevalence of human papillomavirus by immune status and by ethnicity in London. InfectAgentCancer. 2009;4:14.
124. Karagas MR, Waterboer T, Li Z, Nelson HH, Michael KM, Bouwes Bavinck JN, Pawlita M. Genus Beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. BMJ. 2010;341:c2986.
125. Waterboer T, Abeni D, Sampogna F, Rother A, Masini C, Sehr P,... Pawlita M. Serological association of Beta and Gamma human papillomaviruses with squamous cell carcinoma of the skin. BrJDermatol. 2008;159(2):457-9.
126. Plasmeijer EI, Pandeya N, O'Rourke P, Pawlita M, Waterboer T, Feltkamp MC,... Neale RE. The Association between cutaneous squamous cell carcinoma and Betapapillomavirus seropositivity: a cohort study. Cancer EpidemiolBiomarkers Prev. 2011;20(6):1171-7.
127. Paradisi A, Waterboer T, Sampogna F, Tabolli S, Simoni S, Pawlita M, Abeni D. Seropositivity for human papillomavirus and incidence of subsequent squamous cell and basal cell carcinomas of the skin in patients with a previous nonmelanoma skin cancer. BrJDermatol. 2011;165(4):782-91.
128. Casabonne D, Michael KM, Waterboer T, Pawlita M, Forslund O, Burk RD,... Newton R. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. IntJCancer. 2007;121(8):1862-8.
129. Purdie KJ, Surentheran T, Sterling JC, Bell L, McGregor JM, Proby CM,... Breuer J. Human papillomavirus gene expression in cutaneous squamous cell carcinomas from immunosuppressed and immunocompetent individuals. JInvest Dermatol. 2005;125(1):98-107.
130. Dang C, Koehler A, Forschner T, Sehr P, Michael K, Pawlita M,... Nindl I. E6/E7 expression of human papillomavirus types in cutaneous squamous cell dysplasia and carcinoma in immunosuppressed organ transplant recipients. BrJDermatol. 2006;155(1):129-36.
131. Arron ST, Ruby JG, Dybbro E, Ganem D, Derisi JL. Transcriptome sequencing demonstrates that human papillomavirus is not active in cutaneous squamous cell carcinoma. JInvest Dermatol. 2011;131(8):1745-53.
132. Iannacone MR, Gheit T, Waterboer T, Giuliano AR, Messina JL, Fenske NA,... Rollison DE. Case-control study of cutaneous human papillomavirus infection in Basal cell carcinoma of the skin. JInvest Dermatol. 2013;133(6):1512-20.
133. Paolini F, Carbone A, Benevolo M, Silipo V, Rollo F, Covello R,... Venuti A. Human Papillomaviruses, p16INK4a and Akt expression in basal cell carcinoma. JExpClinCancer Res. 2011;30:108.



CHAPTER 3

The presence of Betapapillomavirus antibodies around transplantation predicts the development of keratinocyte carcinoma in organ transplant recipients: a cohort study

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Abstract

Organ transplant recipients (OTR) have an increased risk of developing keratinocyte carcinomas (KC). The aim of this study was to correlate infection with human papillomaviruses (HPV) belonging to the Beta genus (Beta-PV) at transplantation with later development of KC.

In a cohort study, sera collected between one year before and one year after transplantation of OTR transplanted between 1990 and 2006 were tested for antibody responses against the L1 capsid antigen of Beta-PV and other HPV genera (Gamma-, Mu-, Nu-, Alpha-PV) using multiplex serology. The OTR were followed for a maximum of 22 years. Cox regression models with KC, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) as outcome variables were used.

Sixty out of 445 OTR had developed KC: 14 developed only SCC, 24 only BCC and 22 both types of KC. The time-dependent hazard ratio to develop either or both types of KC, adjusted for age, sex and transplanted organ, in OTR tested Beta-PV seropositive around time of transplantation compared to Beta-PV seronegative OTR was 2.9 (95%CI 1.3-6.4). The hazard ratio for SCC was 2.9 (95%CI 0.99-8.5) and for BCC 3.1 (95%CI 1.2-8.0). There was also an association between Mu-PV seropositivity and KC, but there were no significant associations between other HPV genera tested and KC.

A positive seroresponse for Beta-PV around transplantation significantly predicted the development of KC in OTR up to 22 years later, providing additional evidence that infection with Beta-PV plays a role in KC carcinogenesis.

Introduction

Keratinocyte carcinomas (KC) are the most prevalent malignancies seen in organ transplant recipients (OTR). The incidence of squamous cell carcinoma (SCC) is 60-250 times increased compared to the general population, and for basal cell carcinomas (BCC) this is 10-40 times.[1-4] In OTR, SCC are more frequently observed than BCC with an SCC:BCC ratio of 5:1, compared to the general population where an SCC:BCC ratio of 1:4 is found.[5]

Life-long immunosuppressive therapy is the most important risk factor for developing KC in OTR. Other important risk factors include sun exposure, male gender, older age, smoking and fair skin with susceptibility to sunburn. These are similar risk factors as in the immunocompetent population.[6,7] A role of human papillomaviruses (HPV) in the development of KC has also been frequently suggested.[8-10]

HPV are small double-stranded DNA viruses that infect epithelia of skin or mucosa. Over 150 different types have been described.[11,12] HPV has been estimated to play a causative role in 5.2% of all human cancers.[13] HPV types are classified in different classes (1 to 4) by the International Agency for Research on Cancer (IARC) based on their carcinogenic potential.[14,15]

The first time that HPV infection was linked with KC was in patients with a rare autosomal recessive disease called epidermodysplasia verruciformis (EV).[16] EV patients have an increased susceptibility to widespread Beta-PV infections of the skin that progress to SCC in one-third of the patients, mainly on sun-exposed sites.[17] In the years after transplantation, OTR start to develop a clinical picture that resembles that of EV patients. Epidemiological and experimental data have advocated a potential carcinogenic role of cutaneous HPV infection in skin cancer, especially of SCC in the immunosuppressed population.[18,19]

There are different methods to detect Beta-PV infection, based on measurement of viral protein, viral DNA or serum antibody responses.[20] Serological responses are considered as the most consistent marker of biologically relevant Beta-PV infection, reflecting a past or present infection strong enough to evoke an immune response.[8,21]

Most studies investigating the association between serological responses to HPV and SCC are cross-sectional or case-control studies, were carried out in the immunocompetent population and showed an association between detection

of Beta-PV antibodies and SCC.[22-27] As far as we know, cohort studies investigating the association between serological responses to HPV and later development of a first KC were not carried out in OTR.[20] Therefore, we designed a retrospective follow-up study to establish if there is a relationship between the presence of serological responses to HPV, in particular Beta-PV, around transplantation and the development of KC (SCC and BCC) in the years after the organ transplantation.

Material and methods

Study design

Between 1966 and 2006 a total of 2136 patients were transplanted in the Leiden University Medical Center (LUMC) (Supplementary Figure 2). OTR consisted of both kidney transplant recipients (KTR) as well as simultaneous pancreas-kidney transplant recipients (SPKTR).

During the past 25 years sera were collected from a considerable number of OTR. The most extensive source of sera consisted of samples collected from 1989 for clinical care-related screening and diagnostic purposes, which were systematically stored in the department of Medical Microbiology of the LUMC. In the scope of earlier case-control studies, additional sera had been collected and stored.[28,29] From 101 patients who participated in a prospective study pre-transplant sera were retraced from the Eurotransplant serumbank.[30] Altogether, 7912 sera were available from 1269 OTR (Supplementary Figure 2). Because of practical reasons we had to limit the number of sera to be tested to a maximum of 3000, which number of sera was thought to provide enough statistical power to answer our questions.

We selected 648 representative sera from all 164 OTR who developed KC during follow-up of which at least one serum sample was available. For each of these OTR we selected between 3 and 5 control OTR without skin cancer. These patients were matched for type of transplantation (KTR or SPKTR), sex and age at transplantation, which resulted in the selection of 2112 sera from 534 OTR without skin cancer (Supplementary Figure 2). These sera had been collected between 7 years before and 42 years after the transplantation.

Since we were specifically interested in the association between seroresponses to HPV around the transplantation and the later development of KC we further

restricted our selection to OTR who had sera collected between 1 year before and 1 year after the transplantation. This resulted in a final cohort consisting of 1880 sera from 445 OTR, of whom 60 had developed KC (14 only SCC, 24 only BCC and 22 both types of skin cancer) during the 22-year follow-up period (Figure 2). The number of sera collected in these patients ranged from 1 to 11 (mean: 4.2, median: 4.0). The remaining sera will be analyzed in a separate study.
Data about development of KC were retracted from the LUMC oncologic database, the LUMC pathology database and by hand searching of the medical files. The study was approved by the medical ethical committee of the LUMC.

Serum processing and analysis

Serum samples were obtained from -20˚C freezers at the department of Medical Microbiology. They were defrosted and pipetted in 96-wells plates. Some selected serum samples were excluded from further analysis, because either they could not be found, were not properly centrifuged initially or contained too little volume (Supplementary Figure 7). The 96-wells plates were shipped on dry ice to the German Cancer Research Center (DKFZ), Heidelberg, Germany for analysis using Luminex® technology.
This multiplex serology technique is an antibody detection method based on glutathione S-transferase capture ELISA in combination with fluorescent bead technology. Positive serology cut-off points are standardized at 200 MFI (mean fluorescence intensity), targeted on antibodies against major capsid antigen L1 of the various HPV types.[31,32] Sera were tested for seven Alpha-PV (HPV2, 3, 6, 7, 13, 16, 27b), sixteen Beta-PV (HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 93, 96), eight Gamma-PV (HPV4, 48, 50, 60, 65, 95, 101, 103), two Mu-PV (HPV1, 63), and one Nu-PV (HPV41).
The OTR were considered seropositive for specific HPV genera or specific HPV types if at least one of the sera tested between 1 year before and 1 year after the transplantation had a positive antibody response against these specific HPV genera or specific HPV types.

Statistical analyses

Baseline characteristics are given in percentages and simple means calculation. Kaplan-Meier survival analyses were used in conjunction with multivariable cox proportional hazard methods to calculate corresponding hazard ratios (HR) with 95%

confidence intervals (CI) for development of KC in relation to HPV serology. The date of transplantation was used as opening date. As censoring dates we used the date of diagnosis of first KC, the date of the patient's death, the date of last follow-up, the date lost to follow-up or the date of end of study (29th October 2012). Factors considered as potential confounders were sex, type of transplantation and age at transplantation. The Cox models were adjusted for these variables. Since the variable of HPV seropositivity had a time-scope of one year before and after the transplantation (starting point) time dependent Cox regression analyses were performed. The analyses of SCC were performed regardless of the presence of BCC among the cases and controls and the analyses of BCC were performed regardless of the presence of SCC among the cases and controls.

To investigate whether there were Beta-PV types that were the driving force causing the positive association between Beta-PV seropositivity and the development of KC we formed three subgroups: a) patients who were seronegative for any Beta-PV type, b) patients who were seropositive for the specific Beta-PV type regardless of the other Beta-PV types and c) patients who were seronegative for the specific Beta-PV type regardless of the other Beta-PV types. The cumulative incidence of KC, SCC and BCC was calculated with Kaplan-Meier survival analyses. Additionally, we performed conditional step forward logistic regression to get a rough estimation which individual Beta-PV may possibly contribute to the association between seropositivity and the development of KC, SCC and/or BCC. Starting with no variables in the model all Beta-PV types, age, sex and type of organ were introduced into the model, starting with the most relevant one until the addition of new variables did not improve the model any longer.

All analyses were done with SPSS 20.0 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA).

Results

The baseline characteristics of the 445 OTR included in the study are shown in Table 1. The majority of patients were male (65%) and most patients (79%) underwent kidney transplantation. The mean age of the patients at transplantation was 47.3 years and the mean follow-up after transplantation was 11.8 year. A mean of 4.2 sera (range 1 to 17) were available from 1 year

Table 1. Baseline characteristics of the organ transplant recipients who were included in this study.

	All patients	Keratinocyte Carcinoma		Squamous cell carcinoma, irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
		+	-	+	-	+	-
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Transplantation type							
Kidney	345 (78.0)	41 (68.3)	304 (79.0)	22 (61.1)	323 (79.0)	30 (65.2)	315 (78.9)
Kidney & pancreas	100 (22.0)	19 (31.7)	81 (21.0)	14 (38.9)	86 (21.0)	16 (34.8)	84 (21.1)
Gender							
Male	291 (65.4)	40 (66.7)	251 (65.2)	28 (77.8)	263 (64.3)	29 (63.0)	262 (65.7)
Female	154 (34.6)	20 (33.3)	134 (34.8)	8 (22.2)	146 (35.7)	17 (37.0)	137 (34.3)
Age (years) at transplantation							
Mean-median (Range)	47.3-46.8 (19.9-76.4)	47.5-47.7 (19.9-68.7)	47.3-46.7 (21.2-76.4)	50.0-50.0 (31.8-68.7)	47.1-46.5 (19.9-76.4)	45.4-46.1 (19.9-64.2)	47.5-47.0 (21.2-76.4)
Number sera*							
Mean-median (Range)	4.22-4 (1-17)	5.87-7 (1-17)	3.97-4 (1-10)	6.92-8 (1-17)	3.99-4 (1-10)	5.76-6 (1-17)	4.05-4 (1-10)
Time from Tx to first KC							
Mean - median (Range)		7.7-6.9 (0.28-19.8)		9.0-8.3 (0.78-18.6)		8.0-6.6 (0.28-19.8)	

Abbreviations: KC, keratinocyte carcinoma; Tx, transplantation.* sera available and tested for HPV antibody presence between 1 year before until 1 year after transplantation.

before to 1 year after transplantation. Among the patients who developed KC, the mean time from transplantation until the first KC was 7.7 years.

Supplementary Table 1 shows the percentages of OTR who were seropositive for any HPV type tested between one year before and one year after the transplantation, for all OTR together and stratified for those with KC, SCC and BCC. Table 2 summarizes supplementary Table 1 and provides the percentages of OTR who were seropositive for at least one HPV type per HPV genus (Beta-, Gamma-, Mu-, Nu or Alpha-PV). Seroresponses against the Beta genus were more frequently observed in the patients with KC compared to patients without KC, which reached statistical significance ($p = 0.005$) (Table 2). Patients who were seropositive for at least one HPV type of a specific genus had a greater probability for being seropositive for another HPV genus (supplementary Table 2).

Table 2. Numbers and percentages of organ transplant recipients with positive antibody responses against one or more HPV types of different HPV genera*.

	All patients	Keratinocyte carcinoma		Squamous cell, carcinoma irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
		+	-	+	-	+	-
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Any Beta-PV							
Negative	119 (26.7)	7 (11.7)	112 (29.1)	4 (11.1)	115 (28.1)	5 (10.9)	114 (28.6)
Positive	326 (73.3)	53 (88.3)	273 (70.9)	32 (88.9)	294 (71.9)	41 (89.1)	285 (71.4)
Any Gamma HPV							
Negative	113 (25.4)	12 (20.0)	101 (26.2)	6 (16.7)	107 (26.2)	10 (21.7)	103 (25.8)
Positiv	332 (74.6)	48 (80.0)	284 (73.8)	30 (83.3)	302 (73.8)	36 (78.3)	296 (74.2)
Any Mu HPV							
Negative	151 (33.9)	12 (20.0)	139 (36.1)	6 (16.7)	145 (35.5)	9 (19.6)	142 (35.6)
Positive	294 (66.1)	48 (80.0)	246 (63.9)	30 (83.3)	264 (64.5)	37 (80.4)	257 (64.4)
Nu HPV							
Negative	367 (82.5)	46 (76.7)	321 (83.4)	27 (75.0)	340 (83.1)	37 (80.4)	330 (82.7)
Positive	78 (17.5)	14 (23.3)	64 (16.6)	9 (25.0)	69 (16.9)	9 (19.6)	69 (17.3)
Any Alpha-HPV							
Negative	106 (23.8)	13 (21.7)	93 (24.2)	8 (22.2)	98 (24.0)	10 (21.7)	96 (24.1)
Positive	339 (76.2)	47 (78.3)	292 (75.8)	28 (77.8)	311 (76.0)	36 (78.3)	303 (75.9)

* HPV types tested; seven Alpha- (HPV2, 3, 6, 7, 13, 16, 27b), sixteen Beta- (HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 93, 96), eight Gamma- (HPV4, 48, 50, 60, 65, 95, 101, 103), two Mu-types (HPV1, 63), and one Nu-type (HPV41).

The cumulative incidence of KC in Beta-PV seronegative and Beta-PV seropositive OTR is depicted in Figure 1 and supplementary Figure 1. Log rank tests were performed and showed a significant difference between Beta-PV-seropositive and seronegative OTR ($p = 0.043$). The Kaplan Meier plots for SCC and BCC showed similar curves as the plots for KC (supplementary Figures 2 and 3). Table 3 shows the time-dependent non-adjusted hazard ratios and the hazard ratios adjusted for age at transplantation, sex and type of transplantation for the development of KC, SCC and BCC. A positive association was found between seropositivity for at least one Beta-PV type detected between one year before and one year after the organ transplantation and the development of KC, SCC and/or BCC. After adjustment, also

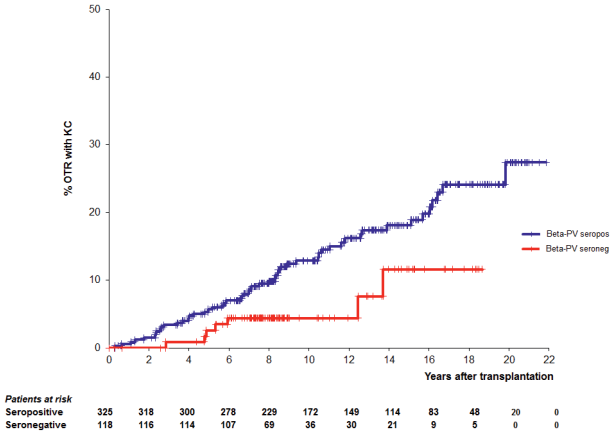


Figure 1.
Kaplan-Meier plot for development of KC in relation to HPV serology.
The cumulative incidence of keratinocyte carcinomas (KC) is statistically significantly higher in organ transplant recipients (OTR) who were seropositive against one or more HPV types of the Beta-PV genus between 1 year before and 1 year after the transplantation (p = 0.043).

Mu-PV seropositivity was associated with KC (Table 3). The Cox proportional hazard analyses (Table 3) and the Kaplan Meier plots of cumulative incidence of KC, SCC and/or BCC in HPV seronegative and seropositive OTR for the other HPV genera tested (Gamma-, Nu- and Alpha-PV) showed no significant association between seropositivity against any of these HPV genera (supplementary Figures 1, 2 and 3).
Beta-PV species 1 (specifically HPV8 and HPV20) and Beta-PV species 3 (specifically HPV75 and HPV76) were most relevant for the positive association between Beta-PV seropositivity and the development of KC (supplementary Figure 4). The potential importance of these Beta-PV types was confirmed for HPV8 and HPV75 by conditional step forward regression analyses and a statistically significant negative association was observed for HPV96 (Table 4).
Beta-PV species 1 (HPV8 and HPV20) and Beta-PV species 3 (HPV76) were also associated with the development of SCC (supplementary Figure 5), which was confirmed by conditional step forward regression analyses (Table 4). A statistically significant negative association was found for HPV24 and HPV38 (Table 4).
Beta-PV species 2 (HPV15) and species 3 (HPV75 and HPV76) were relevant for the development of BCC (supplementary Figure 6), which was confirmed in the conditional step forward regression analyses for HPV15 and HPV26, whereas there was a statistically significant negative association with HPV9 (Table 4).

Table 3. Time dependent Cox proportional Hazard ratios for keratinocyte carcinoma, squamous cell carcinoma and basal cell carcinoma in organ transplant recipients related to HPV genus serostatus between 1 year before and one year after the transplantation.

	Keratinocyte carcinoma		Squamous cell, carcinoma irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
	Unadjusted hazard ratio	**Adjusted* hazard ratio**	**Unadjusted hazard ratio**	**Adjusted* hazard ratio**	**Unadjusted hazard ratio**	**Adjusted* hazard ratio**
Beta-PV	2.2 (1.0-4.9)	2.8 (1.3-6.4)	2.1 (0.75-6.1)	2.9 (0.99-8.5)	2.3 (0.92-6.0)	3.1 (1.2-8.0)
G	1.1 (0.59-2.1)	1.3 (0.66-2.4)	1.2 (0.51-3.0)	1.7 (0.68-4.1)	0.97 (0.48-2.0)	1.1 (0.54-2.2)
Mu-PV	1.8 (0.95-3.4)	2.0 (1.0-3.7)	2.1 (0.86-5.0)	2.4 (0.98-5.7)	1.8 (0.86-3.7)	1.9 (0.93-4.0)
Nu-PV	1.2 (0.68-2.2)	1.5 (0.80-2.7)	1.3 (0.61-2.7)	1.8 (0.84-4.0)	1.0 (0.46-2.0)	1.1 (0.53-2.3)
Alpha-PV	0.93 (0.50-1.7)	0.99 (0.53-1.8)	0.84 (0.38-1.9)	0.97 (0.44-2.2)	0.92 (0.46-1.9)	0.97 (0.48-2.0)

* Adjusted for sex, age at transplantation and type of transplantation.

Discussion

We have found a statistically significant association between the development of KC, SCC and BCC after transplantation and antibody responses to one or more Beta-PV types, measured in serum drawn in a window between one year before and one year after organ transplantation. The antibody response against Beta-PV types was specific for the Beta genus since there were no significant associations for positive seroresponses against the Gamma, Nu and Alpha genera with the development of KC, SCC or BCC. Interestingly we found also a positive association between KC and Mu-PV seropositivity, an HPV genus not recognized previously as potentially associated with KC development.

So far, most studies investigating HPV seroresponses in association with KC development concerned cross-sectional and case-control studies in the immunocompetent population.[20] We are aware of only a few published cohort studies that were performed in the immunocompetent population. A Scandinavian study described a weak association for overall Beta-PV seropositivity prior to SCC diagnoses, whereas a significant association was observed for Beta-PV species 2 (OR = 1.3, 95% CI: 1.1, 1.7), and for serum samples taken more than 18 years

Table 4. Association between 16 different Beta-PV types and skin cancer.

	Keratinocyte carcinoma		Squamous cell carcinoma		Basal cell carcinoma	
	All variables in the regression model#	**Forward regression final model**	**All variables in the regression model**	**Forward regression final model**	**All variables in the regression model**	**Forward regression final model**
Species 1						
HPV5	1.3 (0.57;3.1)		1.7 (0.54;5.1)		1.2 (0.46;3.1)	
HPV8	**2.6 (1.2;5.6)**	**2.4 (1.3;4.6)**	**3.4 (1.3;8.8)**	**3.4 (1.4;8.3)**	2.2 (0.96;5.1)	
HPV20	**2.6 (1.1;5.9)**		**2.9 (1.1;8.0)**	**2.6 (1.0;6.6)**	**2.6 (1.0;6.6)**	
HPV24	0.43 (0.15;1.2)		**0.19 (0.05;0,71)**	**0.20 (0.07;0.63)**	0.68 (0.22;2.1)	
HPV36	0.50 (0.18;1.4)		0.61 (0.18;2.1)		0.40 (0.13;1.3)	
HPV93	0.48 (0.17;1.4)		0.35 (0.09;1.5)		0.27 (0.07;1.1)	
Species 2						
HPV9	**0.37 (0.13;0.99)**		0.50 (0.15;1,7)		**0.25 (0.08;0.81)**	**0.29 (0.11;0.76)**
HPV15	1.5 (0.60;3.7)		1.3 (0.38;4.2)		2.3 (0.86;6.2)	**2.7 (1.2;6.3)**
HPV17	0.82 (0.35;1.9)		0.72 (0.24)		0.95 (0.37;2.4)	
HPV23	1.3 (0.53;3.3)		**3.1 (1.0;9.8)**	2.4 (0.92;6.4)	1.4 (0.53;4.0)	
HPV38	0.94 (0.38;2.3)		**0.23 (0.06;0.83)**	**0.21 (0.07;0.66)**	0.86 (0.31;2.4)	
Species 3						
HPV49	1.4 (0.56;3.6)		1.9 (0.62;5.9)		1.4 (0.48;3.9)	
HPV75	**2.8 (1.1;7.4)**	**3.1 (1.5;6.6)**	1.6 (0.47;5.6)		2.2 (0.74;6.7)	
HPV76	1.8 (0.67;4.8)		2.3 (0.70;7.8)	**2.6 (1.1;6.5)**	1.9 (0.64;5.6)	**2.9 (1.3;6.5)**
Species 4						
HPV92	1.2 (0.49;3.1)		1.5 (0.45;4.9)		0.82 (0.28;2.4)	
Species 5						
HPV96	0.41 (0.17;1.0)	**0.38 (0.18;0.83)**	0.56 (0.19;1.7)		0.63 (0.24;1.7)	
Age*	1.0 (0.98;1.0)		1.0 (1.0;1.1)	**1.1 (1.0;1.1)**	0.99 (0.96;1.0)	
Sex*	0.87 (0.47;1.6)		1.4 (0.56;3.3)		0.81 (0.40;1.6)	
Organ*	**2.2 (1.1;4.6)**	**2.1 (1.1;4.0)**	**4.7 (1.8;12.3)**	**4.9 (2.0;12.1)**	**2.2 (1.0;4.9)**	**2.4 (1.2;4.7)**

logistic regression model
*Age: per year; *Sex: men compared to women; *Organ: combined pancreas and kidney compared to kidney. The bold numbers are statistically significant.

before diagnosis (OR = 1.8, 95% CI: 1.1, 2.8).[8] A population-based cohort study in Australia revealed no associations between the presence of any Beta-PV antibodies and the occurrence of SCC.[33] However, among people who were less

than 50 years old in 1992, the presence of Beta-PV antibodies was associated with a two-fold increased risk of SCC.[33] In the United Kingdom a prospective pilot study found no significant differences in seropositivity of any of 38 HPV types.[34] One study investigated the possible role of seropositivity for different HPVs in the incidence of a subsequent KC in 107 patients with a follow-up of 5 years who were enrolled in a previous case-control study. HPV seropositivity at baseline was strongly associated with the risk of developing a second SCC after 5 years for a number of Beta- and Gamma PV types, but no association was found for developing a second BCC.[35] As far as we know, our study is the first cohort study with a prolonged follow-up period in OTR in which the relationship between HPV serology before and just after the organ transplantation was studied in relation to later development of a first KC.

In 2011 Proby and coworkers conducted a case-control study in OTR from the Netherlands, United Kingdom, France and Italy, to investigate the association between SCC development and seroresponses against Beta-PV combined with detection of Beta-PV DNA in plucked eyebrow hairs.[10] A positive association was found for concordant DNA and seropositivity for HPV36 (OR 2.4; CI 1.0-5.4), with similar, but not statistically significant associations for HPV5, HPV9 and HPV24. Concordant Beta-PV DNA presence in hairs and a serological antibody response for at least one HPV type was significantly associated with SCC risk (OR 1.6; CI 1.1-2.5). Another case-control study conducted in immunocompetent patients in Florida U.S.A. revealed a positive association between seropositivity to increasing numbers of Beta-PV types and SCC (OR 1.9; 95% CI 1.2–3.0).[36] A similar association for Beta-PV types DNA in eyebrow hairs was found in another clinic-based case-control study (4 types vs. HPV-negative: OR 2.0, 95% CI 1.1-3.8).[37] Waterboer and colleagues performed a case-control study in an Italian immunocompetent population and found a significant two to three-fold risk of SCC with seropositivity for any species 2 Beta-PV type (OR 3.3, 95%CI 1.2-8.7) and for the Gamma-PV types (OR 3.1, 95%CI 1.1-8.6).[23] In a multi-national case-control study in immunocompetent subjects, a positive antibody response against four or more Beta-PV types was associated with a doubled risk for SCC in Australia and the Netherlands.[38]

Little data are published about the association between HPV infection and the development of BCC and the findings are contradictory. A relationship between seroresponses to Beta-PV and the development of BCC has been described

previously.[8,24,39,40] Interestingly, Paolini et al reported a high frequency (11.5%) of HPV15 DNA in a small cohort of BCC patients, which is in agreement with the observed positive association between HPV15 and BCC in our study.[41] However, some other studies did not report a positive association between Beta-PV seroreactivity and BCC.[22,25,42,43] A recent systematic review showed no association between Beta-PV serological responses and BCC.[27] In a subgroup of patients in Florida U.S.A. with Beta-PV DNA positive BCC, however, an association between a serological response against Beta-PV and BCC was observed.[43] In a population-based case-control study of patients with SCC, BCC and controls from New Hampshire U.S.A., it was found that seropositivity to Beta-PV (OR = 1.5, 95% CI = 1.0 to 2.1) was significantly associated with SCC. This risk increased with positivity for multiple HPV types. No associations were found with BCC risk.[22] Karagas et al. confirmed these findings in a larger group of 2366 skin cancer cases and controls.[25] We cannot exclude, however, that the OTR who developed BCC in our study are at increased risk of developing SCC later on, because BCC often precede the development of SCC in these patients.

Besides the advantages, the retrospective design of our study also has some disadvantages. For instance, the sera we tested were not systematically collected with the purpose to study a relationship with KC, but were randomly selected from routinely archived sera collected for HLA antibody screening and other diagnostic purposes. The hazard ratios we calculated were adjusted for sex, age at transplantation and type of transplantation.[5] We could not adjust for other risk factors for KC, like sun exposure, sunburns, skin type and type of immunosuppression, because these data were not systematically collected for all patients. These risk factors are associated with an increased prevalence of HPV DNA in eyebrow hairs, but it is not known whether this is also the case for Beta-PV seroresponses.[19,44-47]

A drawback of our study was that data about seroconversion were not systematically available. It can be speculated that seroconversion in the years following transplantation in OTR that were seronegative around transplantation, might have increased the risk of tumor development in the seronegative group, which, if true, could have led to a stronger association between Beta-PV type seropositivity and KC.

In general, a drawback of studies reporting the association between serological responses and skin cancer, is that, because of differences in the employed methodologies (initially starting with western blotting, followed by HPV-type-

specific ELISAs using L1 virus-like particles, and more recently by high throughput multiplex fluorescent bead based assays enabling the simultaneous detection of antibody responses in large series of serologic samples against a variety of HPVs) the outcomes between the different studies cannot always reliably be compared. In our study, however, this is only true for the earlier studies referred to (Western blot by Steger et al., 1990 and ELISA technique by Feltkamp et al., 2003; Stark et al., 1998), since the other studies all used the same multiplex serology technique which was performed in the same laboratory in Heidelberg.[24,39,40] Other factors that may explain the different associations between HPV infection and KC are differences in immunosuppressive regimens between centers which may exert different effects on the present HPV types, different exposure to sunlight reflected by the different latitudes of the centers and differences in exposure to different HPV types.

The exact mechanism by which Beta-PV infection predisposes for the development of KC remains unclear. It has been hypothesized that the effects of the virus on UV-induced DNA damage withholds apoptosis and DNA repair by accumulation of mutations.[48] This is supported by the fact that the E6 and E7 proteins from Beta-PV types are capable of inhibiting UV induced cell-cycle checkpoints and DNA repair mechanisms.[49-52] This potentially deregulates cell cycle control and growth in keratinocytes.[36,53] Some Beta-PV types could up-regulate the p16INK4a and Akt/P13K pathway and might play a role in the carcinogenesis of BCC.[41] The epidemiological evidence that HPV is involved in BCC carcinogenesis, however, is much weaker than the possible involvement of HPV in cutaneous SCC carcinogenesis. In conclusion, a detectable seroresponse for one or more Beta-PV types around the time of transplantation predicts an increased risk for the development of KC in OTR. This study provides additional evidence that infection with Beta-PV types plays a role in keratinocyte carcinogenesis. Additional studies will be necessary to confirm the observed association between Mu-PV infection and KC development.

Supplementary files can be found in the online version of this article

References

1. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. NEnglJMed. 2003;348(17):1681-91.
2. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in the Netherlands. Transplantation. 1990;49(3):506-9.
3. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. BrJDermatol. 2006;154(3):498-504.
4. Tessari G, Naldi L, Boschiero L, Nacchia F, Fior F, Forni A,... Girolomoni G. Incidence and clinical predictors of a subsequent nonmelanoma skin cancer in solid organ transplant recipients with a first nonmelanoma skin cancer: a multicenter cohort study. ArchDermatol. 2010;146(3):294-9.
5. Wisgerhof HC, van der Boog PJ, de Fijter JW, Wolterbeek R, Haasnoot GW, Claas FH,... Bouwes Bavinck JN. Increased risk of squamous-cell carcinoma in simultaneous pancreas kidney transplant recipients compared with kidney transplant recipients. JInvest Dermatol. 2009;129(12):2886-94.
6. Bouwes Bavinck JN, Euvrard S, Naldi L, Nindl I, Proby CM, Neale R,... Harwood CA. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in the Netherlands, United Kingdom, Germany, France, and Italy. JInvest Dermatol. 2007;127(7):1647-56.
7. Terhorst D, Drecoll U, Stockfleth E, Ulrich C. Organ transplant recipients and skin cancer: assessment of risk factors with focus on sun exposure. BrJDermatol. 2009;161 Suppl 3:85-9.
8. Andersson K, Michael KM, Luostarinen T, Waterboer T, Gislefoss R, Hakulinen T,... Dillner J. Prospective study of human papillomavirus seropositivity and risk of nonmelanoma skin cancer. AmJEpidemiol. 2012;175(7):685-95.
9. Neale RE, Weissenborn S, Abeni D, Bouwes Bavinck JN, Euvrard S, Feltkamp MC,... Pfister H. Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. Cancer EpidemiolBiomarkers Prev. 2013;22(4):719-27.
10. Proby CM, Harwood CA, Neale RE, Green AC, Euvrard S, Naldi L,... Bouwes Bavinck JN. A case-control study of Betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. AmJTransplant. 2011;11(7):1498-508.
11. Bernard HU, Burk RD, Chen Z, Van DK, zur HH, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010;401(1):70-9.
12. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur HH. Classification of papillomaviruses. Virology. 2004;324(1):17-27.
13. Parkin DM. The global health burden of infection-associated cancers in the year 2002. IntJCancer. 2006;118(12):3030-44.
14. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El GF,... Cogliano V. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009;10(4):321-2.
15. Human papillomaviruses. IARC Monogr EvalCarcinogRisks Hum. 2007;90:1-636.
16. Jablonska S, Dabrowski J, Jakubowicz K. Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. Cancer Res. 1972;32(3):583-9.
17. Lutzner MA. Epidermodysplasia verruciformis. An autosomal recessive disease characterized by viral warts and skin cancer. A model for viral oncogenesis. BullCancer. 1978;65(2):169-82.
18. Bouwes Bavinck JN, Plasmeijer EI, Feltkamp MC. Beta-papillomavirus infection and skin cancer. JInvest Dermatol. 2008;128(6):1355-8.
19. de Koning MN, Weissenborn SJ, Abeni D, Bouwes Bavinck JN, Euvrard S, Green AC,... Feltkamp MC. Prevalence and associated factors of Betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma. JGenVirol. 2009;90(Pt 7):1611-21.
20. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? DermatolSurg. 2013;39(1 Pt 1):1-23.
21. Struijk L, Hall L, van der Meijden E, Wanningen P, Bouwes Bavinck JN, Neale R,... Feltkamp MC. Markers of cutaneous human papillomavirus infection in individuals with tumor-free skin, actinic keratoses, and squamous cell carcinoma. Cancer Epidemi-

olBiomarkers Prev. 2006;15(3):529-35.
22. Karagas MR, Nelson HH, Sehr P, Waterboer T, Stukel TA, Andrew A,... Pawlita M. Human papillomavirus infection and incidence of squamous cell and basal cell carcinomas of the skin. JNatlCancer Inst. 2006;98(6):389-95.
23. Waterboer T, Abeni D, Sampogna F, Rother A, Masini C, Sehr P,... Pawlita M. Serological association of Beta and Gamma human papillomaviruses with squamous cell carcinoma of the skin. BrJDermatol. 2008;159 (2):457-9.
24. Feltkamp MC, Broer R, di Summa FM, Struijk L, van der Meijden E, Verlaan BP,... Bouwes Bavinck JN. Seroreactivity to epidermodysplasia verruciformis-related human papillomavirus types is associated with nonmelanoma skin cancer. Cancer Res. 2003;63(10):2695-700.
25. Karagas MR, Waterboer T, Li Z, Nelson HH, Michael KM, Bouwes Bavinck JN, Pawlita M. Genus Beta human papillomaviruses and incidence of basal cell and squamous cell carcinomas of skin: population based case-control study. BMJ. 2010;341:c2986.
26. Bouwes Bavinck JN, Stark S, Petridis AK, Marugg ME, ter Schegget J, Westendorp RG,... Pfister H. The presence of antibodies against virus-like particles of epidermodysplasia verruciformis-associated humanpapillomavirus type 8 in patients with actinic keratoses. BrJDermatol. 2000;142(1):103-9.
27. Bzhalava D, Guan P, Franceschi S, Dillner J, Clifford G. A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology. 2013;445(1-2):224-31.
28. Bouwes Bavinck JN, Gissmann L, Claas FH, van der Woude FJ, Persijn GG, ter Schegget J,... Steger G. Relation between skin cancer, humoral responses to human papillomaviruses, and HLA class II molecules in renal transplant recipients. JImmunol. 1993;151 (3):1579-86.
29. Bouwes Bavinck JN, Vermeer BJ, van der Woude FJ, Vandenbroucke JP, Schreuder GM, Thorogood J,... Claas FH. Relation between skin cancer and HLA antigens in renal-transplant recipients. NEnglJMed. 1991;325(12):843-8.
30. Antonsson A, Waterboer T, Bouwes Bavinck JN, Abeni D, de KM, Euvrard S,... Neale RE. Longitudinal study of seroprevalence and serostability of 34 human papillomavirus types in European organ transplant recipients. Virology. 2013;436(1):91-9.
31. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H,... Pawlita M. Seroprevalence of 34 human papillomavirus types in the German general population. PLoSPathog. 2008;4(6):e1000091.
32. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO,... Pawlita M. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. ClinChem. 2005;51(10):1845-53.
33. Plasmeijer EI, Pandeya N, O'Rourke P, Pawlita M, Waterboer T, Feltkamp MC,... Neale RE. The Association between cutaneous squamous cell carcinoma and Betapapillomavirus seropositivity: a cohort study. Cancer EpidemiolBiomarkers Prev. 2011;20 (6):1171-7.
34. Casabonne D, Michael KM, Waterboer T, Pawlita M, Forslund O, Burk RD,... Newton R. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. IntJCancer. 2007;121(8):1862-8.
35. Paradisi A, Waterboer T, Sampogna F, Tabolli S, Simoni S, Pawlita M, Abeni D. Seropositivity for human papillomavirus and incidence of subsequent squamous cell and basal cell carcinomas of the skin in patients with a previous nonmelanoma skin cancer. BrJDermatol. 2011;165(4):782-91.
36. Iannacone MR, Gheit T, Waterboer T, Giuliano AR, Messina JL, Fenske NA,... Rollison DE. Case-control study of cutaneous human papillomaviruses in squamous cell carcinoma of the skin. Cancer EpidemiolBiomarkers Prev. 2012;21(8):1303-13.
37. Iannacone MR, Gheit T, Pfister H, Giuliano AR, Messina JL, Fenske NA,... Rollison DE. Case-control study of genus-Beta human papillomaviruses in plucked eyebrow hairs and cutaneous squamous cell carcinoma. IntJCancer. 2014;134(9):2231-44.
38. Bouwes Bavinck JN, Neale RE, Abeni D, Euvrard S, Green AC, Harwood CA,... Feltkamp MC. Multicenter study of the association between Betapapillomavirus infection and

cutaneous squamous cell carcinoma. Cancer Res. 2010;70(23):9777-86.
39. Stark S, Petridis AK, Ghim SJ, Jenson AB, Bouwes Bavinck JN, Gross G,... Pfister H. Prevalence of antibodies against virus-like particles of Epidermodysplasia verruciformis-associated HPV8 in patients at risk of skin cancer. JInvest Dermatol. 1998;111(4):696-701.
40. Steger G, Olszewsky M, Stockfleth E, Pfister H. Prevalence of antibodies to human papillomavirus type 8 in human sera. JVirol. 1990;64(9):4399-406.
41. Paolini F, Carbone A, Benevolo M, Silipo V, Rollo F, Covello R,... Venuti A. Human Papillomaviruses, p16INK4a and Akt expression in basal cell carcinoma. JExpClinCancer Res. 2011;30:108.
42. Andersson K, Waterboer T, Kirnbauer R, Slupetzky K, Iftner T, de Villiers EM,... Dillner J. Seroreactivity to cutaneous human papillomaviruses among patients with nonmelanoma skin cancer or benign skin lesions. Cancer EpidemiolBiomarkers Prev. 2008;17(1):189-95.
43. Iannacone MR, Gheit T, Waterboer T, Giuliano AR, Messina JL, Fenske NA,... Rollison DE. Case-control study of cutaneous human papillomavirus infection in Basal cell carcinoma of the skin. JInvest Dermatol. 2013;133(6):1512-20.
44. Alotaibi L, Provost N, Gagnon S, Franco EL, Coutlee F. Diversity of cutaneous human papillomavirus types in individuals with and without skin lesion. JClinVirol. 2006;36 (2):133-40.
45. Antonsson A, Forslund O, Ekberg H, Sterner G, Hansson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensalic nature of these viruses. JVirol. 2000;74(24):11636-41.
46. Chen AC, McMillan NA, Antonsson A. Human papillomavirus type spectrum in normal skin of individuals with or without a history of frequent sun exposure. JGenVirol. 2008;89(Pt 11):2891-7.
47. Iannacone MR, Michael KM, Giuliano AR, Waterboer T, Pawlita M, Rollison DE. Risk factors for cutaneous human papillomavirus seroreactivity among patients undergoing skin cancer screening in Florida. JInfectDis. 2010;201(5):760-9.
48. Bouwes Bavinck JN, Feltkamp MC. Milk of human kindness?--HAMLET, human papillomavirus, and warts. NEnglJMed. 2004;350(26):2639-42.
49. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes & development. 2000;14(23):3065-73.
50. Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. Oncogene. 2000;19(4):592-8.
51. Struijk L, van der Meijden E, Kazem S, ter Schegget J, de Gruijl FR, Steenbergen RD, Feltkamp MC. Specific Betapapillomaviruses associated with squamous cell carcinoma of the skin inhibit UVB-induced apoptosis of primary human keratinocytes. The Journal of general virology. 2008;89(Pt 9):2303-14.
52. Underbrink MP, Howie HL, Bedard KM, Koop JI, Galloway DA. E6 proteins from multiple human Betapapillomavirus types degrade Bak and protect keratinocytes from apoptosis after UVB irradiation. Journal of virology. 2008;82(21):10408-17.
53. zur Hausen H. Immortalization of human cells and their malignant conversion by high risk human papillomavirus genotypes. SeminCancer Biol. 1999;9(6):405-11.



CHAPTER 4

p16 Immunostaining in keratinocytic neoplasia in organ transplant recipients: Bowen's disease shows a characteristic pattern

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Abstract

For selecting therapy it is important to distinguish different types of keratinocytic neoplasia. It is sometimes difficult to make histopathologic diagnosis, especially in organ transplant recipients (OTR) who develop numerous lesions.
To investigate p16 immunostaining in different types of keratinocytic neoplasia in OTR, we studied 59 actinic keratoses (AK), 51 Bowen' s disease (BD), 63 squamous cell carcinomas (SCC), 16 benign keratotic lesions (BKL) from 31 OTR patients and 25 controls (eczema and psoriasis). Tissue sections were stained for H&E and p16. We scored intensity, proportion and distribution of p16 positive lesional cells.
In 19% of AK, 92% of BD, 35% of SCC and 12% of BKL more than fifty percent of lesional cells were p16-positive. In 16% of AK, 80% of BD, 18% of SCC and 13% of BKL strong p16 staining was observed. BKL, AK and SCC showed focal and patchy staining, BD showed diffuse pattern with strong staining of all atypical cells. Sparing of the basal layer was predominantly seen in BD. No control specimen showed p16-overexpression.
P16 immunostaining shows a characteristic pattern in BD, but not in AK, SCC and BKL. It appears useful in recognising BD, but not in differentiating between other keratinocytic neoplasia.

Introduction

The spectrum of premalignant and invasive keratinocytic neoplasia includes actinic keratosis (AK), Bowen's disease (BD) and squamous cell carcinoma (SCC). AK can show various grades of intraepidermal dysplasia while BD can be considered as squamous cell carcinoma in situ.[1] For AK and BD a three-tier grading system has been proposed analogous to Cervical Intraepithelial Neoplasia (CIN), named Keratinocytic Intraepidermal Neoplasia, grades KIN I, KIN II and KIN III.[2,3] Both AK and BD can progress into SCC.

Histologic differentiation between pre-cancerous lesions and SCC can be difficult, since the basement membrane can be hard to define. This can be caused by fragmented tissue, small specimen size or severe inflammatory infiltration.[4,5] Correct diagnosis is important since the treatment options depend on the stage of the neoplasia.[6,7] Immunostaining that facilitated differentiation between malignant and premalignant keratinocytic lesions would be of great value.

Immunostaining for the p16 tumor suppressor protein is used as an immunohistochemical marker for anogenital intraepithelial neoplasia induced by high-risk oncogenic human papilloma virus (HPV) genotypes, where its expression is related to the grade of intraepithelial neoplasia.[8-10] P16 up regulation in HPV infected dysplastic cells results from a feedback mechanism consequent on the interaction between the HPV-E7 gene protein and cellular pRB. However, in skin neoplasia the relation between keratinocyte neoplasia and HPV is not as clear.[11] There is evidence that in OTR HPV plays a role in development of keratinocyte neoplasia . However, no significant association between p16 protein expression and the presence of HPV has been found.[12-14] The mechanism of p16 expression in keratinocytic neoplasia is not fully understood. Cutaneous expression of p16 protein has been associated with ultraviolet B irradiation, but this association has not been confirmed.[15-17]

Previous studies using staining for p16 in keratinocytic neoplasia have shown discrepant results and reports on keratinocytic neoplasms in organ transplant recipients are few.[17,18] In the present study we investigated p16 immunostaining patterns in a spectrum of well defined premalignant and invasive keratinocytic neoplasia from organ transplant recipients. The main goal was to find out if differences in p16 immunostaining might be useful to differentiate between these different keratinocytic neoplasia.

Methods

Specimen selection

A total of 189 paraffin-embedded formalin fixed tissue blocks from 31 organ transplant recipients were retrieved from the pathology archive from the Leiden University Medical Center. The blocks were from 59 AK, 51 BD, 63 SCC and 16 benign keratinocytic lesions (BKL). Skin biopsies of psoriasis (n=11) and eczema (n=14) from immunocompetent patients were included as controls.

H&E and p16 immunostaining

All slides were reviewed by a specialized dermatopathologist and classified according to established criteria.[19] Immunostaining was performed using heat-induced epitope retrieval with citrate buffer and a primary mouse monoclonal anti-p16INK4a antibody clone JC8 (Immunologic, Duiven, the Netherlands) followed by visualization using Dako EnVision™ detection system (Dako, Glostrup, Denmark).[20]

P16 scoring

The percentage and distribution of p16 positive lesional cells and the intensity of lesional p16 staining were determined by microscopic evaluation at low scanning power, with visualization of the entire lesion. The proportion of positive lesional cells was scored as: no staining; 1% – 10%; 11% – 50%; and > 50% p16 positive lesional cells. The intensity of lesional cell staining was scored as weak, moderate or strong staining. Positivity was defined as moderate or strong staining for p16 in more than 10 percent of lesional cells.[21]

The study was performed in accordance with the Declaration of Helsinki and the Dutch Code for Proper Secondary Use of Human Tissue, approved by the medical ethics committee of the LUMC.

Results

Actinic keratosis

This group included 27 KIN I and 32 KIN II lesions.

P16 immunostaining showed patchy staining pattern with clusters of p16 positive cells in the suprabasal layers (Figure 1), while the basal layer was frequently negative (Figure 2) .

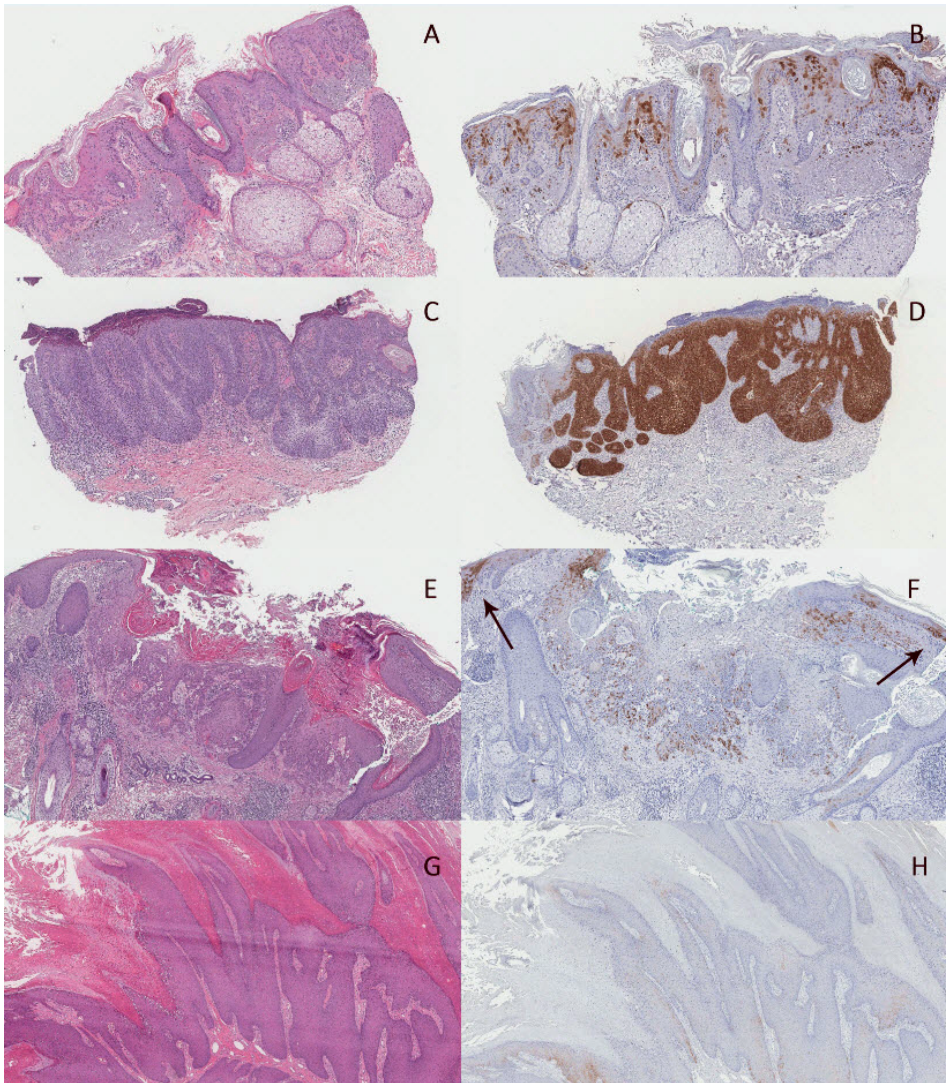


Figure 1. (A-B) Actinic keratosis; keratinocytic intraepidermal neoplasia grade 1 (A) low power view H&E. (B) P16 immunostaining shows a patchy distribution. (C-D) Bowen's disease (C) low power view H&E. (D) P16 immunostaining shows strong staining of lesional cells. (E-F) Squamous cell carcinoma (E) low power view H&E. (F) P16 immunostaining shows a patchy distribution pattern with variable intensity. Note positive cells in the adjacent epidermis compatible with actinic keratosis (ARROW). (G-H) Benign keratotic lesion; case of verruca vulgaris (G) medium power view H&E. (H) P16 immunostaining shows a non-specific pattern with few scattered keratinocytes with low or moderate staining intensity in the lower 1/3 of the epidermis.

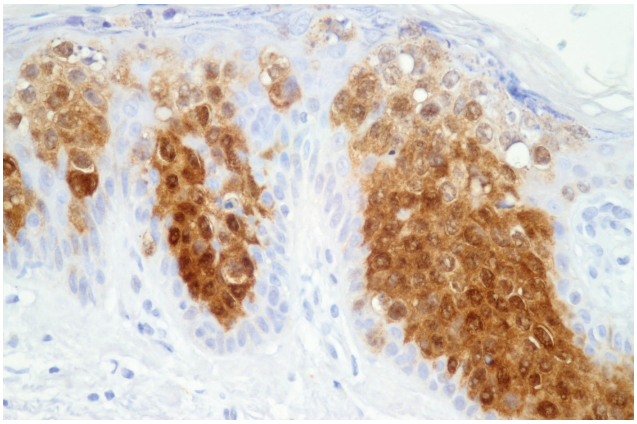


Figure 2.
Actinic keratosis, KINII, showing abundant p16 immunostaining but sparing of the basal layer.

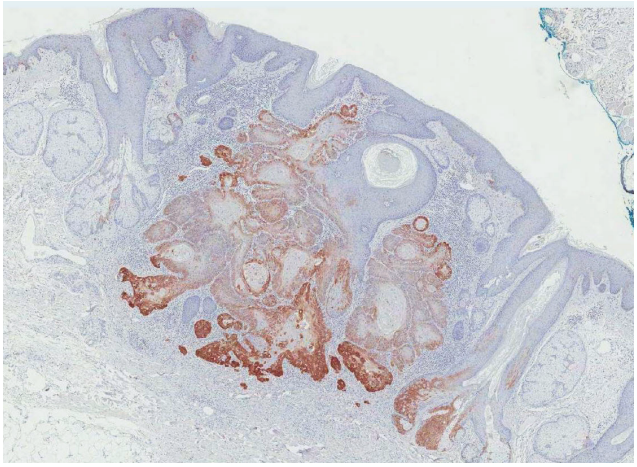


Figure 3.
Well differentiated squamous cell carcinoma with accentuated p16 immunostaining at the invasive peripheral border with the dermis.

Positivity of p16 was seen in 23 of 59 (39%) AK and in 11 of 59 (19%) cases more than 50% of the lesional cells were p16 positive (Table 1). Eight AK were completely negative. The percentage of p16 positive cells were somewhat higher in KIN II than in KIN I lesions, but this difference was not statistically significant. Most AK (63%, n=37) showed moderate intensity of p16 staining in lesional cells.

Bowen's disease.

Diffuse full thickness positivity with strong staining of all atypical cells in the lesional epidermis was seen in 39 of 51 (76%) cases. However, most cases showed focal sparing of the palisaded cells in the basal layer (Figure 1).

Forty-nine of 51 cases (96.1%) showed positivity of p16 immunostaining. In 47 cases (92%) more than 50% of positive lesional cells was seen. None of the BD were completely negative. In 41 of 51 cases (80%) the intensity of p16 staining was strong.

Squamous cell carcinoma

The group of 63 SCC consisted of 45 well differentiated, 14 moderately differentiated, 4 poorly differentiated SCC (Table 1).

Focal staining by p16 in a patchy or scattered pattern, with variable intensity, similar to that observed in AK, was seen in most cases (Figure 1). Also accentuation of the peripheral border of tumor nests was seen in SCC (Figure 1 and Figure 3). Positivity of p16 immunostaining was seen in 40 of 64 cases (62%). Staining of more than 50% of the lesional cells was found in 21 of 64 (33%) cases. Three SCC did not show any positive cells. No (significant) differences were found between well differentiated, moderately and poorly differentiated SCC. In the majority of SCC (75%) the staining intensity was moderate.

Benign keratotic lesion (BKL) and other benign controls

The group of BKL consisted of 5 verrucae vulgaris and 11 seborrheic keratosis.

In most cases a patchy or scattered pattern, with variable intensity was seen (Figure 1). Positivity of p16 was seen in 5 of 16 (31.3%) cases. Only 2 cases, both seborrheic keratosis, showed more than 50 % positive lesional cells. In most cases (75%) the staining intensity was moderate.

In 5 of 14 (36%) eczema and 3 of 11 (27%) psoriasis lesions, a few scattered cells (1-10%) were p16 positive. We observed this in the coil and duct of the eccrine sweat glands, but also some in the seborrheic glands and reactive epidermis. None of these lesions showed positivity of p16. In the other 17 lesions p16 staining was completely negative.

Discussion

This study evaluated p16 immunostaining patterns in different types of keratinocytic neoplasia from organ transplant recipients. P16 staining was most pronounced in BD with positivity (>10% p16 positive lesional cells) in 47 of 51 (92%) cases. The characteristic staining pattern in BD with strong staining of all lesional cells

Table 1. p16 immunostaining in keratinocytic neoplasia.

	Percentage p16 positive cells				Intensity of p16 positive cells*			Positivity**
	0%	**1-10%**	**11-50%**	**>50%**	**Weak**	**Moderate**	**Strong**	
AK (59)	8 (13.6%)	26 (44.1%)	14 (23.7%)	11 (18.6%)	6 (11.8%)	37 (72.5%)	8 (15.7%)	23 (39.0%)
KIN I (27)	4 (14.8%)	14 (51.9%)	8 (29.6%)	1 (3.7%)	3 (13.0%)	19 (82.6%)	1 (4.4%)	8(29.6%)
KIN II (32)	4 (12.5%)	12 (37.5%)	6 (18.8%)	10 (31.3%)	3 (10.7%)	18 (64.3%)	7 (25.0%)	15 (46.9%)
BD (KIN III) (51)	0	1 (2.0%)	3 (5.9%)	47 (92.2%)	2 (3.9%)	8 (15.7%)	41 (80.4%)	49 (96.1%)
SCC (63)	3 (4.8%)	21 (35.0%)	18 (30.0%)	21 (35.0%)	2 (3.3%)	47 (78.4%)	11 (18.3%)	40 (63.5%)
Well differentiated (45)	1 (2.2%)	13 (28.9%)	16 (35.6%)	15 (33.3%)	1 (2.2%)	37 (84.1%)	6 (13.7%)	31 (68.9%)
Moderately differentiated (14)	1 (7.1%)	7 (50.0%)	2 (14.3%)	4 (28.6%)	1 (7.7%)	9 (69.2%)	3 (23.1%)	6 (42.9%)
Poor differentiated (4)	1 (25.0%)	1 (25.0%)	0	2 (50.0%)	0	1 (33.3%)	2 (66.7%)	2 (50.0%)0
BKL (16)	1 (6.3%)	10 (62.5%)	3 (18.7%)	2 (12.5%)	1 (6.7%)	12 (80.0%)	2 (13.3%)	5 (31.3%)
Psoriasis (11)	8 (72.7%)	3 (27.3%)	0	0	1 (33.3%)	2 (66.7%)	0	0
Eczema (n=14)	9 (64.3%)	5 (35.7%)	0	0	0	5 (100.0%)	0	0

*Percentage is calculated in p16 positive cases.
** Positivity of p16 immunostaining was defined as more than 10% moderate or strong staining of lesional cells.
Abbreviations: AK; actinic keratosis, KIN; keratinocyte intraepidermal neoplasia, BD; Bowen's disease, SCC; squamous cell carcinoma, BKL; benign keratotic lesion

accompanied often by sparing of the basal layer, may be considered as a useful adjunct in confirming the diagnosis of BD and in differentiating BD from SCC. In AK and SCC the extent, percentage and intensity of p16 positive cells was much less than in BD, much more variable and did not allow clear-cut distinction between these conditions.

Previous studies of p16 immunostaining in premalignant and malignant keratinocytic lesions showed discrepant results.[12,13,17,18,22-29] Comparison between these different studies is difficult, since different scoring methods were used. Only two studies included lesions from organ transplant patients and their numbers were relatively small.[17,18] Most previous studies of BD and Bowenoid AK report strong p16 staining of the entire epidermis with or without sparing of the basal layer. However, in some studies a considerable proportion of BD, varying between 15% and 40%, did not show positivity of p16. In contrast, in our study staining of less than 10% of the lesional cells was observed in only 1 of 51 (2%) BD cases. Comparison with another study including keratinocytic neoplasms in OTR is impossible, because the results of OTR were not specified.[18] Blokx et al found similar results, with none of the BD (n=14) in OTR negative for p16, while 2 out of 14 BD in immunocompetent patients were p16 negative.[17]

It is reported that progression to SCC occurs in approximately 5 % of BD cases.[19] Such SCC shows poor differentiation and is considered to be of high risk.[30] It is unknown if these SCC show the same extensive p16 immunostaining as observed in BD. The present study contained 1 case of SCC originating from a BD and was poorly differentiated. Remarkably, in this case both SCC and the small remaining area of BD were p16 negative.

Previous studies on p16 immunostaining in AK and SCC reported conflicting results with positivity of p16 varying between 0% and 68 % for AK, and between 10% and 75% for SCC.[12,17,18,22-28] The results of the present study show a gradual increase in the percentage of cases showing positivity of p16 from 34% for KIN I to 50% for KIN II and 63% for SCC. P16 expression by more than 50% of the lesional cells was found in only 1 of 11 (4%) KIN I lesions, compared to 10 of 32 (31%) KIN II lesions and 21 of 64 (33%) SCC. However, staining patterns and intensity were variable in these groups and the differences were not statistically significant, indicating that p16 immunostaining is not useful as a differential diagnostic marker.

For benign keratinocytic lesions (BKL), our results differ from previous reports.[18,23,25,26,28] We grouped the diagnoses of verruca vulgaris and seborrheic

keratosis as BKL in our study. Previous studies that have reported on immunostaining in BKL have studied most frequently seborrheic keratosis and to lesser extent verrucae or benign keratosis not further specified. In two studies, no positivity of p16 in BKL was found.[18,28] Three studies showed positive p16 immunostaining in seborrheic keratosis and benign keratosis in up to 67% of cases.[23,25,26] Those results were consistent with our finding that p16 immunostaining is frequent, but not extensive or strong. All control specimens of psoriasis and eczema did not show positivity of p16 with only focal staining.
Besides extent and intensity of p16 immunostaining, pattern of staining can be informative. This was most distinct in BD. Diffuse full thickness positivity with strong staining of all atypical cells in the lesional epidermis was only seen in BD. Focal staining in a patchy or scattered pattern, with variable intensity and distribution across the lesion was the dominant pattern in AK, SCC and BKL. Two studies have mentioned sparing of palisaded cells in the basal layer while the upper layers show distinct p16 immunostaining as a characteristic finding in BD.[23,29] We observed this pattern predominantly in BD, but it was also seen in AK and SCC. In SCC accentuation of p16 immunostaining at the border of tumor nests with the dermis was observed.

In conclusion p16 immunostaining shows a very distinct staining pattern in most BD, characterised by strong staining of all abnormal cells with sparing of the basal layer cells. This can be useful in confirming a diagnosis of BD. However, the distribution, proportion and intensity of p16 positive cells is very variable in AK, SCC and BKL, suggesting that p16 immunostaining cannot be considered as a useful adjunct in differentiating between these conditions.

References

1. Weedon D. Tumors of the epidermis. In: D. W, editor. Skin pathology. London: Churchill Livingstone; 2002. p. 761-72.
2. Cockerell CJ. Pathology and pathobiology of the actinic (solar) keratosis. BrJDermatol. 2003;149 Suppl 66:34-6.
3. Ramos-Ceballos FI, Ounpraseuth ST, Horn TD. Diagnostic concordance among dermatopathologists using a three-tiered keratinocytic intraepithelial neoplasia grading scheme. JCutanPathol. 2008;35(4) :386-91.
4. Cooper SM, Wojnarowska F. The accuracy of clinical diagnosis of suspected premalignant and malignant skin lesions in renal transplant recipients. ClinExpDermatol. 2002;27(6):436-8.
5. Jenni D, Hofbauer GF. Keratinocyte cancer and its precursors in organ transplant patients. CurrProblDermatol. 2015;46:49-57.
6. Feldman SR, Fleischer AB, Jr. Progression of actinic keratosis to squamous cell carcinoma revisited: clinical and treatment implications. Cutis. 2011;87(4):201-7.
7. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part I. Epidemiology of skin cancer in solid organ transplant recipients. JAmAcadDermatol. 2011;65(2):253-61.
8. Cubilla AL, Lloveras B, Alejo M, Clavero O, Chaux A, Kasamatsu E,... Bosch FX. Value of p16(INK)(4)(a) in the pathology of invasive penile squamous cell carcinomas: A report of 202 cases. AmJSurgPathol. 2011;35(2):253-61.
9. de Koning MN, Quint WG, Pirog EC. Prevalence of mucosal and cutaneous human papillomaviruses in different histologic subtypes of vulvar carcinoma. ModPathol. 2008;21(3):334-44.
10. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U,... von Knebel DM. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. IntJCancer. 2001;92(2):276-84.
11. Quint KD, Genders RE, de Koning MN, Borgogna C, Gariglio M, Bouwes Bavinck JN,... Feltkamp MC. Human Beta-papillomavirus infection and keratinocyte carcinomas. JPathol. 2015;235(2):342-54.
12. Kusters-Vandevelde HV, de Koning MN, Melchers WJ, Quint WG, de Wilde PC, de Jong EM,... Blokx WA. Expression of p14ARF, p16INK4a and p53 in relation to HPV in (pre-)malignant squamous skin tumours. JCell MolMed. 2009;13(8B):2148-57.
13. Murao K, Yoshioka R, Kubo Y. Human papillomavirus infection in Bowen disease: negative p53 expression, not p16(INK4a) overexpression, is correlated with human papillomavirus-associated Bowen disease. JDermatol. 2014;41(10):878-84.
14. Svajdler M, Jr., Mezencev R, Kaspirkova J, Kacerovska D, Kazakov DV, Ondic O, Michal M. Human Papillomavirus Infection and p16 Expression in Extragenital/Extraungual Bowen Disease in Immunocompromised Patients. AmJDermatopathol. 2016.
15. Chazal M, Marionnet C, Michel L, Mollier K, Dazard JE, Della V, V,... Basset-Seguin N. P16(INK4A) is implicated in both the immediate and adaptative response of human keratinocytes to UVB irradiation. Oncogene. 2002;21(17):2652-61.
16. Pavey S, Conroy S, Russell T, Gabrielli B. Ultraviolet radiation induces p16CDKN2A expression in human skin. Cancer Res. 1999;59(17):4185-9.
17. Blokx WA, de Jong EM, de Wilde PC, Bulten J, Link MM, Ruiter DJ, van de Kerkhof PC. P16 and p53 expression in (pre)malignant epidermal tumors of renal transplant recipients and immunocompetent individuals. ModPathol. 2003;16(9):869-78.
18. Conscience I, Jovenin N, Coissard C, Lorenzato M, Durlach A, Grange F,... Bernard P. P16 is overexpressed in cutaneous carcinomas located on sun-exposed areas. EurJDermatol. 2006;16(5):518-22.
19. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification--part two. JCutanPathol. 2006;33(4):261-79.
20. Molijn A, Jenkins D, Chen W, Zhang X, Pirog E, Enqi W,... Chinese HPVTG. The complex relationship between human papillomavirus and cervical adenocarcinoma. International journal of cancer. 2016;138(2):409-16.
21. Lesnikova I, Lidang M, Hamilton-Dutoit S, Koch J. p16 as a diagnostic marker of cervical neoplasia: a tissue microarray study of 796 archival specimens. DiagnPathol. 2009;4:22.

22. Bagazgoitia L, Cuevas J, Juarranz A. Expression of p53 and p16 in actinic keratosis, bowenoid actinic keratosis and Bowen's disease. JEurAcadDermatolVenereol. 2010;24(2):228-30.
23. Harvey NT, Leecy T, Wood BA. Immunohistochemical staining for p16 is a useful adjunctive test in the diagnosis of Bowen's disease. Pathology. 2013;45(4):402-7.
24. Hodges A, Smoller BR. Immunohistochemical comparison of p16 expression in actinic keratoses and squamous cell carcinomas of the skin. ModPathol. 2002;15(11): 1121-5.
25. Kaabipour E, Haupt HM, Stern JB, Kanetsky PA, Podolski VF, Martin AM. p16 expression in keratoacanthomas and squamous cell carcinomas of the skin: an immunohistochemical study. ArchPatholLab Med. 2006;130(1):69-73.
26. Mortier L, Marchetti P, Delaporte E, Martin de Lassalle E, Thomas P, Piette F,... Danze PM. Progression of actinic keratosis to squamous cell carcinoma of the skin correlates with deletion of the 9p21 region encoding the p16(INK4a) tumor suppressor. Cancer letters. 2002;176(2):205-14.
27. Murao K, Kubo Y, Ohtani N, Hara E, Arase S. Epigenetic abnormalities in cutaneous squamous cell carcinomas: frequent inactivation of the RB1/p16 and p53 pathways. BrJDermatol. 2006;155(5):999-1005.
28. Salama ME, Mahmood MN, Qureshi HS, Ma C, Zarbo RJ, Ormsby AH. p16INK4a expression in actinic keratosis and Bowen's disease. BrJDermatol. 2003;149(5):1006-12.
29. Willman JH, Heinz D, Golitz LE, Shroyer KR. Correlation of p16 and pRb expression with HPV detection in Bowen's disease. JCutanPathol. 2006;33(9):629-33.
30. Kao GF. Carcinoma arising in Bowen's disease. ArchDermatol. 1986;122(10):1124-6.



CHAPTER 5

Pain identifies squamous cell carcinoma in organ transplant recipients: The SCOPE-ITSCC PAIN study

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Abstract

Organ transplant recipients are at high risk for cutaneous squamous cell carcinomas (SCC). We aimed to define clinically meaningful patient-reported warning signals predicting the presence of invasive SCC. Patient-reported signs and symptoms of 812 consecutively biopsied skin lesions from 410 organ transplant recipients were determined by questionnaire and physical examination and related to the subsequent biopsy proven diagnoses. Receiver-operating-characteristic (ROC) curve analyses were used as a measure of distinction between the predictive values of patient-reported warning signals and the occurrence of SCC.

Pain was an independent predictive patient-reported warning signal for a biopsy proven invasive SCC. The odds ratio from the fully adjusted model predicting SCC was 4.4 (95% confidence interval: 2.4–8.2). Higher scores on the visual analog scale for pain were associated with a greater likelihood for the presence of SCC compared to none or mild pain. The odds ratio for scores on the visual analog scale from 1 to 3, 4 to 6 and 7 to 10 were 4.9 (2.2–10.5), 2.3 (0.96–5.5), and 16.5 (3.6–75.8).

Pain is the most powerful patient-reported warning signal for invasive cutaneous SCC in organ transplant recipients. Empowerment of patients by education could accelerate diagnosis and treatment of cutaneous SCC.

Introduction

In organ transplant recipients (OTR), the risk of invasive cutaneous squamous cell carcinomas (SCC) is 50 to 100-fold increased compared with the immunocompetent population.[1-8] More than half of the OTR will be affected during their long-term course.[1] Invasive cutaneous SCC in OTR derives from in-situ SCC (also called Bowen's disease) or actinic keratosis. These intraepidermal precursor lesions grow faster in OTR compared to immunocompetent patients.[9,10] After a first invasive SCC, multiple subsequent SCC will develop in 60 to 80% of these patients within 3 years.[2,7,11,12]
In the immunocompetent population keratoacanthomas are rapidly growing, low-grade skin tumors that rarely invade or metastasize and often spontaneously involute, and they are considered as "keratoacanthoma-like" invasive SCC. However, in OTR the distinction between invasive SCC and keratoacanthoma cannot be made by clinical or histological examination and, therefore, keratoacanthomas in OTR are considered equivalent to invasive cutaneous SCC.[1]
OTR with SCC usually have numerous keratotic skin lesions, such as actinic keratoses, common warts, seborrheic warts and hyperkeratotic papillomas and they may mimic invasive cutaneous SCC hampering timely diagnosis making.[13] Validated patient-reported warning signals are needed to facilitate rapid screening of large numbers of keratotic skin lesions in OTR to identify invasive SCC for early diagnosis and treatment. Presence of pain has been proposed as a warning signal and a diagnostic criterion to distinguish malignant from benign lesions, but its validity has not been studied.[14-16] Thus, the aim of our study was to identify clinically meaningful independent patient-reported signs and symptoms predicting the presence of invasive cutaneous SCC in OTR.

Patients and Methods

Study design

Our study was performed between July 2008 and February 2011 in 10 centers dedicated to surveillance of OTR with skin problems in Europe and the United States. The investigator-sponsored study was designed by the principal investigators and emerged from a joint initiative of SCOPE (Skin Care in Organ transplant Patients Europe, http://www.scopenetwork.org/) and ITSCC (International Transplant Skin

Cancer Collaborative, http://www.itscc.org/). Each site's independent ethics committee or institutional review board approved the protocol and participants gave informed consent in accordance with the Declaration of Helsinki.

Study procedures

Patients were eligible for enrollment if they received a solid organ and underwent skin biopsy. Consecutive patients requiring a skin biopsy for medical, cosmetic or other reasons were enrolled during the study period. The first SCC in this study was not necessary the first SCC in an individual patient. Tumor size and localization as well as clinical features (hyperkeratosis, central keratotic plug, ulceration, and erythema) were identified by visual inspection, scored and recorded during the physical examination by a dermatologist at the point at which the biopsy was performed and histological diagnosis thereby established. Medical history of diabetes, neurological disorders, and analgesic drug use as well as baseline clinical data from the medical chart were recorded.

SCC and keratoacanthomas are often considered to be part of the same skin disease and are treated alike.[1] Therefore, the histological diagnoses were grouped into invasive cutaneous SCC and/or keratoacanthoma and all other histological diagnoses (in situ SCC, actinic keratoses, seborrheic warts, common warts, hyperkeratotic papillomas, basal cell carcinomas, and miscellaneous skin disorders).

Patient questionnaire

The study procedures included a detailed patient questionnaire recording signs and symptoms of individual lesions at the time of biopsy. The questionnaire was developed in Leiden, the Netherlands and an initial pilot study was performed to qualitatively assess patient understanding, clinician usability / data entry and comprehensiveness of information collected. Based upon this pilot study, amendments were made to the questionnaire and a second pilot study was performed in Zürich, Switzerland. The pilot questionnaire was used to improve and refine the questions asked in the study and not to determine how many patients were needed to ensure adequate power for the various VAS scores. The questions covered different aspects of pain: spontaneous pain (without touch or palpation); tenderness by touching or palpation (no spontaneous pain), intensity of pain on a visual-analog scale from 0 to 10 where 0 denotes no pain and 10 strongest pain, pain disturbing sleep as well as itching and bleeding of the lesion. The time between initial

appearance of the lesion and onset of pain was recorded. The complete standard operating procedures and questionnaire are available as supplement 1.

Statistical methods and analyses

We randomly allocated the biopsies 1:1 into a testing and a validation set: the testing set with 406 biopsies was used for model training and building, selection of candidate symptoms and testing of robustness and sensitivity, and the second set with 406 biopsies was used to validate the results derived from the training set. The models derived from the validation set were then applied to the complete data set to estimate the independent association of patient-reported signs and symptoms with biopsy proven invasive cutaneous SCC.

Receiver-operating-characteristic (ROC) curve analyses were used to calculate the area under the curve (AUC) with 95% confidence intervals as a measure of distinction between the predictive values of patient-reported signs and symptoms and biopsy-proven invasive cutaneous SCC. An AUC with a value between 1 (100%) and 0.5 (0%) indicates a positive predictive symptom and between 0 (100%) and 0.5 (0%) a negative predictive symptom.[17] An AUC greater than 0.9 indicates high accuracy, while 0.7 – 0.9 indicates moderate accuracy, 0.5 – 0.7 low accuracy and 0.5 a chance result.[17]

To estimate the independent association of patient-reported warning signals and invasive cutaneous SCC we used univariable and multivariable logistic regression models for patients in whom only the first biopsy was analyzed and for the complete dataset. Odds ratios (OR) and 95% confidence intervals (CI) were provided as measures of strength of association and precision, respectively. Univariable analyses were performed and three models were tested: in model 1 the variable of interest was adjusted for age, gender and center; in model 2 we also adjusted for time since transplantation and number of prior squamous cell carcinomas and in model 3 we also adjusted for diabetes, neurological disease and analgesic drug use.

Sensitivity analyses were performed to assess the impact of the number of biopsies per patient and of the presence of keratoacanthomas on the association between warning signals and invasive SCC.

For the analyses we used the software package IBM SPSS Statistics, release 20.0.0 (2011).

Results

We enrolled 410 patients undergoing 812 skin biopsies; 289 (70.7%) kidney, 17 (4.2%) combined kidney and pancreas, 2 (0.5%) combined kidney and lung, 1 (0.2%) combined kidney and heart, 46 (11.2%) heart, 28 (6.8%) liver, 22 (5.4%) lung and 9 non-specified transplant recipients with a male predominance (69.3%). Figure 1 displays the histological diagnosis distribution of the 812 skin biopsies: 266 invasive SCC, 34 keratoacanthomas, 135 basal cell carcinomas, 144 intraepithelial skin lesions (76 in situ SCC and 68 actinic keratoses), 95 benign keratotic skin lesions (34 seborrheic warts, 24 common warts and 37 hyperkeratotic papillomas) and 138 miscellaneous skin biopsies. We did not observe perineural invasion in SCC biopsies. Table 1 shows the patient characteristics stratified by the presence of invasive SCC and Table 2 the histological characteristics of the biopsies at enrollment. SCC occurred later after transplantation and often in patients with a medical history of multiple SCC. The frequency of diabetes, neurological disease and analgesic drug use were similar in patients with and without SCC (Table 1 and supplementary Tables S1a and S1b).

Figure 2 shows the ROC curve analyses for pain (yes vs. no) with the training and validation sets. The presence and intensity of pain were the most important patient-reported signs and symptoms predicting SCC or keratoacanthoma (supplementary Figures S1a – S1c and S2). Spontaneous pain and tenderness on palpation predicted a 4-fold and more increased relative risk of SCC or keratoacanthomas. Figure 3 and supplementary Tables S2 and S3 display adjusted odds ratios for these factors associated with SCC. For the fully adjusted model, the odds ratio for pain and tenderness combined among patients undergoing the first skin biopsies was 4.4 (95% confidence interval: 2.4 – 8.2), and for all biopsies 4.9 (95% confidence interval: 3.3 - 7.1). Higher scores on the visual analog scale for pain were associated with a greater likelihood for the presence of malignant skin cancer compared none or mild pain. For the fully adjusted model, the odds ratio for scores on the visual analog scale from 1 to 3, 4 to 6 and 7 to 10 among patients undergoing the first skin biopsies were 4.9 (95% confidence interval: 2.2 – 10.5), 2.3 (95% confidence interval: 0.96 – 5.5), and 16.3 (95% confidence interval: 3.6 – 75.8), and for all biopsies 4.3 (95% confidence interval: 2.6 - 7.1), 3.6 (95% confidence interval: 2.1 – 6.1), and 10.2 (95% confidence interval: 4.9 – 20.9), respectively. All other signs depicted in supplementary Figures S1d to S1m had a lower or no predictive accuracy compared to pain, e.g. erythema, itchiness and bleeding.

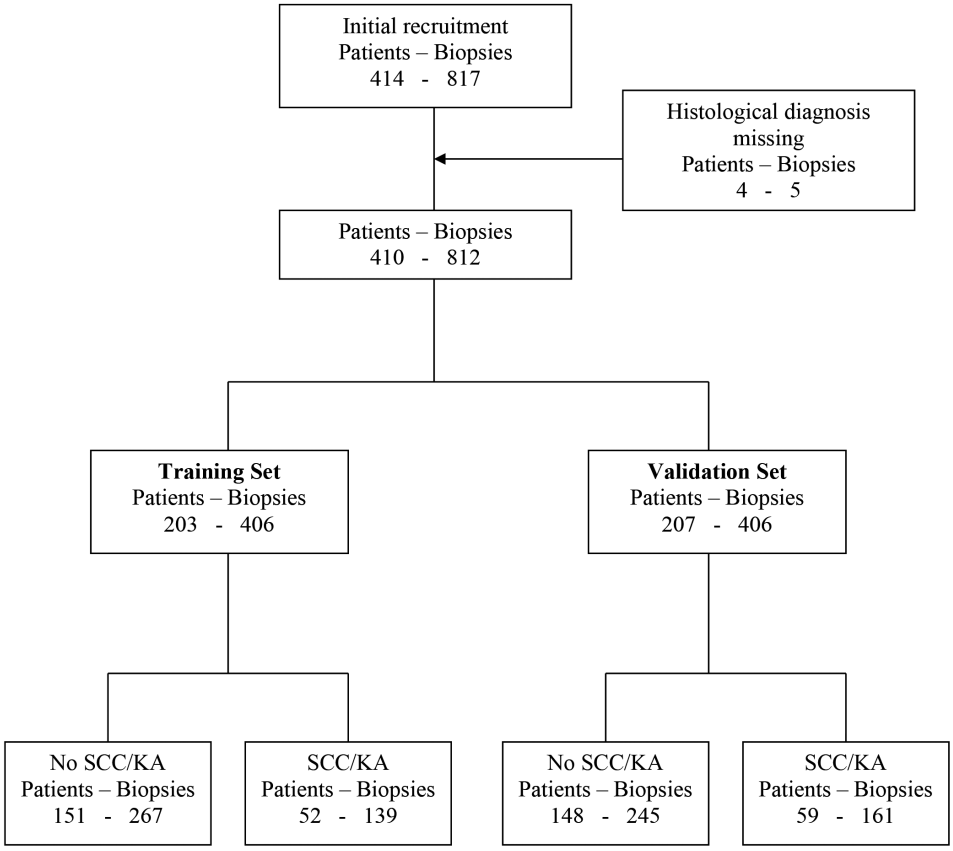


Figure 1. Study profile. SCC depicts invasive squamous cell carcinoma and KA keratoacanthoma.

Table 1. Baseline characteristics of the patients at the first biopsy.

	All patients	Skin biopsies without SCC/KA	Skin biopsies with SCC/KA	p-Value
Number of patients	410	299	111	
Sex: N (%)				
Women	125 (30.5)	90 (30.1)	35 (31.5)	
Men	285 (69.5)	209 (69.9)	76 (68.5)	0.780
Age (25%; median; 75%)				
At transplantation (years)	32.7; 44.6; 56.3	35.1; 47.2; 57.2	28.1; 37.1; 51.4	<0.0001
At biopsy (years)	51.9; 61.7; 67.3	51.5; 61.2; 67.3	54.2; 62.6; 68.1	0.122
Time since transplantation (years)	5.9; 13.7; 22.1	4.9; 11.1; 18.9	13.0; 21.6; 27.8	<0.0001
Number of earlier SCC by medical history				
(minimum; mean; SD; maximum) (25%; median; 75%)	0; 5.1; 11.5; 100 0; 0; 4	0; 3.3; 8.7; 65 0; 0; 2	0; 9.4; 15.6; 100 1; 3; 9	<0.0001
No. of biopsies per patient in this study: N (%)				
1	252 (61.5)	198 (66.2)	54 (48.7)	
2 - 4	131 (32.0)	92 (30.8)	39 (35.1)	<0.0001
5 and more	27 (6.5)	9 (3.0)	18 (16.2)	
Diabetes: N (%)				
No diabetes	334 (82.2)	240 (81.1)	94 (85.5)	
Diabetes type I	25 (6.2)	21 (7.1)	4 (3.6)	0.406
Diabetes type II	47 (11.6)	35 (11.8)	12 (10.9)	
Neurological disease: N (%)				
No	367 (90.6)	268 (90.8)	99 (90.0)	
Yes	38 (9.4)	27 (9.2)	11 (10.0)	0.795
Analgesic drug use: N (%)				
No	307 (74.9)	228 (76.3)	79 (71.2)	
Yes	103 (25.1)	71 (23.7)	32 (28.8)	0.292

Abbreviations: SCC: squamous cell carcinoma, KA: keratoacanthoma, SD: standard deviation.
The numbers do not always add up to the total number, because of missing values.
The p-values of the categorical variables (sex, no. of biopsies, diabetes, neurological disease and analgesic drug use) are calculated with the Chi-square test, the p-values of the continuous variables (age and number of earlier SCC) by ANOVA.

Table 2. Characteristics of the first biopsies.

	Skin biopsies without SCC/KA	Skin biopsies with SCC/KA	Rate of SCC/KA (%)	p-Value
Number of first biopsies	299	111	27.1	
Reason of visit (when indicated): N (%)				
Routine follow-up visit	134	27	16.8	
Because of the biopsied lesion	35	18	34.0	0.028
Because of other lesion	6	2	25.0	
Reason of biopsy: N (%)				
Suspicion SCC/KA	100	100	50.0	
Suspicion BCC	63	3	4.5	
Pain	6	4	40.0	<0.0001
Annoying for patient	47	1	2.1	
Other reason	74	2	2.2	
Patients' reported features				
Pain				
No pain	241	41	14.5	
Tenderness	29	38	56.7	<0.0001
Spontaneous pain	29	32	52.5	
T and S combined	58	70	54.7	<0.0001
Intensity pain (VAS)				
VAS 0 (no pain)	227	39	14.7	
VAS 1-3	33	34	50.7	
VAS 4-6	21	23	52.3	<0.0001
VAS 7-10	3	11	78.6	
Pain perceived at night				
No	225	79	26.0	
Yes	9	19	67.9	<0.0001
Awakened by pain	0	1	100.0	
Itchiness				
No	213	67	23.9	
Yes	77	42	35.3	0.020
Prior bleeding of the lesion				
No	241	74	23.5	
1-4 times	40	27	40.3	0.005
5 and more times	12	9	42.9	
Duration of pain				
No pain	242	41	14.5	
2 months and longer pain	20	14	41.2	<0.0001
Less than 2 months pain	24	43	64.2	
Duration of lesion				
2 months and longer	187	50	21.1	
Less than 2 months	42	48	53.3	<0.0001

Table 2. Continued

	Skin biopsies without SCC/KA	Skin biopsies with SCC/KA	Rate of SCC/KA (%)	p-Value
Features observed by doctor				
Location skin lesion				
Trunk	79	12	13.2	
Head and neck	110	45	29.0	
Legs and feet	29	12	29.3	
Upper arms	23	2	8.0	<0.0001
Forearms	22	17	43.6	
Hands and fingers	20	22	52.4	
Other	9	0	0	
Size of the lesion				
0 – 1 cm	224	73	24.6	
1 and more cm	71	38	34.9	0.039
Hyperkeratosis				
No	184	27	12.8	
Yes	113	84	42.6	<0.0001
Central keratotic plug				
No	272	65	19.3	
Yes	20	46	69.7	<0.0001
Erosion				
No	200	46	18.7	
Yes	40	39	49.4	<0.0001
Erythema				
No	112	21	15.8	
Yes	58	26	31.0	0.008

Abbreviations: SCC: squamous cell carcinoma, KA: keratoacanthoma, BCC: basal cell carcinoma, VAS: visual analog scale, SD: standard deviation. The numbers do not always add up to the total number, because of missing values. The p-values of the categorical variables (all variables in this Table) are calculated with the Chi-square test.

Additional analyses showed that the odds ratios for the ability of tenderness on palpation and spontaneous pain to distinguish SCC or keratoacanthoma from basal cell carcinoma were higher, namely 14.3 (95% confidence interval: 7.1 - 28.8) and 16.5 (95% confidence interval: 7.3 - 37.3), respectively. Odds ratios for the ability of these features to distinguish actinic keratoses and Bowen's disease were 2.5 (95% confidence interval: 1.6 - 4.0) and 3.9 (95% confidence interval: 2.2 - 6.8), respectively. These analyses are detailed in supplementary Tables S4 and S5.

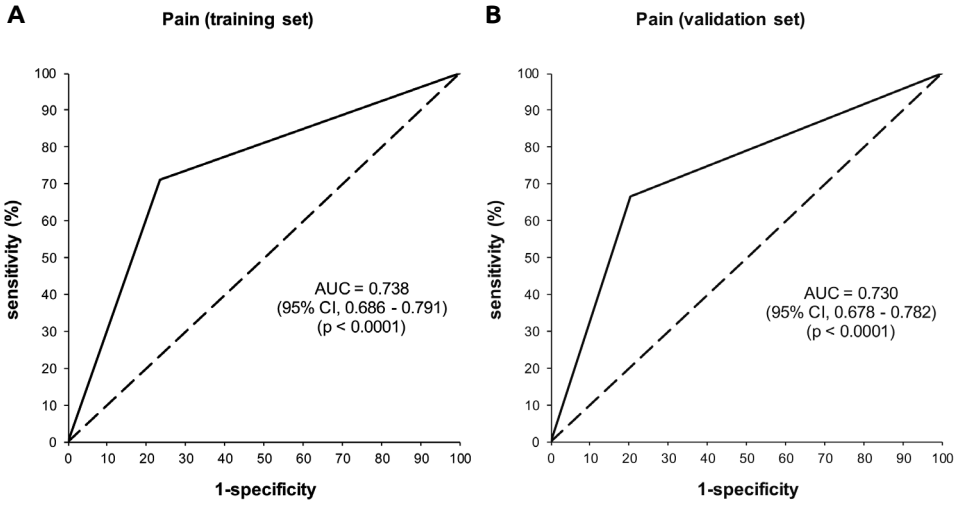


Figure 2. Receiver-operating-characteristic (ROC) curves indicating sensitivity and specificity of the self-reported symptom of pain (yes vs. no and on a visual analog scale) in the training (panel A) and validation (panel B) set to predict the histological presence of invasive squamous cell carcinoma or keratoacanthoma. AUC denotes area under the curve, and CI confidence interval.

ROC curve analyses for pain (yes vs. no and on a visual analogue scale) with the training and validation sets combined restricted to 410 patients and for the complete set of 812 biopsies are provided in supplementary Figure S2, showing that the analyses of the complete set of biopsies is consistent with the analyses of first biopsies. Analyses stratified for specific body sites (supplementary Table S6), hyperkeratotic and non-hyperkeratotic lesions (supplementary Table S7), and lesions with or without a central keratotic plug (supplementary Table S8) consistently showed that pain predicted SCC or keratoacanthoma in all strata. The outcomes of sensitivity analyses were similar compared to the primary statistical analysis. Also when only analyzing patients with five or more biopsies, the results were similar compared to results derived from patients with one biopsy (supplementary Table S9).

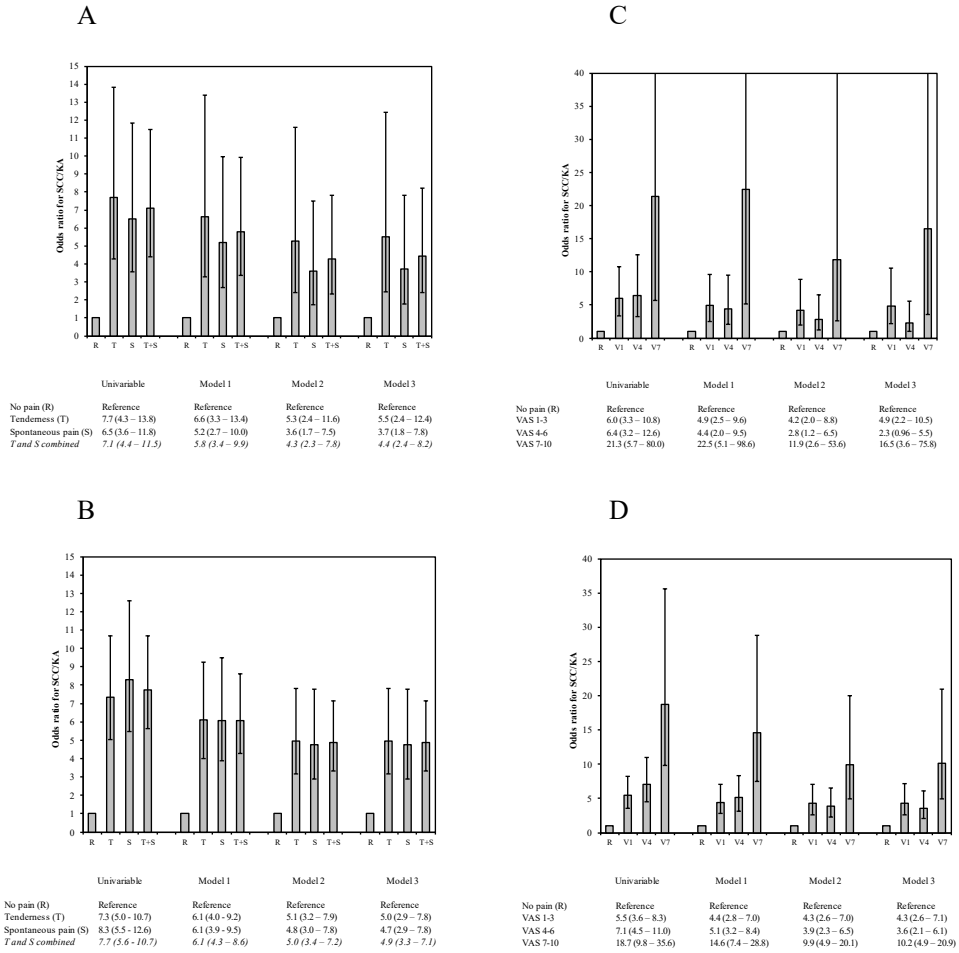


Figure 3. Univariable and multivariable adjusted odds ratios with 95% confidence intervals for squamous cell carcinoma or keratoacanthoma compared to no pain (R) according to tenderness (T), spontaneous pain (S) and the combination of tenderness and spontaneous pain (panels A and B) and according to a visual analogue scale (VAS) for pain (panels C and D). Model 1 is adjusted for age, gender and center; model 2 is also adjusted for time since transplantation and number of prior squamous cell carcinomas and model 3 is also adjusted for diabetes, neurological disease and analgesic drug use. In panels A and C only the first biopsy per patient is depicted; in panels B and D all biopsies are depicted. The exact numbers and other potential warning signals and clinical features are provided in the supplementary Tables S2 and S3.

Discussion

Pain predicts invasive SCC or keratoacanthoma in OTR. Presence of the lesion for less than 2 months and location on the forearms, hands and fingers were other recognizable symptoms for the patients. Bleeding of the lesions did not predict the presence of a cutaneous SCC but was a characteristic of basal cell carcinomas (data not shown). OTR need to be educated that they should seek medical advice immediately if they percept a painful skin lesion.

Clinically, hyperkeratosis and/or a central keratotic plug often characterize SCC and keratoacanthomas in OTR. However, pain was also a useful warning signal for SCC or keratoacanthoma prediction even if hyperkeratosis and/or a central keratotic plug were absent. Thus, the consulting clinician should have a low threshold for obtaining a histological diagnosis in patients presenting with painful lesions. Although symptoms may provide important clinical clues when evaluating suspicious lesions, histopathologic evaluation remains the definitive method of distinguishing intraepithelial and invasive neoplasms.[14]

Our results in OTR are similar compared with the immunocompetent population in whom SCC are also significantly more likely to present with tenderness when compared with melanoma, basal cell carcinoma, seborrheic keratosis, or actinic keratoses.[14,16,18] However, the presence of pain appears to be more marked among OTR.[15]

The reason why SCC are often painful is poorly understood. There is a reported incidence of perineural spread of 1% for basal cell carcinomas and 2–14% for SCC.[19] Patients usually present with paresthesia, followed by neuropathic pain in the area previously affected by the skin cancer.[19] Although perineural invasion may be of importance, we did not observe this histologic feature in any of the 266 SCC diagnosed. This observation is limited by the fact that only routine histological sections for histopathological diagnosis were analyzed and serial sections encompassing the totality of tumors were not performed. Some perineural invasion may thus have gone unnoticed; however, perineural invasion on its own is unlikely to explain the high rate of pain and tenderness in our patients.

Tumor growth may affect cutaneous nerves.[14] Beyond perineural invasion, modulators of cutaneous sensation such as nerve growth factor and semaphorin, receptors such as μ opiate or Mrgpr receptors have been identified in mostly inflammatory skin diseases.[20-22] Mediators of itch activate unmyelinated afferent neurons originating

as free nerve endings in the epidermis.[23] Compared with basal cell carcinomas, SCC are more often characterized by inflammation.[24] Hydrogen ions, purines, lipids (for example, prostanoids), and immune-related agents (cytokines and chemokines) induced during inflammation may sensitize nociceptors (pain-sensitive cells) and evoke hyperalgesia or increased sensitivity to noxious stimuli.[25] Inflammatory skin diseases are frequently characterized by cutaneous sensations such as itch in atopic dermatitis where IL-31 seems an important mediator, or burning and stinging in dermatitis herpetiformis (Morbus Duhring).[26,27] Breaching of the basement membrane by tumors seems an important factor. While inflammation is present in the intraepithelial lesions of in situ SCC and actinic keratosis pain and tenderness dominate in invasive SCC.[28] The inflammatory microenvironment of invasive SCC may thus favor the sensation of tenderness by as yet undefined pathways.[29]

The strength of this study consists in the large numbers of biopsies that were prospectively collected in multiple countries from kidney, heart, pancreas, liver and lung recipients, and thus yielding robust and valid estimations of pain as a predicting factor for the occurrence of skin malignancy. Our results have to be interpreted in the context of the study design. The study design did not allow assessing the impact on patient education on morbidity and mortality; however, patient education will possibly shorten time until diagnosis and therefore widen the window for preventive and therapeutic measures.

In conclusion, the symptom of pain is a powerful patient-reported warning signal of cutaneous SCC in OTR and the presence of pain should prompt the patients to seek their physician's attention and the doctor to have a high level of suspicion and a low threshold for histological confirmation of the diagnosis. Adherence to these rules will possibly change our current practice in OTR.

Acknowledgement

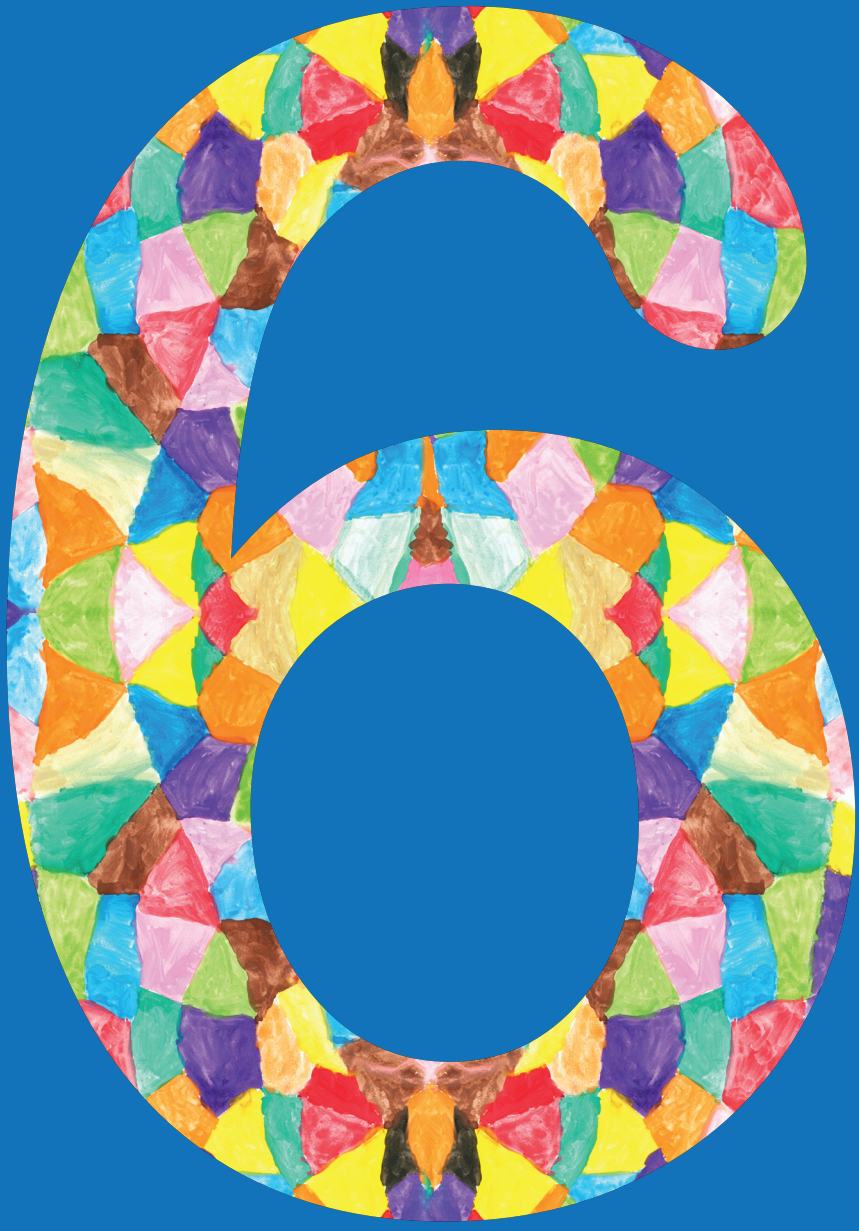
We acknowledge all patients who participated in this observational study.

Supplementary files can be found in the online version of this article

References

1. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. NEnglJMed. 2003;348(17):1681-91.
2. Euvrard S, Kanitakis J, Decullier E, Butnaru AC, Lefrancois N, Boissonnat P,... Chapuis F. Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. Transplantation. 2006;81(8):1093-100.
3. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in the Netherlands. Transplantation. 1990;49(3):506-9.
4. Ramsay HM, Reece SM, Fryer AA, Smith AG, Harden PN. Seven-year prospective study of nonmelanoma skin cancer incidence in U.K. renal transplant recipients. Transplantation. 2007;84(3):437-9.
5. Vajdic CM, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M,... Grulich AE. Cancer incidence before and after kidney transplantation. JAMA. 2006;296(23): 2823-31.
6. Villeneuve PJ, Schaubel DE, Fenton SS, Shepherd FA, Jiang Y, Mao Y. Cancer incidence among Canadian kidney transplant recipients. Am J Transplant. 2007;7(4):941-8.
7. Wisgerhof HC, Edelbroek JR, de Fijter JW, Haasnoot GW, Claas FH, Willemze R, Bouwes Bavinck JN. Subsequent squamous- and basal-cell carcinomas in kidney-transplant recipients after the first skin cancer: cumulative incidence and risk factors. Transplantation. 2010;89(10):1231-8.
8. Wisgerhof HC, van der Geest LG, de Fijter JW, Haasnoot GW, Claas FH, le CS,... Bouwes Bavinck JN. Incidence of cancer in kidney-transplant recipients: a long-term cohort study in a single center. Cancer Epidemiol. 2011;35(2):105-11.
9. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. NEnglJMed. 2001;344(13):975-83.
10. Marks R. Squamous cell carcinoma. Lancet. 1996;347(9003):735-8.
11. Harwood CA, Mesher D, McGregor JM, Mitchell L, Leedham-Green M, Raftery M,... Proby CM. A surveillance model for skin cancer in organ transplant recipients: a 22-year prospective study in an ethnically diverse population. AmJTransplant. 2013;13(1):119-29.
12. Krynitz B, Edgren G, Lindelof B, Baecklund E, Brattstrom C, Wilczek H, Smedby KE. Risk of skin cancer and other malignancies in kidney, liver, heart and lung transplant recipients 1970 to 2008--a Swedish population-based study. International journal of cancer. 2013;132(6):1429-38.
13. Bouwes Bavinck JN, Euvrard S, Naldi L, Nindl I, Proby CM, Neale R,... Harwood CA. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: a case-control study in the Netherlands, United Kingdom, Germany, France, and Italy. JInvest Dermatol. 2007;127(7):1647-56.
14. Cook BA, Gravely AA, Nelson DB, Warshaw EM. Is tenderness a reliable predictor for differentiating squamous cell carcinomas from actinic keratoses? J Am Acad Dermatol. 2011;65(1):211-2.
15. Kwatra SG, Mills KC, Zeitany A, Pearce DJ, Williford PM, D'Agostino RB, Jr., Yosipovitch G. Pain and nonmelanoma skin cancer in transplant patients. J Am Acad Dermatol. 2012;67(6):1387-8.
16. Mills KC, Kwatra SG, Feneran AN, Pearce DJ, Williford PM, D'Agostino RB, Yosipovitch G. Itch and pain in nonmelanoma skin cancer: pain as an important feature of cutaneous squamous cell carcinoma. Arch Dermatol. 2012;148(12):1422-3.
17. Akobeng AK. Understanding diagnostic tests 3: Receiver operating characteristic curves. Acta Paediatr. 2007;96(5):644-7.
18. Askari SK, Schram SE, Wenner RA, Bowers S, Liu A, Bangerter AK, Warshaw EM. Evaluation of prospectively collected presenting signs/symptoms of biopsy-proven melanoma, basal cell carcinoma, squamous cell carcinoma, and seborrheic keratosis in an elderly male population. J Am Acad Dermatol. 2007;56(5):739-47.
19. Viken J, Bendtsen L, Hansen K, Katholm M, Laursen H, Hastrup N,... Ashina M. Facial pain and multiple cranial palsies in a patient with skin cancer. J Headache Pain. 2011;12(3):381-3.
20. Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A,... Dong X. Sensory neuron-specific GPCR Mrgprs are itch receptors medi-

ating chloroquine-induced pruritus. Cell. 2009;139(7):1353-65.
21. Steinhoff M, Bienenstock J, Schmelz M, Maurer M, Wei E, Biro T. Neurophysiological, neuroimmunological, and neuroendocrine basis of pruritus. The Journal of investigative dermatology. 2006;126(8):1705-18.
22. Tominaga M, Kamo A, Tengara S, Ogawa H, Takamori K. In vitro model for penetration of sensory nerve fibres on a Matrigel basement membrane: implications for possible application to intractable pruritus. Br J Dermatol. 2009;161(5):1028-37.
23. Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. Nat Rev Neurosci. 2006;7(7):535-47.
24. Dziunycz P, Hofbauer GF. Immune phenotype of peripheral blood cells and skin squamous cell carcinoma in organ transplant recipients. Expert Rev Clin Immunol. 2010;6(3):359-62.
25. Dawes JM, Calvo M, Perkins JR, Paterson KJ, Kiesewetter H, Hobbs C,... McMahon SB. CXCL5 mediates UVB irradiation-induced pain. Sci Transl Med. 2011;3(90):90ra60.
26. Bolotin D, Petronic-Rosic V. Dermatitis herpetiformis. Part I. Epidemiology, pathogenesis, and clinical presentation. J Am Acad Dermatol. 2011;64(6):1017-24; quiz 25-6.
27. Nobbe S, Dziunycz P, Muhleisen B, Bilsborough J, Dillon SR, French LE, Hofbauer GF. IL-31 expression by inflammatory cells is preferentially elevated in atopic dermatitis. Acta Derm Venereol. 2012;92(1):24-8.
28. Muhleisen B, Petrov I, Gachter T, Kurrer M, Scharer L, Dummer R,... Hofbauer GF. Progression of cutaneous squamous cell carcinoma in immunosuppressed patients is associated with reduced CD123+ and FOXP3+ cells in the perineoplastic inflammatory infiltrate. Histopathology. 2009;55(1):67-76.
29. Kosmidis M, Dziunycz P, Suarez-Farinas M, Muhleisen B, Scharer L, Lauchli S,... Hofbauer GF. Immunosuppression affects CD4+ mRNA expression and induces Th2 dominance in the microenvironment of cutaneous squamous cell carcinoma in organ transplant recipients. J Immunother. 2010;33(5):538-46.



CHAPTER 6

Metastasis of cutaneous squamous cell carcinoma in organ transplant recipients and the immunocompetent population: is there a difference? A systematic review and meta-analysis

J Eur Acad Dermatol Venereol. 2019;33(5):828-841

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Abstract

Organ transplant recipients (OTR) have a higher risk of developing cutaneous squamous cell carcinoma (cSCC) compared to the immunocompetent population. Immunosuppression is often stated as a risk factor for metastasis. However, evidence for this is scarce.

To investigate the cSCC metastasis risk in OTR and the immunocompetent population, a systematic review of the literature was performed up to January 2018 using: Medline; Embase; Web of Science and ISI Science Citation Index. Studies assessing cSCC metastasis risk in ORT or immunocompetent cohorts were considered. A pooled risk estimate for metastasis was calculated for the immunocompetent population and OTR separately.

The pooled metastasis risk estimate for OTR was respectively 7.3% (95% CI 6.2-8.4) for cSCC on total body, and 11.0% (95% CI 7.7-14.8) for cSCC of the head and neck area. For the immunocompetent population reported risk estimate analysis showed a pooled metastatic risk of 3.1% (95% CI 2.8-3.4) in total body cSCC and of 8.5% (95% CI 7.3-9.8) in cSCC of the head and neck area.

Pooled risk estimate per single cSCC in OTR were 1.3% (95% CI 1.0-1.7) in total body cSCC and 4.0% (95% CI 2.7-5.5) in cSCC of the head and neck area. In the immunocompetent population these pooled risk estimates were respectively 2.4% (95% CI 2.1-2.6) and 6.7% (95% CI 5.7-7.8).

OTR show a higher overall risk of cSCC metastasis compared to the immunocompetent population. Metastasis risks per single cSCC were substantially lower in both groups. However, due to heterogeneity and differences between studies, comparisons are difficult. Comprehensive follow-up studies with defined cohorts are necessary to adequately asses the risk for cSCC metastasis.

Introduction

Keratinocyte carcinoma (KC) is the most prevalent cancer worldwide, consisting of cutaneous squamous cell carcinoma (cSCC) and basal cell carcinoma (BCC).[1] Approximately 20-25% of the KCs are cSCC.[2,3] With a lifetime risk for cSCC between 7 to 14% and a rising incidence, cSCC is considered a major health problem in white populations, at substantial costs.[4-9] In high risk populations like solid organ transplant recipients (OTR) the reported cSCC risk is considerably higher, 60 to 250 times increased compared to the immunocompetent population.[10,11] The incidence of cSCC in OTR is directly related to the level and duration of immunosuppressive medication.[10,12-14] Important risk factors for both the immunocompetent and the transplant population are cumulative ultraviolet radiation, older age and male sex and probably human papillomavirus infection.[15-19]

Metastases occur in approximately 5% (range 0.1-9.9%) of cSCC, usually to regional lymph nodes.[15,16,18,20-25] Low risk cSCC (<2cm, depth not beyond dermis, good differentiation grade) metastasize only in 0-3%.[26-29] Presence of risk factors, like large tumor size (>2cm), deep infiltration, location on the lip or ear, poor differentiation grade and perineural and lymphovascular invasion increase the risk for metastasis up to 40%.[2,16,23,30,31] Currently, tumor depth is identified as the most important risk factor for metastasis.[32] For OTR patients a higher risk (13%) for metastatic disease has been described compared to immunocompetent patients with cSCC.[16] Furthermore, it seems that OTR present more commonly with aggressive cSCC (thicker tumors, poorly differentiated and infiltrative), irrespective of size, with a higher predilection for metastasis and worse outcome.[33-37] Furthermore, in studies reporting only on metastasized cSCC, around 5 to 23% of patients were immunosuppressed.[21,38,39]

However, data regarding the metastatic risk of cSCC in OTR is relatively scarce and mostly based on small studies. This current systematic review investigates whether the risk for cSCC metastasis is increased for OTR compared to immunocompetent patients.

Methods

Search strategy and Study selection

The Cochrane Database of Systematic Reviews was searched for a systematic review on this topic, but none was found. An electronic database search was performed up

to 01 January 2018 using the following data sources: Medline; Embase; Web of Science and the ISI Science Citation Index. No restrictions were applied with regards to language or calendar year.
The following search terms and equivalents were used: "squamous cell carcinoma, malignancy, non-melanoma, skin, immunosuppression, transplantation, metastasis". Detailed search strings for PubMed, Embase and Web of Science are shown in supplementary file 1. Relevant citations were checked by browsing the references of review articles and relevant publications of primary investigations were included. Titles and abstracts from retrieved articles were screened by 2 authors (RG and EP). Subsequently full-texts of potentially relevant articles were assessed for eligibility by the same 2 authors. Any discrepancy was resolved by consensus.

Eligibility criteria
Initially, a search was performed for studies that directly compared metastasis risk in OTR and in the immunocompetent population, but direct comparison of metastatic risk in both groups was scarce. Therefore, we broadened the eligibility criteria to include cohort studies and also single arm cohort studies of populations diagnosed with cSCC in: (1). a population of OTR or (2). the immunocompetent population, without immunosuppressed patients, and in which occurrence of metastasis was reported. Studies were included when they had 25 or more patients with a cSCC in any location in both immunocompetent patients and OTR. We excluded studies reporting on populations with solely metastasized cSCC. When more than one report was published on the same population or subpopulation, we included the report with the largest number of cSCC or with the longest follow-up.

Data collection process and risk of bias assessment
The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist was followed for reporting of the review.[40] Inclusion criteria and methods of data analysis were specified in advance. A data extraction sheet was developed and piloted. Two reviewers extracted data independently (RG and EP) and cross-checked each other's results and disagreements were resolved by discussion. The following information was extracted from each included study; (1) study characteristics (e.g. design, location, centre, years of data collection, aim), (2) population characteristics (including number of patients with a solid organ transplant and immunocompetent population patients, gender, age, transplantation characteristics) and tumor data (including

number of cSCC, length of follow-up) (3) type of outcome measure (metastasis; nodal, in-transit, systemic).
A component based approach to assess risk of bias based on the Newcastle-Ottawa Quality assessment scale was used.[41] Relevant items of this scale were used and adjusted for our cohort. Important items relevant to the topic of this review were added. The following design elements were assessed; (1) whether the outcome metastasis was noted in the study aim, (2) statement of absence of immunosuppressive patients in the immunocompetent population studies, (3) inclusion of all consecutive cSCC in patients (or a random sample) during a distinct study period, (4) adequacy of follow-up, (5) use of standardized diagnostic protocol for metastasis, (6) number of low and high risk tumors, (7) presence of high risk features of cSCC. Quality assessment was scored positive bullet for each item if it was mentioned in the article. For high risk features at least two features had to be present.

Statistical analysis
The risk of metastasis of cSCC was the primary outcome measure. We estimated a pooled risk of metastasis in the two populations separately: in studies reporting on the immunocompetent population and in studies of OTR. Descriptive statistics were used to calculate metastasis risk. A subdivision was made for cSCC studied on the total body and the head and neck area. Cohorts consisting of specific cSCC (e.g. only high risk or on specific anatomic locations) were excluded from analysis.
Summary estimates were calculated for the proportion of patients with metastasis and summary estimates were calculated for studies that reported the exact number of cSCC in their cohort.
Statistical analysis was performed using STATA 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Search results
Details of the selection process for eligible studies are shown in Figure 1. A total of 10396 publications were retrieved and 53 studies fulfilled the inclusion criteria. Two cohort studies described the metastasis risk in both OTR and immunocompetent population and will be described in more detail. Fourteen studies were performed solely in OTR and 37 solely in the immunocompetent population.

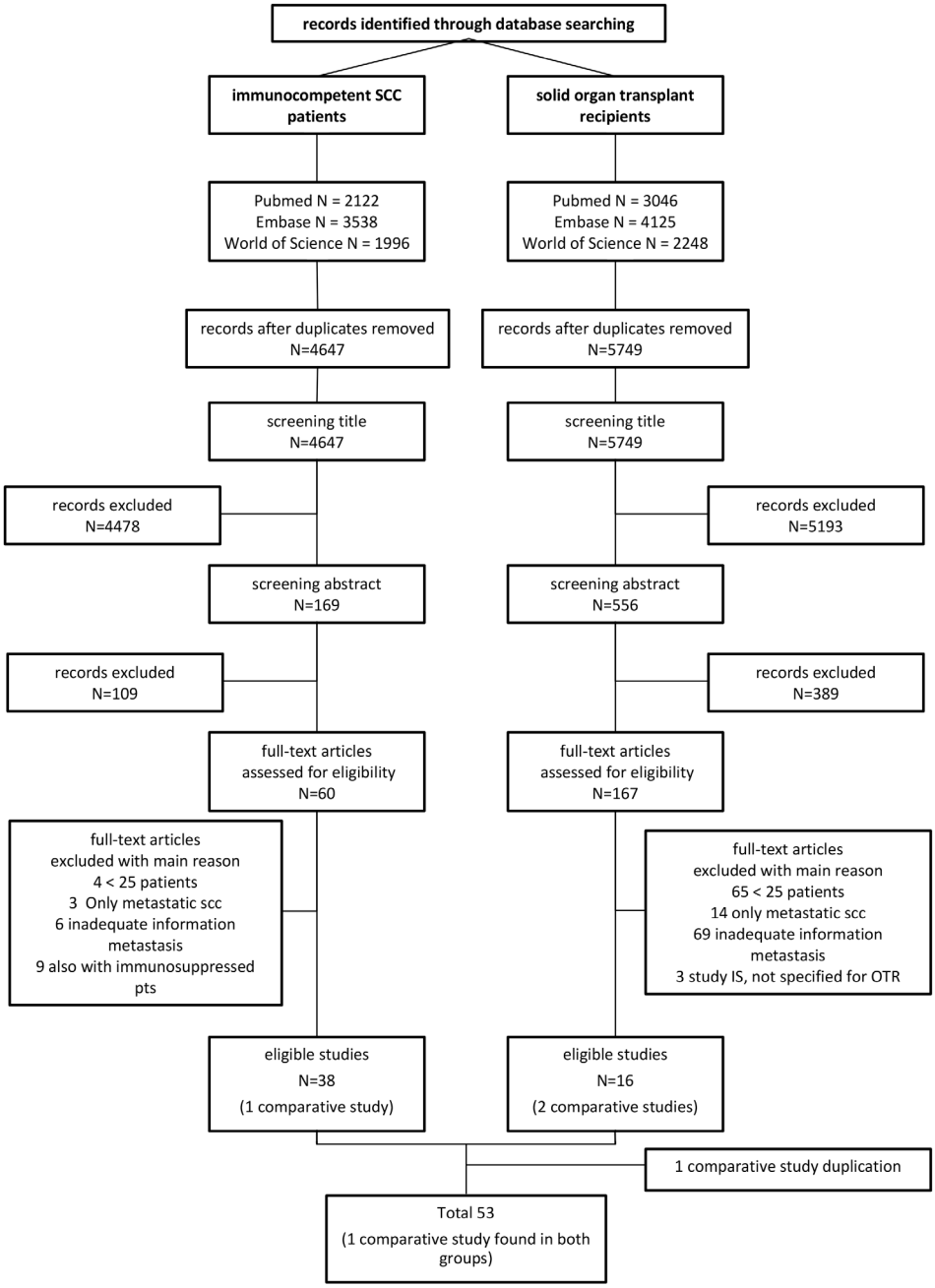


Figure 1. Flow chart. Details of the selection process for eligible studies.

Studies in OTR

Sixteen studies reported on the metastasis risk of cSCC in OTR. The included studies were published between 1980 and 2017; the number of included patients ranged from 34 to 796 (Table 1).[42-57] These studies were performed in the USA, New-Zealand, Australia, the Netherlands, France, Portugal and Israel. Follow-up in these studies was mainly calculated from time of transplantation instead of follow-up from cSCC development. Only 5 studies informed about follow-up time of cSCC. Thirteen studies included patients with cSCC on the total body,[42-49,51-53,56,57] three studies reported on patients with a cSCC in the head and neck area.[50,54,55]

Studies in the immunocompetent population

Thirty-nine studies reported on metastasis risk of cSCC in the immunocompetent population. Nineteen studies reported on special cohorts in the immunocompetent population, like specific anatomical locations or only reporting on specific high risk cSCC or cSCC derived in burns.[58-76] The results of these specific cohorts are not taken into account in the pooled metastasis risk analyses. The details of these studies are shown in Table 2.

Studies included for analyses were published between 1957 and 2017; the number of included patients ranged from 40 to 6164 (Table 1).[22-25,30,44,47,77-89] These studies were performed in the USA, New-Zealand, Australia, the Netherlands , Denmark, the UK, Greece, Turkey and Israel. Median follow-up time ranged from 24 to 81 months. Overall follow-up ranged from 0-312 months.

Fourteen studies included patients with cSCC on the total body [22-24,30,44,47,78-81,83,84,87,88], six studies with patients with cSCC in the head and neck area.[25,77,82,85,86,89] One study also included specified data on 10 OTR, that were excluded for this study as it were less than 25 patients.[88]

Two studies comparing immunocompetent population and OTR

Both studies were performed in the USA and studied total body cSCC.[44,47]

One study retrospectively compared 153 OTR with cSCC with 154 numerically matched cSCC patients in immunocompetent patient that were randomly chosen from a pool of patients.[47] Follow-up time was shorter in the control group (mean 37 months) compared to the OTR group (mean 65 months). The reported risk for metastasis in the OTR population was 4.6%, in the control population 1.3%. No significant differences between OTR and the control population were found for lymph node

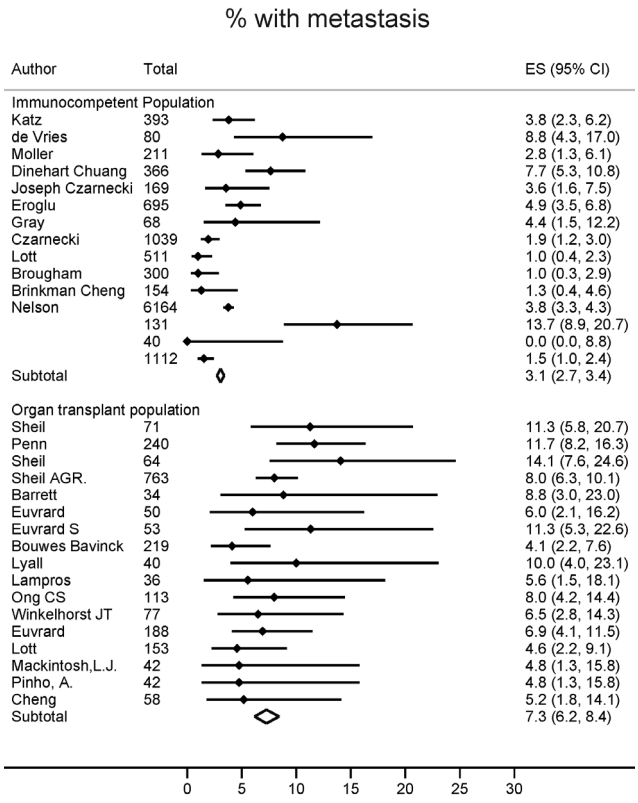


Figure 2.
Forest plot of metastasis risk of total body cutaneous squamous cell carcinoma per patient stratified by organ transplant population and immunocompetent population.

spread, although they stated that there was as a trend toward significance (p=0.10) and suggested that OTR are 3.5 times more likely to have lymph node spread.
The second retrospective cohort study compared 58 OTR and 40 high risk immunocompetent patients (defined as patients with more than 1 cSCC in the past).[44] The OTR and immunocompetent groups were comparable regarding race and sex, patient care, follow-up time, numbers of skin lesions, and field cancerization and chemo preventive therapies. This study included a total follow-up of 369 patient-years for both OTR and immunocompetent patients. Two OTR were diagnosed with regional lymph node metastases. No metastases were found in the control group.

Risk of bias assessment

Risk of bias assessment was based upon a component based approach for studies in OTR and normal population regarding total body and head and neck cSCC (Table 1).

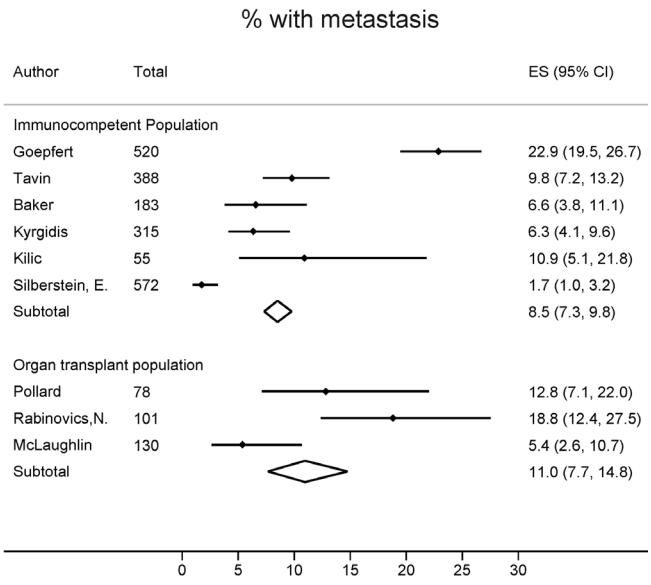


Figure 3.
Forest plot of metastasis risk of cutaneous squamous cell carcinoma of the head and neck area per patient stratified by organ transplant population and immunocompetent population.

6

The results were based upon the studies included in the pooled risk analysis and figures. In general, studies reporting on the immunocompetent population gave more details about the study population and the cSCC in the cohort.

The majority of studies reported on consecutive cSCC in their patients during the study period, but seven did not.[24,30,52,54,83,85] Seven studies reported on loss of follow-up [30,45,50,57,81,82,86] with a loss of follow-up of more than 5% in 6/7.[30,45,50,57,81,86] One study excluded patients with follow-up less than 6 months [50], and one study included only patients with a minimum follow-up of more than one year.[86] One study stated that a protocol was followed annually to detect metastasis with radiological imaging.[86] Four studies reported on clinical follow-up of patients.[42,44,50,55] In seven studies the distribution of T-stage among the cSCC was mentioned.[50,55,78,81,86,87,89] In 21 studies, one or more tumor related potential high risk features factors for metastasis, such as size, location, depth of invasion or perineural invasion, were taken into account. One study in the immunocompetent population specifically stated that no OTR were included in the cohort of the immunocompetent population.[30] One study reported separately on 10 OTR patients in their cohort.[88] Two studies compared OTR to the immunocompetent population.[44,47] In 14 studies, the outcome of metastasis was specifically noted in the study aim.[22,25,44,48,50,78-82,84,87-89]

Table 1. Details of studies included in the analyses.

STUDY CHARACTERISTICS														
Author, year, country	**Study period**	**N pt cSCC (%male)**	**N cSCC**	**Age pt median (mean) range in years**	**Treatment cSCC**	**FU cSCC median (mean) range in months**	**Total N pt metastasis**	**N in transit meta**	**N nodal meta**	**N distant meta**	**% meta per pt**	**% meta per cSCC**	**Meta risk factors**
TOTAL BODY cSCC														
OTR														
Barrett, 1993, USA	1968-1993	34 (nm)	nm	47	Surg	(27)	3	nm	3	1	8,8	-	nm	
Bouwes Bavinck, 1996, AU	1969-1994	219 (66)	2042	(50), 15-72	nm	nm	9	nm	nm	nm	4,1	0,4	nm	
Cheng,, 2017, US,	2008-2015	58 (76)	167	(70)	nm	46	3	2	1	nm	5,2	1,8	nm	
Euvrard, 2006, FR	1966-2004	188 (nm)	476	nm	nm	(21)	13	nm	nm	nm	6,9	2,1	nm	
Lampros, 1998, US	1985-1996	36 (nm)	172	(55)	nm	nm	2	nm	nm	nm	5,6	1,2	nm	
Lott, 2010, US	1997-2008	153 (73)	978	nm	nm	36	7	nm	7	nm	4,6	0,7	nm	
Lyall, 1998, NA	1972-1997	40 (nm)	nm	nm	nm	nm	4	nm	4	nm	10,0	-	nm	
Mackintosh, 2012, UK	2005-2008	42 (nm)	151	nm	nm	nm	2	nm	2	nm	4,8	1,3	nm	
Ong, 1999, AU	1984-1998	113 (nm)	849	54	nm	nm	9	nm	nm	nm	8,0	1,1	nm	
Penn, 1980, US	1968-1993	240 (nm)	nm	nm	nm	nm	28	nm	24	4	11,7	-	nm	
Pinho, 2016, PT	2004-2013	42 (nm)	43	nm	nm	nm	2	nm	2	nm	4,8	4,7	nm	
Sheil, 1992, AU/NZ	1963-1992	796 (nm)	nm	nm	nm	nm	61	nm	nm	nm	8,0	-	nm	
Winkelhorst, 2001, NL	1968-1998	77 (nm)	nm	(53), 29-72	nm	nm	5	nm	nm	nm	6,5	-	nm	
Immunocompetent population														
Brinkman, 2013, NL	2001-2008	131 (66)	155	(73), 19-96	Surg	81, 27-125	18	0	12	6	13,7	11,6	nm	
Brougham, 2012, NZ	1997-2007	6164 (57)	8997	(74), 21–108	Surg	71, 31-121	232	8	251	23	3,8	2,6	location, size, diff , PNI	
Cheng, 2017, US	2008-2015	40 (60)	111	(70)	nm	46	0	nm	nm	nm	0,0	0,0	nm	
Chuang, 1990, US	1976-1984	169 (60)	169	72 (71)	Surg	46	6	1	5	0	3,6	3,6	nm	
Czarnecki, 1994, AU	1988-1989	68 (75)	68	(72)	nm	> 36	3	0	1	2	4,4	4,4	nm	

	RISK OF BIAS / QUALITY ASSESSMENT							
	Metastasis noted in study aim	Statement IS present	Consecutive cSCC	Loss to FU in %	Diagnostic protocol meta	% T stage	cSCC features	Score
	no	OTR cohort	yes	nm	yes	nm	nm	●●●○○○○
	no	OTR cohort	yes	nm	nm	nm	nm	●●○○○○○
	no	OTR cohort	yes	nm	yes	nm	11% > 2mm depth, 1% PNI	●●●●○○○
	no	OTR cohort	yes	6	nm	nm	nm	●●●○○○○
	no	OTR cohort	yes	nm	nm	nm	70% Head and neck	●●○○○○○
	no	OTR cohort	yes	nm	nm	nm	9% > subdermal, 8% PNI, 3% LVSI	●●●○○○○
	yes	OTR cohort	yes	nm	nm	nm	nm	●●●○○○○
	no	OTR cohort	yes	nm	nm	nm	nm	●●○○○○○
	no	OTR cohort	yes	nm	nm	nm	nm	●●○○○○○
	no	OTR cohort	no	nm	nm	nm	nm	●○○○○○○
	no	OTR cohort	yes	nm	nm	nm	7% > 2cm, 16% poor diff, 30% > 4mm depth, 30% ear/lip/ anogenital, 2% PNI	●●●○○○○
	no	OTR cohort	yes	nm	nm	nm	nm	●●○○○○○
	no	OTR cohort	yes	8	nm	nm	nm	●●●○○○○
	yes	nm	yes	nm	nm	1:46, 2:25, 3:6, NS: 24 (AJCC 6th)	13% poor diff, 68% HN	●●●●○○○
	yes	nm	yes	nm	nm	nm	10% >2cm, 8% poor diff, 49% HN, 1% PNI, 1% LVSI	●●●○○○○
	no	yes, present	yes	nm	yes	nm	0% PNI	●●●○○○○
	no	nm	no	nm	nm	nm	79% HN	○○○○○○○
	yes	yes, not present	no	13	nm	nm	nm	●●○○○○○

Table 1. Continued

Author, year, country	Study period	N pt cSCC (%male)	N cSCC	Age pt median (mean) range in years	Treatment cSCC	FU cSCC median (mean) range in months	Total N pt metastasis	N in transit meta	N nodal meta	N distant meta	% meta per pt	% meta per cSCC	Meta risk factors
STUDY CHARACTERISTICS													
Czarnecki, 2000, AU	1988-1998	300 (67)	nm	nm	Surg	6-120	3	nm	nm	nm	1,0		nm
Dinehart, 1989, US	1979-1988	366 (77)	366	(67)	Surg	(20) 2-94	27	nm	23	4	7,7	7,7	nm
Eroglu, 1996, TR	1980-1989	1039 (nm)	nm	65, 15-97	Surg/ RT/CT	28, 6-149	20	nm	20	nm	1,9		nm
Gray, 1997, US	1984-1992	511 (54)	511	(74), 10-101		52	5	nm	nm	nm	1,0	1,0	nm
Katz, 1957, US	1946-1950	393 (70)	577	nm	Surg/ RT	60	15	nm	nm	nm	3,8	2,6	nm
Lott, 2010, US	1997-2008	154 (66)	256	nm	nm	36	2	nm	2	nm	1,3	0,8	nm
Moller, 1979, DK	1950-1959	211 (73)	211	(65)	Surg/ RT	204-312	11	0	9	4	5,2	5,2	nm
Nelson, 2017, UK	2005-2014	1122 (64)	1495	78, 44-102	Surg	79, 24-143	18	nm	nm	nm	1,6	1,2	size, depth
de Vries, 1969, NL	1962-1967	80 (nm)	80	nm	Surg/ RT	6-60	7	nm	7	nm	8,8	8,8	nm
cSCC OF THE HEAD AND NECK AREA													
OTR													
McLaughlin, 2017, USA	2005-2015	130	383	(62)	Surg	(40)	7	nm	7	n nm	5.4	1.8	Scalp, subdermal growth
Pollard, 2000, US	1968-1998	78 (nm)	214	nm	nm	nm	10	nm	nm	nm	12,8	4,7	nm
Rabinovics, 2013, IL	1992-2010	101 (84)	198	nm	nm	nm	19	nm	17	2	18,8	9,6	nm
Immunocompetent population													
Baker, 2001, UK	1990-1995	183 (73)	227	78	Surg	24	12	nm	12	nm	6,6	5,3	nm
Goepfert, 1984, USA	1970-1979	520	967	64	nm	24	119	nm	93	26	22.9	12.3	nm
Kilic, 2014, TR	2010-2012	55	55	56, 29-89	Surg	24	6	nm	6	nm	10,9	10,9	nm
Kyrgidis, 2010, GR	1996-2006	315 (46)	nm	72. 26–95	Surg/ RT/CT	47, 12-124	20	nm	nm	5	6,3		nm
Silberstein, 2015, IL	1998-2005	572 (59)	725	(72)	Surg	72, 24-x	10	nm	10	0	1,7	1,4	T stage
Tavin, 1996, US	1961-1992	388	388	nm	Surg/ RT		38	nm	40	8	9,8	9,8	nm

Abbreviations: N; number, pt; patients, cSCC; cutaneous squamous cell carcinoma, FU; follow-up, meta; metastasis, IS; immunosuppression, OTR; organ transplant recipients, nm; not mentioned, Surg; surgery, RT; radiotherapy, CT; chemotherapy, diff; differentiation grade, USA; United states of America, AU; Australia, FR; France, NZ; New Zealand, UK; United Kingdom, PT; Portugal, NL; the Netherlands, TR; Turkey, DK; Denmark, IL; Israel, GR; Greece

RISK OF BIAS / QUALITY ASSESSMENT							
Metastasis noted in study aim	Statement IS present	Consecutive cSCC	Loss to FU in %	Diagnostic protocol meta	% T stage	cSCC features	Score
no	nm	yes	nm	nm	nm	nm	●○○○○○○
yes	nm	no	nm	nm	nm	85% HN, 2% PNI	●●○○○○○
yes	nm	yes	> 10	nm	1:10, 2:21, 3:32, 4:28 (AJCC 5th)	9% poor diff	●●●●○○○
no	nm	no	nm	nm	nm	78% HN	○○○○○○○
yes	nm	yes	nm	nm	nm	58% HN	●●○○○○○
no	yes, present	yes	nm	nm	nm	2% subdermal. 1% PNI	●●●○○○○
yes	nm	yes	nm	nm	1:2, 2:38, 3:8, 4:1 (AJCC 3rd)	75% HN	●●●○○○○
yes	yes, present	yes	nm	nm	nm	11% poor diff, mean size 15mm, mean depth 4.5mm, 65% HN	●●●●○○○
yes	nm	yes	nm	nm	nm	85% HN	●●○○○○○
yes	OTR cohort	yes	10.3	Clinical FU protocol	1:73, 2: 27 (AJCC), 1 69, 2a 24, 2b: 7 (BWH)	12% subdermal,	●●●●●○○
no	OTR cohort	no	nm	nm	nm	nm	●○○○○○○
no	OTR cohort	yes	nm	yes	1:69, 2:27, 3+4: 4, Other: 3% (AJCC)	69% stage 1+2	●●●●○○○
no	nm	yes	nm	nm	nm	26% ear, 6% lip	●○○○○○○
yes	no	yes	2	nm	nm	14% PNI	●●●○○○○
no	nm	no	nm	nm	nm	36% ear, 1% periocular, 3% lip	○○○○○○○
no	nm	yes	8	yes	1:51, 2:20, 3:29 (AJCC 7th)	mean size 2.2 cm, mean depth 5mm, 18% poor diff, 13% periocular, 24% ear, 20% PNI	●●●●●○○
yes	nm	yes	nm	nm	0:3, 1:89. 2:8, 3:1, 4:1 (nm)	17% ear, 6.3% peri-ocular	●●●○○○○
yes	nm	yes	nm	nm	nm	26% ear, 6% lip	●●○○○○○

Table 2. Details of studies regarding special locations or high risk tumors

STUDY CHARACTERISTICS													
Author, year, country	**Study period**	**N pt cSCC (%male)**	**N cSCC**	**Age pt median (mean) range in years**	**Treatment cSCC**	**FU cSCC median (mean) range in months**	**Total N pt metastasis**	**N in transit meta**	**N nodal meta**	**N distant meta**	**% meta per pt**	**% meta per cSCC**	**Meta risk factors**
Auricle													
Mayo, 2017, UK[66]	2007-2012	192 (100)	192	(81), 41-98	SURG/ RT	24-60	4	nm	4	nm	2,1	2,1	diff, PNI
Shiffman, 1975, CA[72]	1952-1973	52 (96)	nm	(73), 28-96	SURG/ RT	(23)	7	1	5	3	13,5	nm	nm
Eyelid													
Faustina, 2004, USA[62]	1952-2000	111 (80)	nm	64, 31-91	SURG/ RT	77, 6-484	33	nm	27	7	29,7	nm	nm
Nasser, 2014, USA[69]	1999-2011	65 (62)	nm	67, 41-89	SURG/ RT	27, 1-150	6	nm	6	0	9,2	nm	T stage, size
Soysal, 2007, TR[73]	1997-2006	76 (54)	76	67, 11-93	SURG/ RT	nm	5	nm	5	nm	6,6	6,6	nm
Lip													
Boddie, 1977, USA[58]	1943-1973	56 (nm)	56	9-39	SURG/ RT	60	18	nm	18	nm	32,1	32,1	nm
Cerezo, 1993, ES[59]	1976-1985	117 (87)	nm	68, 31-93	SURG/ RT	65, 11-160	8	nm	8	nm	6,8	nm	T stage
McCombe, 2000, AU[67]	1979-1988	323 (87)	nm	65, 18-94	SURG/ RT	94	16	nm	16	nm	5,0	nm	T stage, RT, age
Unsal, 2017, USA[74]	1973-2013	14901 (82)	14901	68	SURG/ RT	nm	131	nm	119	12	0,9	0,9	nm
de Visscher, 1998, NL[61]	1979-1992	184 (90)	nm	(66)	SURG	(56), 24-x	12	nm	12	1	6,5	nm	depth, PNI
Scalp													
Jenkins, 2014, UK[64]	2005-2009	101 (78)	nm	(82)	SURG	nm	7	nm	7	nm	6,9	6,9	no
Hand													
Bean, 1984, USA[57]	1963-1983	51 (80)	64	(72), 41-92	SURG/ RT	nm	5	nm	4	1	9,8	7,8	nm
Trunk and extremities													
Friedman, 1985, USA[63]	1965-1975	63 (73)	71	(65), 33-97	nm	100, 2-215	5	nm	5	1	7,9	7,0	nm
Joseph, 1992, AU[65]	1977-1987	695 (90)	695	(68), 51-84	SURG	48, 12-216	34	0	33	1	4,9	4,9	nm
de Lima Vasquez, 2008, BR[60]	1987-2005	57 (60)	57	nm	SURG/ RT	23	22	nm	22	4	38,6	38,6	nm
Ribeiro, 2006 BR[70]	1995-1999	36(16)	43	(74), 50-95	nm	nm	0	nm	nm	nm	0,0	0,0	nm

	RISK OF BIAS / QUALITY ASSESSMENT							
	Metastasis noted in study aim	Statement IS present	Consecutive cSCC	Loss to FU in %	Diagnostic protocol meta	% T stage	cSCC features	Score
	yes	yes, absent	no	1%	nm	nm	17% poor diff, 21% subdermal, 5% PNI, 2% LVSI	●●●●●○○
	no	nm	yes	nm	nm	nm	31%> 2cm, 21% catillage,	●●○○○○○
	yes	nm	yes	nm	nm	nm	8% PNI	●●○○○○○
	yes	nm	yes	nm	yes	1:9, 2:46, 3:38, 4:6 (AJCC 7th)	25% PNI	●●●○○○○
	no	nm	yes	nm	nm	nm	size mean 24mm, 12% poor diff, 24% PNI	●●○○○○○
	no	nm	yes	1	nm	1:53, 2:21, 3:7, 4:5% 52.6% UK:15 (nm)	3% PNI	●●●○○○○
	no	nm	yes	7%	nm	1:86, 2:13, 3:1 (UICC 1987)	size mean 10mm	●●●○○○○
	no	nm	yes	nm	nm	1:85, 2:10, 3-4:1 (AJCC 4th)	size mean 12mm, 4% poor diff	●●●○○○○
	no	nm	yes	nm	nm	1:80, 2:12, 3:4, 4:4 (AJCC 8th)	7% poor diff	●●○○○○○
	yes	nm	yes	nm	nm	1:93, 2:5, 3:2, 4:1 (AJCC, 4th)	size 7%>20mm, 5% poor diff, 5% PNI, 1% LVSI	●●●●○○○
	yes	nm	yes	nm	nm	nm	size mean 30mm, 25% poor diff, 41% > 10mm depth,	●●●○○○○
	no	yes, present	yes	nm	nm	nm	size mean 16mm,	●●○○○○○
	yes	nm	no	nm	nm	nm	10% poor diff, 12% subdermal	●●○○○○○
	yes	nm	yes	nm	nm	nm	nm	●●○○○○○
	no	nm	yes	18%	nm	1:-, 2:-, 3:63, 4:37 (AJCC, 6th)	nm	●●●○○○○
	no	nm	yes	12%	nm	nm	nm	●●○○○○○

Table 2. Continued

STUDY CHARACTERISTICS													
Author, year, country	**Study period**	**N pt cSCC (%male)**	**N cSCC**	**Age pt median (mean) range in years**	**Treatment cSCC**	**FU cSCC median (mean) range in months**	**Total N pt metastasis**	**N in transit meta**	**N nodal meta**	**N distant meta**	**% meta per pt**	**% meta per cSCC**	**Meta risk factors**
High risk SCC only													
Salmon, 2011,USA/ NZ[71]	1988-2008	72 (64)	73	76, 45-91	SURG/ RT	36	0	nm	nm	nm	0,0	0,0	nm
Burns													
Ames, 1982, USA[56]	1944-1976	1118 (nm)	1118	nm	nm	nm	106	0	75	15	1,4	1,4	nm
Metwally, 2017, EG[68]	2004-2015	26 (61)	26	(47)	SURG	> 12	6	nm	6	3	23,1	23,1	diff

Abbreviations: N; number, pt; patients, cSCC; cutaneous squamous cell carcinoma, FU; follow-up, meta; metastasis, IS; immunosuppression, OTR; organ transplant recipients, nm; not mentioned, Surg; surgery, RT; radiotherapy, CT; chemotherapy, diff; differentiation grade, UK; United Kingdom, CA; Canada, USA; United states of America, , TR; Turkey, ES; Spain, AU; Australia, NL; the Netherlands, BR; Brazil, NZ; New Zealand, EG; Egypte

Metastasis risk: pooled analysis

Summary estimates were calculated for the proportion of patients with metastasis in the studies reporting on total body (Figure 2) and cSCC of the head and neck area (Figure 3).

For cSCC on total body, the pooled metastasis risk estimate for OTR was 7.3% (95% CI 6.2-8.4), with a range of 4.1 to 14.1% reported in these studies. For the immunocompetent population reported risk estimates in individual studies ranged from 0 to 13.7% and analysis showed a pooled risk of 3.1% (95% CI 2.7-3.4).

For studies reporting on cSCC of the head and neck area, the pooled metastasis risk estimate for OTR was 11.0% (95% CI 7.7-14.8), with a range of 5.4 to 18.8% reported in these studies. For the immunocompetent population reported risk estimates in individual studies ranged from 1.7 to 22.9% and analysis showed a pooled risk of 8.5% (95% CI 7.3-9.8).

In addition, estimates were calculated for studies that reported the exact number of cSCC with metastasis as patients can have multiple primary cSCC.

	RISK OF BIAS / QUALITY ASSESSMENT							
	Metastasis noted in study aim	Statement IS present	Consecutive cSCC	Loss to FU in %	Diagnostic protocol meta	% T stage	cSCC features	Score
	no	nm	yes	nm	nm	nm	6% poor diff, depth mean 3.6mm, 90% HN, 73% PNI, 0% LVSI	●●○○○○
	yes	nm	yes	nm	nm	nm	nm	●●○○○○
	no	nm	yes	nm	yes	nm	0% poor diff	●●○○○○

Eight studies in the immunocompetent population reported the same number of cSCC as patients. Five of those were excluded from the risk analysis of metastasis per cSCC because the cSCC were not included consecutively.

For cSCC on total body, the pooled metastasis risk estimate for a single cSCC in OTR was 1.3% (95% CI 1.0-1.7), with a range of 0.4 to 7.7% reported in these studies. For the immunocompetent population analysis showed a pooled risk of 2.4% (95% CI 2.1-2.6) and reported risk estimates in individual studies ranged from 0 to 11.6% (Figure 4).

For studies reporting on cSCC of the head and neck area, the pooled metastasis risk estimate for a single cSCC in OTR was 4.0% (95% CI 2.7-5.5), with a range of 1.8 to 9.6% reported in these studies. For the immunocompetent population analysis showed a pooled risk of 6.7% (95% CI 5.7-7.8) and reported risk estimates in individual studies ranging from 1.4 to 12.3% (Figure 5).

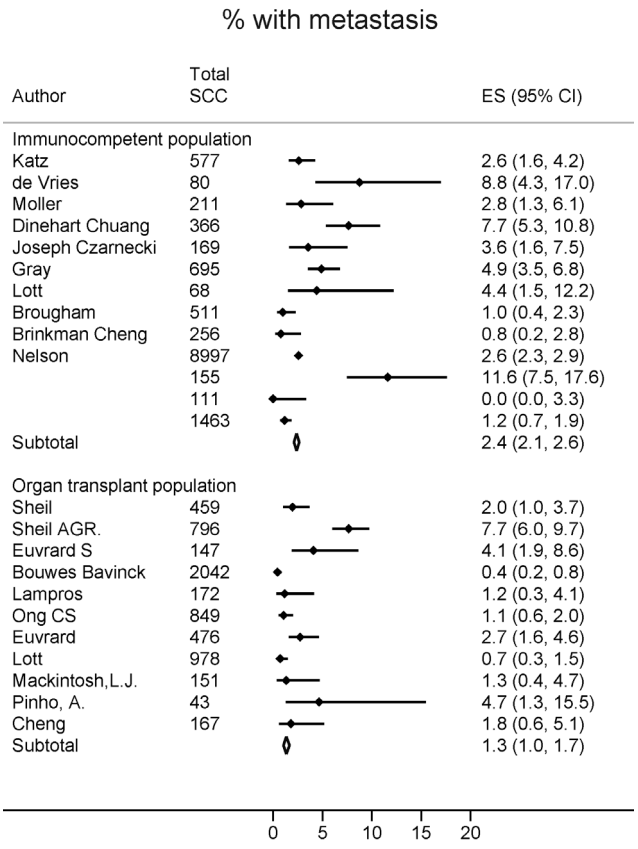


Figure 4.
Forest plot of metastasis risk of total body cutaneous squamous cell carcinoma per tumor stratified by organ transplant population and immunocompetent population.

Discussion

The aim of this study was to estimate the overall risk for cSCC to develop metastasis in OTR versus the immunocompetent population. This review suggests a low overall metastasis risk in cSCC, in both the immunosuppressed organ transplant population and the immunocompetent population, and a higher overall metastasis risk in OTR.

We found a risk of metastasis of cSCC on total body skin per patient in OTR patients of 7.3%, versus a pooled risk of 3.1% in the immunocompetent population. For cSCC of the head and neck area these estimates were higher, 11.0% in OTR and 8.5% in the immunocompetent population. These findings are in line with the hypothesis that OTR are at higher risk of metastasis.[16,33-37] The chronic use of immunosuppressive

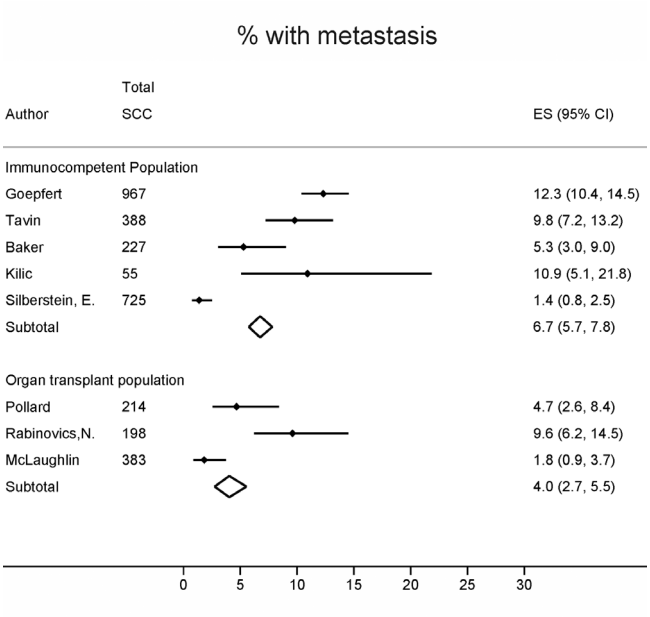


Figure 5.
Forest plot of metastasis risk of cutaneous squamous cell carcinoma of the head and neck area per tumor stratified by organ transplant population and immunocompetent population.

medication is thought to be a driver of this increased risk, earlier studies reported that patients on immunosuppression tend to have more cSCC and a higher proportion of aggressive cSCC that are less differentiated and thicker tumors.[18,37] Immunosuppressive drugs (e.g. azathioprine, calcineurin inhibitors) are linked to aberrant production of cytokines that promote tumor growth, angiogenesis and metastasis.[90] Immunosuppressive drugs have a potential oncogenic action in cells or by facilitating tumor cell escape from immunosurveillance.[91] Immunosuppression is not included as a risk factor for upgrading tumor stage in the tumor TNM classification system. Instead, it is described as a prognostic factor to take into account for clinical practice.[92-94]

However, the pooled risk of metastasis per cSCC was lower, as patients often develop multiple lesions, especially OTR. In this analyses, the risk for a single cSCC to metastasize in OTR is lower compared to the immunocompetent population. Therefore, immunosuppressive state in OTR does not seem to increase the risk of metastasis in a single cSCC, but could be an overall risk factor for metastasis due to the multiplicity of cSCC in OTR. A possible explanation for the lower metastatic risk of a single cSCC in the OTR group might be due to the frequent skin checks in OTR. Those skin checks enable the detection of cSCC in OTR in an earlier stage compared to some cSCC in the immunocompetent population. However, since OTR patients develop more cSCC compared to immunocompetent patients, the cumulative risk for the total number of cSCC per patient is higher in the OTR group.

The definition of an immunosuppressed patient is often not clear and in studies regarding risk factors for metastasis different criteria are used. Ideally, immunosuppression should be defined more precise in studies. OTR are subject to lifelong immunosuppressive therapy and therefore an ideal population for studying the influence of immunosuppression on cSCC behaviour. Nevertheless, also patients with hematologic malignancies, HIV or chronic diseases on immunosuppressive drugs are to a certain extent immunosuppressed.[95-100]

The presence of cSCC high risk features was taken into account for our risk of bias assessment. High risk features mentioned in literature are size, depth of invasion, location on head and neck, differentiation grade and perineural invasion.[101] Not all studies report on these items. In addition, most studies do not mention proportion of high risk cSCC in their cohort. The proportion of high risk tumors obviously influences the metastasis rate in a cohort. Another important factor to take into account is age, as older age is a risk factor for metastasis.[102] In the OTR, the age of cSCC patients is lower compared to the immunocompetent population and therefore could contribute to the lower metastasis risk per cSCC in these group.

This systematic review has some limitations. The quality of evidence is limited due to diversity in study design, types of patients studied and data reporting. Because of this considerable heterogeneity between studies, limiting both the interpretation and scope of this review, the outcomes have to be interpreted with caution. Second, the studies reporting on the immunocompetent population could include some non-reported OTR, diluting the effect. Lastly, but most importantly, follow-up time is variable. Although most cSCC metastasize within the first two years, longer follow-up allows more cSCC to metastasize and might therefore influence the results of

studies with a longer follow up.[103] Unfortunately the majority of studies in OTR did not provide adequate follow up time per cSCC. Follow-up time started at time of transplantation, and not at time of cSCC diagnosis. Also differences in study periods influence the rate of cSCC, especially the risk of metastasis per cSCC
Concluding, we found a difference in metastasis risk for OTR and immunocompetent cSCC patients. Prospective follow-up studies, with distinct cohorts of risk groups are necessary to adequately assess the risk for metastasis rate of cSCC, mainly in organ transplant patients and other immunosuppressed patients.

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Supplementary files can be found in the online version of the article

References

1. Almahroos M, Kurban AK. Ultraviolet carcinogenesis in nonmelanoma skin cancer. Part I: incidence rates in relation to geographic locations and in migrant populations. Skinmed. 2004;3(1):29-35.
2. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. NEnglJMed. 2001;344(13): 975-83.
3. Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. Lancet. 2010;375(9715): 673-85.
4. Deady S, Sharp L, Comber H. Increasing skin cancer incidence in young, affluent, urban populations: a challenge for prevention. BrJDermatol. 2014;171(2):324-31.
5. Hollestein LM, de VE, Nijsten T. Trends of cutaneous squamous cell carcinoma in the Netherlands: increased incidence rates, but stable relative survival and mortality 1989-2008. EurJCancer. 2012;48(13):2046-53.
6. Karia PS, Han J, Schmults CD. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. JAmAcadDermatol. 2013;68(6):957-66.
7. Robsahm TE, Helsing P, Veierod MB. Cutaneous squamous cell carcinoma in Norway 1963-2011: increasing incidence and stable mortality. Cancer Med. 2015.
8. Yoong C, De'Ambrosis B. Cutaneous invasive squamous cell carcinoma: 10-year experience and recommendations for follow up. AustralasJDermatol. 2009;50(4):261-5.
9. Green AC, Olsen CM. Cutaneous squamous cell carcinoma: an epidemiological review. Br J Dermatol. 2017;177(2):373-81.
10. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. BrJDermatol. 2006;154(3):498-504.
11. Tessari G, Naldi L, Boschiero L, Nacchia F, Fior F, Forni A,... Girolomoni G. Incidence and clinical predictors of a subsequent nonmelanoma skin cancer in solid organ transplant recipients with a first nonmelanoma skin cancer: a multicenter cohort study. ArchDermatol. 2010;146(3):294-9.
12. Bordea C, Wojnarowska F, Millard PR, Doll H, Welsh K, Morris PJ. Skin cancers in renal-transplant recipients occur more frequently than previously recognized in a temperate climate. Transplantation. 2004;77(4):574-9.
13. Hartevelt MM, Bouwes Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in the Netherlands. Transplantation. 1990;49(3):506-9.
14. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part I. Epidemiology of skin cancer in solid organ transplant recipients. JAmAcadDermatol. 2011;65(2):253-61.
15. Brantsch KD, Meisner C, Schonfisch B, Trilling B, Wehner-Caroli J, Rocken M, Breuninger H. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. Lancet Oncol. 2008;9(8):713-20.
16. Rowe DE, Carroll RJ, Day CL, Jr. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. JAmAcadDermatol. 1992;26(6):976-90.
17. Terhorst D, Drecoll U, Stockfleth E, Ulrich C. Organ transplant recipients and skin cancer: assessment of risk factors with focus on sun exposure. BrJDermatol. 2009;161 Suppl 3:85-9.
18. Weinberg AS, Ogle CA, Shim EK. Metastatic cutaneous squamous cell carcinoma: an update. DermatolSurg. 2007;33(8):885-99.
19. Quint KD, Genders RE, de Koning MN, Borgogna C, Gariglio M, Bouwes Bavinck JN,... Feltkamp MC. Human Beta-papillomavirus infection and keratinocyte carcinomas. J Pathol. 2015;235(2):342-54.
20. Farasat S, Yu SS, Neel VA, Nehal KS, Lardaro T, Mihm MC,... Liegeois NJ. A new American Joint Committee on Cancer staging system for cutaneous squamous cell carcinoma: creation and rationale for inclusion of tumor (T) characteristics. JAmAcadDermatol. 2011;64(6):1051-9.
21. Veness MJ, Palme CE, Morgan GJ. High-risk cutaneous squamous cell carcinoma of the head and neck: results from 266 treated patients with metastatic lymph node disease. Cancer. 2006;106(11):2389-96.
22. Brougham ND, Dennett ER, Cameron R, Tan ST. The incidence of metastasis from cutaneous squamous cell carcinoma and the impact of its risk factors. JSurgOncol.

2012;106(7):811-5.
23. Chuang TY, Popescu NA, Su WP, Chute CG. Squamous cell carcinoma. A population-based incidence study in Rochester, Minn. ArchDermatol. 1990;126(2):185-8.
24. Dinehart SM, Pollack SV. Metastases from squamous cell carcinoma of the skin and lip. An analysis of twenty-seven cases. JAmAcadDermatol. 1989;21(2 Pt 1):241-8.
25. Tavin E, Persky M. Metastatic cutaneous squamous cell carcinoma of the head and neck region. Laryngoscope. 1996;106(2 Pt 1):156-8.
26. Baum CL, Wright AC, Martinez JC, Arpey CJ, Brewer JD, Roenigk RK, Otley CC. A new evidence-based risk stratification system for cutaneous squamous cell carcinoma into low, intermediate, and high risk groups with implications for management. J Am Acad Dermatol. 2018;78(1):141-7.
27. Rose AM, Nicoll KJ, Moinie A, Jordan DJ, Evans AT, Proby CM, Naasan A. Patients with low-risk cutaneous squamous cell carcinoma do not require extended out-patient follow-up. J Plast Reconstr Aesthet Surg. 2017;70(6):852-5.
28. Veness MJ. Defining patients with high-risk cutaneous squamous cell carcinoma. AustralasJDermatol. 2006;47(1):28-33.
29. Veness MJ, Porceddu S, Palme CE, Morgan GJ. Cutaneous head and neck squamous cell carcinoma metastatic to parotid and cervical lymph nodes. Head Neck. 2007;29(7):621-31.
30. Czarnecki D, Staples M, Mar A, Giles G, Meehan C. Metastases from squamous cell carcinoma of the skin in southern Australia. Dermatology. 1994;189(1):52-4.
31. Breuninger H, Schaumburg-Lever G, Holzschuh J, Horny HP. Desmoplastic squamous cell carcinoma of skin and vermilion surface: a highly malignant subtype of skin cancer. Cancer. 1997;79(5):915-9.
32. Thompson AK, Kelley BF, Prokop LJ, Murad MH, Baum CL. Risk Factors for Cutaneous Squamous Cell Carcinoma Recurrence, Metastasis, and Disease-Specific Death: A Systematic Review and Meta-analysis. JAMA Dermatol. 2016;152(4):419-28.
33. Carucci JA. Squamous cell carcinoma in organ transplant recipients: approach to management. Skin therapy letter. 2004;9 (4):5-7.
34. Veness MJ, Quinn DI, Ong CS, Keogh AM, Macdonald PS, Cooper SG, Morgan GW. Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience. Cancer. 1999;85(8):1758-64.
35. Carucci JA, Martinez JC, Zeitouni NC, Christenson L, Coldiron B, Zweibel S, Otley CC. In-transit metastasis from primary cutaneous squamous cell carcinoma in organ transplant recipients and nonimmunosuppressed patients: clinical characteristics, management, and outcome in a series of 21 patients. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2004;30(4 Pt 2):651-5.
36. Martinez JC, Otley CC, Stasko T, Euvrard S, Brown C, Schanbacher CF, Weaver AL. Defining the clinical course of metastatic skin cancer in organ transplant recipients: a multicenter collaborative study. ArchDermatol. 2003;139(3):301-6.
37. Smith KJ, Hamza S, Skelton H. Histologic features in primary cutaneous squamous cell carcinomas in immunocompromised patients focusing on organ transplant patients. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2004;30(4 Pt 2):634-41.
38. Dinehart SM, Chu DZ, Maners AW, Pollack SV. Immunosuppression in patients with metastatic squamous cell carcinoma from the skin. JDermatolSurgOncol. 1990;16(3):271-4.
39. Khurana VG, Mentis DH, O'Brien CJ, Hurst TL, Stevens GN, Packham NA. Parotid and neck metastases from cutaneous squamous cell carcinoma of the head and neck. AmJSurg. 1995;170(5):446-50.
40. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D,... Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283(15):2008-12.
41. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. [Available from: www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

42. Barrett WL, First MR, Aron BS, Penn I. Clinical course of malignancies in renal transplant recipients. Cancer. 1993;72(7):2186-9.
43. Bouwes Bavinck JN, Hardie DR, Green A, Cutmore S, MacNaught A, O'Sullivan B,... Hardie IR. The risk of skin cancer in renal transplant recipients in Queensland, Australia. A follow-up study. Transplantation. 1996;61(5):715-21.
44. Cheng JY, Li FY, Ko CJ, Colegio OR. Cutaneous Squamous Cell Carcinomas in Solid Organ Transplant Recipients Compared With Immunocompetent Patients. JAMA Dermatol. 2017.
45. Euvrard S, Kanitakis J, Decullier E, Butnaru AC, Lefrancois N, Boissonnat P,... Chapuis F. Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. Transplantation. 2006;81(8):1093-100.
46. Lampros TD, Cobanoglu A, Parker F, Ratkovec R, Norman DJ, Hershberger R. Squamous and basal cell carcinoma in heart transplant recipients. JHeart Lung Transplant. 1998;17(6):586-91.
47. Lott DG, Manz R, Koch C, Lorenz RR. Aggressive behavior of nonmelanotic skin cancers in solid organ transplant recipients. Transplantation. 2010;90(6):683-7.
48. Lyall P, Lynn KL. Metastatic squamous cell carcinoma of skin following renal transplantation. Nephrology. 1998;4(5-6):423-6.
49. Mackintosh LJ, Geddes CC, Herd RM. Skin tumours in the West of Scotland renal transplant population. BrJDermatol. 2012.
50. McLaughlin EJ, Miller L, Shin TM, Sobanko JF, Cannady SB, Miller CJ, Newman JG. Rate of regional nodal metastases of cutaneous squamous cell carcinoma in the immunosuppressed patient. American journal of otolaryngology. 2017;38(3):325-8.
51. Ong CS, Keogh AM, Kossard S, Macdonald PS, Spratt PM. Skin cancer in Australian heart transplant recipients. JAmAcadDermatol. 1999;40(1):27-34.
52. Penn I. Immunosuppression and skin cancer. ClinPlastSurg. 1980;7(3):361-8.
53. Pinho A, Gouveia M, Cardoso JC, Xavier MM, Vieira R, Alves R. Non-melanoma skin cancer in Portuguese kidney transplant recipients - incidence and risk factors. An Bras Dermatol. 2016;91(4):455-62.
54. Pollard JD, Hanasono MM, Mikulec AA, Le QT, Terris DJ. Head and neck cancer in cardiothoracic transplant recipients. Laryngoscope. 2000;110(8):1257-61.
55. Rabinovics N, Mizrachi A, Hadar T, Ad-El D, Feinmesser R, Guttman D,... Bachar G. Cancer of the head and neck region in solid organ transplant recipients. Head Neck. 2013.
56. Sheil AGR. Development of Malignancy Following Renal-Transplantation in Australia and New-Zealand. Transplantation Proceedings. 1992;24(4):1275-9.
57. Winkelhorst JT, Brokelman WJ, Tiggeler RG, Wobbes T. Incidence and clinical course of de-novo malignancies in renal allograft recipients. EurJSurgOncol. 2001;27(4):409-13.
58. Ames FC, Hickey RC. Metastasis from squamous cell skin cancer of the extremities. SouthMedJ. 1982;75(8):920-3, 32.
59. Bean DJ, Rees RS, O'Leary JP, Lynch JB. Carcinoma of the hand: a 20-year experience. South MedJ. 1984;77(8):998-1000.
60. Boddie AW, Jr., Fischer EP, Byers RM. Squamous carcinoma of the lower lip in patients under 40 years of age. South MedJ. 1977;70(6):711-2, 5.
61. Cerezo L, Liu FF, Tsang R, Payne D. Squamous-Cell Carcinoma of the Lip - Analysis of the Princess-Margaret-Hospital Experience. Radiotherapy and Oncology. 1993;28(2):142-7.
62. de LV, V, Sachetto T, Perpetuo NM, Carvalho AL. Prognostic factors for lymph node metastasis from advanced squamous cell carcinoma of the skin of the trunk and extremities. World J SurgOncol. 2008;6:73.
63. de Visscher JGAM, van den Elsaker K, Grond AJK, van der Wal JE, van der Waal I. Surgical treatment of squamous cell carcinoma of the lower lip: Evaluation of long-term results and prognostic factors - A retrospective analysis of 184 patients. Journal of Oral and Maxillofacial Surgery. 1998;56(7):814-20.
64. Faustina M, Diba R, Ahmadi MA, Esmaeli B. Patterns of regional and distant metastasis in patients with eyelid and periocular squamous cell carcinoma. Ophthalmology. 2004;111(10):1930-2.
65. Friedman HI, Cooper PH, Wanebo HJ. Prognostic and therapeutic use of microstaging of cutaneous squamous cell carcinoma of

the trunk and extremities. Cancer. 1985;56(5):1099-105.
66. Jenkins G, Smith AB, Kanatas AN, Houghton DR, Telfer MR. Anatomical restrictions in the surgical excision of scalp squamous cell carcinomas: does this affect local recurrence and regional nodal metastases? IntJ Oral MaxillofacSurg. 2014;43(2):142-6.
67. Joseph MG, Zulueta WP, Kennedy PJ. Squamous cell carcinoma of the skin of the trunk and limbs: the incidence of metastases and their outcome. AustNZJSurg. 1992;62(9):697-701.
68. Mayo E, Sharma S, Horne J, Yuen HM, Lee A, Gulati A. Squamous cell carcinoma of the pinna: which histological features could be used to predict prognosis? The British journal of oral & maxillofacial surgery. 2017;55(5):524-9.
69. McCombe D, MacGill K, Ainslie J, Beresford J, Matthews J. Squamous cell carcinoma of the lip: A retrospective review of the Peter MacCallum Cancer Institute experience 1979-88. Australian and New Zealand Journal of Surgery. 2000;70(5):358-61.
70. Metwally IH, Roshdy A, Saleh SS, Ezzat M. Epidemiology and predictors of recurrence of Marjolin's ulcer: experience from Mansoura Universityxs. Ann R Coll Surg Engl. 2017;99(3):245-9.
71. Nasser QJ, Roth KG, Warneke CL, Yin VT, El Sawy T, Esmaeli B. Impact of AJCC 'T' designation on risk of regional lymph node metastasis in patients with squamous carcinoma of the eyelid. British Journal of Ophthalmology. 2014;98(4):498-501.
72. Ribeiro AM, Curado MP, Filho JB. Cutaneous squamous cell carcinoma of the lower limbs in Goiania, Goias, Brazil. Int J Dermatol. 2006;45(9):1039-42.
73. Salmon PJ, Hussain W, Geisse JK, Grekin RC, Mortimer NJ. Sclerosing squamous cell carcinoma of the skin, an underemphasized locally aggressive variant: a 20-year experience. DermatolSurg. 2011;37(5):664-70.
74. Shiffman NJ. Squamous cell carcinomas of the skin of the pinna. CanJ Surg. 1975;18(3):279-83.
75. Soysal HG, Markoc F. Invasive squamous cell carcinoma of the eyelids and periorbital region. BrJ Ophthalmol. 2007;91(3):325-9.
76. Unsal AA, Unsal AB, Henn TE, Baredes S, Eloy JA. Cutaneous squamous cell carcinoma of the lip: A population-based analysis. Laryngoscope. 2017.
77. Baker NJ, Webb AA, Macpherson D. Surgical management of cutaneous squamous cell carcinoma of the head and neck. BrJ Oral MaxillofacSurg. 2001;39(2):87-90.
78. Brinkman JN, Hajder E, van der Holt B, Den Bakker MA, Hovius SE, Mureau MA. The Effect of Differentiation Grade of Cutaneous Squamous Cell Carcinoma on Excision Margins, Local Recurrence, Metastasis, and Patient Survival: A Retrospective Follow-up Study. AnnPlastSurg. 2014.
79. Czarnecki D, Sutton T, Czarnecki C, Culjak G. A 10-year prospective study of patients with skin cancer. JCutanMedSurg. 2002;6(5):427-9.
80. de Vries NC. Metastases of squamous cell carcinoma of the skin and lip. Dermatologica. 1969;138(4):333-9.
81. Eroglu A, Berberoglu U, Berreroglu S. Risk factors related to locoregional recurrence in squamous cell carcinoma of the skin. Journal of Surgical Oncology. 1996; 61(2):124-30.
82. Goepfert H, Dichtel WJ, Medina JE. Perineural invasion in squamous cell skin carcinoma of the head and neck. American Journal of Surgery. 1984;148(4):642-7.
83. Gray DT, Suman VJ, Su WPD, Clay RP, Harmsen WS, Roenigk RK. Trends in the population-based incidence of squamous cell carcinoma of the skin first diagnosed between 1984 and 1992. Archives of Dermatology. 1997;133(6):735-40.
84. KATZ AD, URBACH F, LILIENFELD AM. The frequency and risk of metastases in squamous-cell carcinoma of the skin. Cancer. 1957;10(6):1162-6.
85. Kilic C, Tuncel U, Comert E, Polat I. Nonmelanoma facial skin carcinomas: methods of treatment. J CraniofacSurg. 2014;25(2): e113-e6.
86. Kyrgidis A, Tzellos TG, Kechagias N, Patrikidou A, Xirou P, Kitikidou K,... Antoniades K. Cutaneous squamous cell carcinoma (SCC) of the head and neck: Risk factors of overall and recurrence-free survival. European Journal of Cancer. 2010;46(9): 1563-72.
87. Moller R, Reymann F, Hou-Jensen K. Metastases in dermatological patients with squamous cell carcinoma. ArchDermatol.

1979;115(6):703-5.
88. Nelson TG, Ashton RE. Low incidence of metastasis and recurrence from cutaneous squamous cell carcinoma found in a UK population: Do we need to adjust our thinking on this rare but potentially fatal event? Journal of surgical oncology. 2017;116(6): 783-8.
89. Silberstein E, Sofrin E, Bogdanov-Berezovsky A, Nash M, Segal N. Lymph Node Metastasis in Cutaneous Head and Neck Squamous Cell Carcinoma. Dermatol Surg. 2015;41(10):1126-9.
90. Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. Transplantation. 2004;77(12):1777-82.
91. Gutierrez-Dalmau A, Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients - A systematic review. Drugs. 2007;67(8):1167-98.
92. In: Amin MB ES, Greene F, et al, eds., editor. AJCC Cancer Staging Manual. 8th ed. ed. New York, NY: Springer; 2017.
93. Karia PS, Morgan FC, Califano JA, Schmults CD. Comparison of Tumor Classifications for Cutaneous Squamous Cell Carcinoma of the Head and Neck in the 7th vs 8th Edition of the AJCC Cancer Staging Manual. JAMA Dermatol. 2018;154(2):175-81.
94. Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF, Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32 (4):327-34.
95. Frierson HF, Jr., Deutsch BD, Levine PA. Clinicopathologic features of cutaneous squamous cell carcinomas of the head and neck in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma. Human pathology. 1988;19(12):1397-402.
96. Mehrany K, Weenig RH, Lee KK, Pittelkow MR, Otley CC. Increased metastasis and mortality from cutaneous squamous cell carcinoma in patients with chronic lymphocytic leukemia. JAmAcadDermatol. 2005;53(6):1067-71.
97. Nguyen P, Vin-Christian K, Ming ME, Berger T. Aggressive squamous cell carcinomas in persons infected with the human immunodeficiency virus. Arch Dermatol. 2002;138(6):758-63.
98. Jennings L, Schmults CD. Management of high-risk cutaneous squamous cell carcinoma. Journal of Clinical and Aesthetic Dermatology. 2010;3(4).
99. Fine JD, Johnson LB, Weiner M, Li KP, Suchindran C. Epidermolysis bullosa and the risk of life-threatening cancers: the National EB Registry experience, 1986-2006. J Am Acad Dermatol. 2009;60(2):203-11.
100. Kinlen LJ. Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. The American journal of medicine. 1985;78(1A):44-9.
101. Nehal KS, Bichakjian CK. Update on Keratinocyte Carcinomas. N Engl J Med. 2018;379(4):363-74.
102. Genders RE, Osinga JAJ, Tromp EE, O'Rourke P, Bouwes Bavinck JN, Plasmeijer EI. Metastasis Risk of Cutaneous Squamous Cell Carcinoma in Organ Transplant Recipients and Immunocompetent Patients. Acta Derm Venereol. 2018;98(6):551-5.
103. Haisma MS, Plaat BE, Bijl HP, Roodenburg JL, Diercks GF, Romeijn TR, Terra JB. Multivariate analysis of potential risk factors for lymph node metastasis in patients with cutaneous squamous cell carcinoma of the head and neck. J Am Acad Dermatol. 2016;75(4):722-30.



CHAPTER 7

Metastasis risk of cutaneous squamous cell carcinoma in organ transplant recipients and immunocompetent patients

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Abstract

Organ transplant recipients (OTR) have a high incidence of cutaneous squamous cell carcinoma (cSCC) and immunosuppression has been reported to be an important risk factor for metastasis.
This study aimed to identify the metastasis risk over a 10- year period for 593 cSCC patients of whom 134 were OTR and 459 were immunocompetent patients.
Metastasis incidence rate (IR) was 1046 (95%CI 524- 2096) per 100.000 person years in OTR and 656 (95%CI; 388-1107) in immunocompetent patients, yielding an IR ratio of 1.6 (95% CI 0.67-3.81). In OTR head/neck location, older age at transplantation and older age at the diagnosis of first cSCC were associated with metastatic risk and seven out of eight metastasized tumors were smaller than 2 centimetres. In immunocompetent patients tumor size and tumor depth were associated with metastasis.
In conclusion, we were not able to demonstrate an increased metastasis incidence rate in OTR compared to immunocompetent patients. However, OTR and immunocompetent patients differed in risk factors for metastasis.

Introduction

Cutaneous squamous cell carcinoma (cSCC) is the second most frequent form of keratinocyte carcinoma (KC).[1,2] The incidence of cSCC varies globally, with a higher incidence closer to the equator. In Finland the incidence is reported to be 6 for men and 4 for women per 100.000 person years . In Australia, these numbers are reported to be 1035 and 472 per 100.000 person years.[3,4]
The incidence of cSCC metastasis varies, with higher incidence often reported from tertiary hospitals.[5-10] The risk of developing metastasis from low-risk cSCC in the general population is between 0.5 and 5% , but may be as high as 45% in high risk cSCC, i.e. tumors located on the lip and ear, large tumors and recurrent tumors.[5,11,12]

The risk of developing cSCC in organ transplant recipients (OTR) is 40-250 times increased compared to that in the general population.[13] In some reports, it has been suggested that cSCC in OTR frequently exhibit aggressive behaviour irrespective of size, that in transit metastases are more frequent and that cSCC in OTR have a worse prognosis than cSCC in immunocompetent patients.[14,15] Between 5 to 23 % of all patients with metastatic cSCC, have been reported to be immunosuppressed, many of whom were OTR and therefore immunosuppression has been reported to be a risk factor for metastasis.[16-18]
In clinical practice, it is difficult to identify high risk cSCC and to detect a metastasis at an early stage, both in immunosuppressed and immunocompetent patients.[19-21] Tumor size, Clark's level, Breslow's thickness, degree of differentiation, perineural invasion and location are associated with the development of metastasis.[22] Staging systems are developed to help determine high-risk tumors.[23-25] The American Joint Committee on Cancer (AJCC) classification is the most commonly used and subject to regular modifications. Since 2010 the AJCC classification is based on several high-risk features of cSCC.[23] Though immunosuppression is mentioned as a risk factor, it does not influence tumor stage in any of the staging systems.
OTR are subject to lifelong immunosuppressive therapy and therefore are an ideal population with regard to analysing the influence of immunosuppression on cSCC metastatic behaviour. Comparative studies between OTR and immunocompetent patients focusing on the metastasis risk of cSCC are scarce. We aimed to identify the risk of cSCC metastasis in a defined cohort of OTR and immunocompetent patients, calculated both per patients as well as per individual tumors.

Methods

For this retrospective study, all consecutive patients with primary cSCC who were diagnosed from January 2004 to December 2013 were identified from the institutional oncology database of the Leiden University Medical Centre. Each patient's medical record was cross-checked with pathology records of cSCC in the same period. Missing patients and/or tumors were added to the database manually. Each tumor was given its own record; one individual patient could have had more records in case of multiple primary cSCC. Detailed histopathological information on all tumors was extracted from the Dutch pathology registry (PALGA). Tumors were categorized by differentiation grade (good, moderate, poor, undifferentiated), presence of lymphovascular invasion, presence of perineural invasion, tumor depth, location and tumor size.[26] All tumors were classified using the TNM staging classification (AJCC 7th edition).

Patient data were extracted from the institutional oncology database. When missing, additional data were collected from patient files. Status and data of immunosuppression were retrieved from the institutional patient database and patient files. Patient files for all patients were checked for information on possible development of metastasis in December 2015, i.e. a minimum follow-up of 2 years. In case of metastasis, the cSCC that most probably was the index tumor was identified. Cases were defined as patients that developed a metastasis from a cSCC that was diagnosed during the study period. Follow-up time was defined as the time of cSCC diagnosis until time of diagnosis of metastasis, death or end of study or to censoring. In our institution OTR were examined at least every 3 months. Immunocompetent patients were followed clinically based on tumor stage according to national guidelines, i.e. twice a year first 1-2 years and once a year in year 3-5 for patients with low-risk cSCC and every three months first year, every 4, 6 and 12 months in year 2, 3 and 4-5, respectively for patients with high-risk cSCC. Screening for metastasis was routinely done by lymph node palpation, and on indication in high-risk tumors by radiologic imaging (ultrasound, X-ray, MRI, CT or PET-scan). Sentinel lymph node procedure was not performed. Descriptive statistics were used for patient and tumor characteristics at baseline. Frequencies, percentages, median, mean and range were calculated, when appropriate. Patients that were immunosuppressed for other reasons than organ transplantation were excluded. Univariable Cox regression analysis was used to identify risk factors for metastasis, excluding cases with missing values for each variable. Multivariable analyses were not carried out due to limited number of events.

Table 1. Baseline characteristics of 593 patients with cutaneous squamous cell carcinoma

	OTR N (%) N=134	**Immunocompetent N (%) N=459**
Sex		
Female	40 (30)	188 (41)
Male	94 (70)	271 (59)
Age first lifetime cSCC (years)		
<60	90 (67)	78 (17)
>=60	44 (33)	381 (83)
Number cSCC study period mean/patient	6.2	1.8
1	39 (29)	357 (78)
2-5	58 (43)	91 (20)
>5	37 (28)	11 (2)
Number lifetime cSCC mean/patient	8.6	2.4
1	32 (24)	313 (68)
2-5	52 (39)	130 (28)
>5	50 (37)	16 (4)
Type of transplantation		
Kidney	110 (82)	NA
Kidney-pancreas	17 (13)	NA
Liver	7 (5)	NA

Abbreviations: OTR: organ transplant recipient; cSCC: cutaneous squamous cell carcinoma; NA: not applicable

A p-value of <0.05 was set as statistically significant for all analyses. Statistical analysis was performed using SPSS version 23 (SPSS, Chicago, Illinois, USA). Incidence rate per person year of metastasis was calculated using STATA 13.1.

Results

During the follow-up time of median 4.0 years (range 1 month-10.8 years), 1792 cSCC were diagnosed in 665 patients, with up to 116 cSCC in one patient. After exclusion of one OTR who had developed a cSCC 1 year before transplantation, 66 patients that were immunosuppressed for other reasons than organ transplantation (inflammatory disease or hematologic malignancy) and six patients who had developed a metastasis from a cSCC diagnosed prior to the study period, the study cohort consisted of 134 OTR and 459 immunocompetent patients (Table 1).

Table 2. Patient and tumor characteristics in 23 patients with metastatic cutaneous squamous cell carcinoma

Sex	Age at cSCC diagnosis	OTR	Location	cSCC size in mm	Differentiation grade	Depth
F	43	kidney/pancreas	cheek	14	good	unknown
M	61	kidney	scalp	10	good	subcutis
M	62	kidney/pancreas	chest	14	undifferentiated	unknown
M	66	Kidney	frontal	15	good	unknown
M	68	kidney	peri-ocular	16	good	dermis
M	68	liver	neck	17	good	dermis
M	70	kidney/pancreas	ear	25	good	dermis
M	71	kidney	scalp	15	good	dermis
F	65	no	finger	10	poor	unknown
M	66	no	temporal	10	undifferentiated	muscle
F	72	no	cutaneous lip	16	good	muscle
M	74	no	temporal	unknown	moderate	dermis
M	75	no	ear	25	good	muscle
M	76	no	scalp	unknown	good	unknown
F	79	no	peri-ocular	38	undifferentiated	unknown
F	84	no	arm	unknown	poor	unknown
F	85	no	temporal	45	good	dermis
M	85	no	frontal	15	moderate	muscle
M	85	no	occipital	45	good	subcutis
M	86	no	neck	15	unclassifiable	dermis
M	88	no	temporal	32	good	subcutis
M	93	no	ear	19	good	cartilage
M	94	no	ear	30	moderate	subcutis

Abbreviations: cSCC = cutaneous squamous cell carcinoma, OTR = organ transplant recipient,

F = female, M = male, PNI = perineural invasion, meta = metastasis.

PNI	T-stage AJCC	Number of lifetime cSCC until metastasis	Type of metastasis	Time between cSCC and metastasis (months)	Death because of metastasis
no	1	8	nodal	27	no
no	1	3	nodal	14	no
no	unknown	8	nodal	3	yes
no	1	1	nodal	3	alive
no	1	3	distant	30	no
no	1	1	nodal	21	cause of death unknown
no	2	15	nodal	6	alive
no	1	15	nodal	38	alive
no	unknown	1	nodal	17	yes
no	3	1	distant	13	yes
no	3	1	nodal & distant	16	yes
no	unknown	1	nodal	7	yes
no	3	1	nodal	21	alive
no	unknown	1	distant	7	cause of death unknown
no	2	1	nodal	6	no
no	unknown	11	nodal	2	no
no	2	1	nodal	7	alive
no	3	1	nodal	39	cause of death unknown
no	2	5	nodal & distant	0	yes
no	1	1	nodal	15	no
yes	2	1	nodal & distant	1	yes
no	3	6	nodal	13	yes
no	2	1	nodal	8	cause of death unknown

Table 3. Univariate hazard ratios for patient characteristics as risk factors for metastasis in patients with cutaneous squamous cell carcinoma

	Organ transplant recipients			Immunocompetent patients		
	total	**with metastasis, n (%)**	**Hazard ratio (95%CI)**	**total**	**with metastasis, n (%)**	**Hazard ratio (95%CI)**
PATIENTS	**N=134**	**N=8**		**N=459**	**N=15**	
Sex						
Female	40	1 (2)	1	188	5 (3)	1
Male	94	7 (7)	4.4 (0.52-36.1)	271	10 (4)	
Age at 1st cSCC (years)						
<60	90	2 (2)	1	78	0	NA
>=60	44	6 (14)	8.3 (1.7-41.8)	381	15 (4)	NA
Number cSCC study period						
1	39	1 (3)	1	357	12 (3)	1
>1	95	7 (7)	1.5 (0.19-12.8)	102	3 (3)	0.75 (0.21-2.7)
Number lifetime cSCC						
1	33	2 (6)	1	314	12 (4)	1
>1	101	6 (6)	0.48 (0.09-2.6)	145	3 (2)	0.49 (0.14-1.7)
Age at TX (years)						
<60	113	5 (4)	1	NA	NA	NA
>=60	21	3 (14)	5.0 (1.2-21.7)	NA	NA	NA

Abbreviations: CI = confidence interval, cSCC = cutaneous squamous cell carcinoma, TX = transplantation, NA = not applicable

In all, 23 patients (3.9%; 95% CI 2.6-5.8) developed a metastasis from a cSCC diagnosed in the study period, eight (6.0; 95% CI 3.1-11.3) OTR and 15 (3.3%; 95% CI 2.0-5.3) immunocompetent patients. The incidence rate (IR) for metastasis was 1046 (95%CI 524-2096) per 100,000 person years in the OTR group and 656 (95%CI; 388-1107) in the immunocompetent patient group, yielding an incidence rate ratio (IRR) between OTR versus immunocompetent patients of 1.6 (95% CI 0.67-3.8). Median time between cSCC diagnosis and metastasis diagnosis was longer in the OTR group than in the immunocompetent group, 17.5 months and 10.5 months, respectively. Distant site metastasis was found in 1 OTR and in 5 immunocompetent patients, of whom 3 also had concomitant nodal metastasis.

Patient, tumor and metastasis characteristics are shown in Table 2, 3 and 4. Univariate hazard ratios for metastasis are listed in Tables 3 and 4. Having a first cSCC at an

Table 4. Univariate hazard ratio of tumor characteristics as risk factors for metastasis in patients with cutaneous squamous cell carcinoma

	Organ transplant recipients			Immunocompetent patients		
	total	**metastatic cSCC, n (%)**	**Hazard ratio (95%CI)**	**total**	**metastatic cSCC, n (%)**	**Hazard ratio (95%CI)**
TUMORS	**N=829**	**N=8**		**N=814**	**N=15**	
Location tumor						
Body	582	1 (1)	1	296	2 (1)	1
Head & neck	247	7 (3)	16.9 (2.1-138.5)	518	13 (3)	3.8 (0.85-16.9)
T-stage						
T1	657	6 (1)	1	584	1 (1)	1
T2-T4	73	1 (1)	1.5 (0.18-12.7)	105	10 (10)	61.4 (7.8-484.9)
Missing	99	1 (1)		125	4 (3)	
Tumor size (diameter)						
<=20mm	726	7 (1)	1	661	6 (1)	1
>20mm	59	1 (2)	1.8 (0.21-14.6)	71	6 (8)	10.1 (3.2-32.1)
Missing	44	0		82	3 (4)	
Differentiation						
Good-moderate	720	7 (1)	1	681	10 (1)	1
Poor-undifferentiated	94	1 (1)	1.1 (0.13-9.0)	120	4 (3)	2.3 (0.71-7.5)
Missing	15	0		13	1 (8)	
Depth/invasion						
Dermis	227	4 (2.0)	1	458	3 (1)	1
Hypodermis	27	1 (4)	1.4 (0.24-8.5)	46	8 (17)	6.5 (3.3-13.0)
Missing	575	3 (1)		310	4 (1)	

Abbreviations: cSCC = cutaneous squamous cell carcinoma, CI = confidence interval.

older age was significantly associated with an increased risk of metastasis in OTR (HR 8.3 95%CI:1.7-41.8). In the immunocompetent group, all patients (15) with metastasis developed their first cSCC after the age of 60 years. In OTR, being transplanted at 60 years or older (HR 5.0, 95%CI 1.2-21.7), increased the risk of metastasis. Having multiple cSCC was not associated with increased risk for metastasis in either group. cSCC located in the head and neck area was a risk factor for metastasis, both in the OTR group (HR 16.9, 95% CI 2.1-138.5) and in the immunocompetent group (HR 3.8, 95% CI 0.85-16.9), but statistically significant only in the OTR group. In the OTR group T stage, tumor size, differentiation grade and depth of invasion were not associated with increased metastasis risk. T stage of 2 or higher, tumor size and tumor depth beyond the dermis were associated with

increased risk of metastasis in the immunocompetent group. In the OTR group, perineural invasion was not found in any metastatic cSCC and in 3 non-metastatic cSCC, but was observed in one metastatic cSCC and in 9 non-metastatic cSCC in the immunocompetent population. Lymphovascular invasion was only seen in one non-metastatic cSCC in OTR and in 2 non-metastatic cSCC in immunocompetent patients.

Discussion

In this study we were not able to demonstrate a statistically significant increased risk for metastasis from cSCC in OTR compared to immunocompetent patients with cSCC. However, the risk factors seemed to be different between the two groups.
With cSCC metastases incidences of 6.0% in OTR and 3.3% in the immunocompetent patients, the incidence of metastasis in our study was lower than in other studies. In OTR metastasis incidences of 7-14% are reported for low-risk tumors and 10-20% for high-risk tumors.[1,9,18,27-30] In the general population, the overall incidence varies between 2.3-9.9%.[31] For low-risk tumors the risk is between 0.5-5% and may increase to more than 20% in high risk cSCC.[5,11,12]
The mean time between diagnosing the cSCC and detecting metastasis was longer in the OTR group than in the immunocompetent group. The OTR group had a median time of 17.5 months to develop metastasis, compared to 10.5 months in the immunocompetent group. This was a surprising finding, considering both the immunosuppressed state leading to decreased immunological tumor surveillance and the increased clinical surveillance of OTR. As the majority of tumors in OTR were not located in the head and neck area, in contrast to the immunocompetent group, this difference in tumor distribution could contribute to the relatively low risk of metastasis in OTR. In other studies, the median time to develop metastasis is reported from 4 to 12 months.[32,33]
In OTR, age over 60 years at transplantation and age over 60 years at time of first cSCC diagnosis were risk factors for metastasis. This is in line with previous studies.[34] As OTR developed their first cSCC at a younger age than immunocompetent patients, this could explain the relatively low number of metastases in OTR. Most OTR in our study were renal transplant recipients and have somewhat different immunosuppressive treatment regimens than other OTR , especially heart transplant recipients.[35-37] This could contribute to different incidence rates of cSCC metastasis across the literature.

In our immunocompetent patients, tumor size and depth, and subsequently T-stage, were significant risk factors for metastasis, as shown in other studies.[5,9,28,38,39] In our OTR we did not find these associations. Tumor size is a well-known risk factor for metastatic lesions with an association between increasing tumor thickness and lesion size.[5,9,18,19,40-43] In our study, seven out of eight cSCC that metastasised in OTR were smaller than 2 cm in diameter and most of the tumors were low risk T1 tumors.
This suggests that OTR with relatively small tumors (<2cm) are already at an increased risk of developing metastasis from cSCC, and are less dependent on specific tumor characteristics.[18,44] OTR have regular clinical follow-ups, and small tumors should therefore be diagnosed before they grow into large tumors. Although it is published that patients with multiple cSCC are at an increased risk of metastasis, we were unable to confirm that having multiple tumors led to a higher risk of metastasis.[45] Given the low number of metastases in our OTR group and with metastases mainly developing from relatively small tumors, frequent follow-up of OTR should continue.
This study has several limitations. First, we had only a small number of patients with metastasising cSCC in the OTR group. It was, therefore, difficult to estimate the effect size for risk factors. We acknowledge that statistical analysis should be considered insufficient and only indicative. We also had too few cases to perform multivariable analyses to control for possible confounders. Second, the Leiden University Medical Centre is a tertiary referral centre for selected cSCC patients. Due to this selection bias, extrapolation of our results is difficult, as one should expect a higher rate of metastases in immunocompetent patients with cSCC referred to the hospital. Finally, the cSCC in our cohort were diagnosed by several pathologists without a systematic re-evaluation of the histopathological slides. This might have introduced some error but probably not bias, as no differences are expected in how pathologists will evaluate tumors from OTR and immunocompetent patients.
To conclude, the metastatic rate of cSCC in OTR and immunocompetent patients in this study is lower than in most other studies. We were not able to demonstrate an increased risk of metastasis in OTR compared to immunocompetent patients. Most tumors that did metastasise in OTR were small tumors without high-risk features. Close and regular inspection of the skin with adequate and rapid diagnosis and treatment of cSCC is important to prevent metastasis.

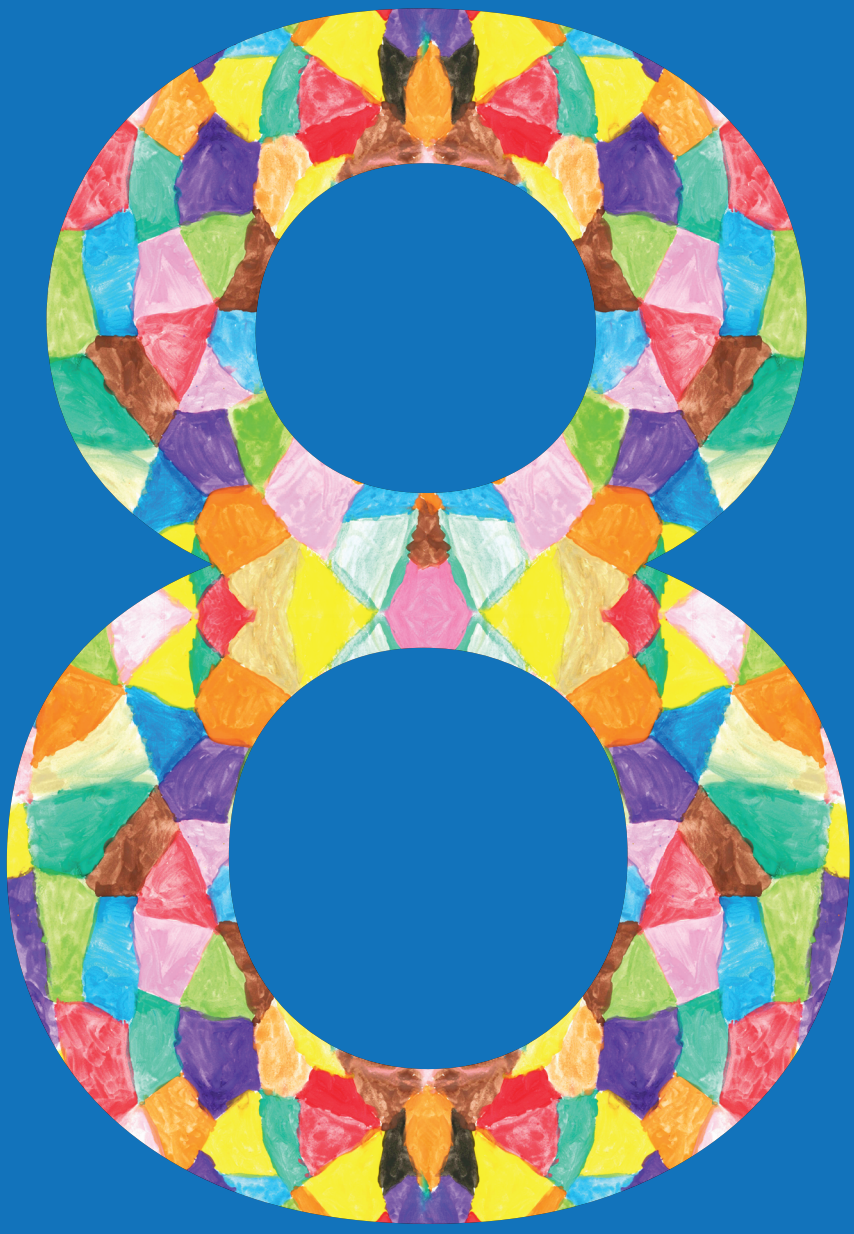
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References

1. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. NEnglJMed. 2001;344(13): 975-83.
2. Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. Lancet. 2010;375(9715): 673-85.
3. Green A, Battistutta D, Hart V, Leslie D, Weedon D. Skin cancer in a subtropical Australian population: incidence and lack of association with occupation. The Nambour Study Group. Am J Epidemiol. 1996;144(11): 1034-40.
4. Hannuksela-Svahn A, Pukkala E, Karvonen J. Basal cell skin carcinoma and other nonmelanoma skin cancers in Finland from 1956 through 1995. Arch Dermatol. 1999;135(7):781-6.
5. Brantsch KD, Meisner C, Schonfisch B, Trilling B, Wehner-Caroli J, Rocken M, Breuninger H. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. Lancet Oncol. 2008;9(8):713-20.
6. Brougham ND, Dennett ER, Cameron R, Tan ST. The incidence of metastasis from cutaneous squamous cell carcinoma and the impact of its risk factors. JSurgOncol. 2012;106(7):811-5.
7. Chuang TY, Popescu NA, Su WP, Chute CG. Squamous cell carcinoma. A population-based incidence study in Rochester, Minn. ArchDermatol. 1990;126(2):185-8.
8. Dinehart SM, Pollack SV. Metastases from squamous cell carcinoma of the skin and lip. An analysis of twenty-seven cases. JAmAcadDermatol. 1989;21(2 Pt 1):241-8.
9. Rowe DE, Carroll RJ, Day CL, Jr. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. JAmAcadDermatol. 1992;26(6):976-90.
10. Tavin E, Persky M. Metastatic cutaneous squamous cell carcinoma of the head and neck region. Laryngoscope. 1996;106(2 Pt 1):156-8.
11. Mourouzis C, Boynton A, Grant J, Umar T, Wilson A, Macpheson D, Pratt C. Cutaneous head and neck SCCs and risk of nodal metastasis - UK experience. JCraniomaxillofacSurg. 2009;37(8):443-7.
12. O'Hara J, Ferlito A, Takes RP, Rinaldo A, Strojan P, Shaha AR,... Paleri V. Cutaneous squamous cell carcinoma of the head and neck metastasizing to the parotid gland--a review of current recommendations. Head Neck. 2011;33(12):1789-95.
13. Proby CM, Wisgerhof HC, Casabonne D, Green AC, Harwood CA, Bouwes Bavinck JN. The epidemiology of transplant-associated keratinocyte cancers in different geographical regions. Cancer Treat Res. 2009;146:75-95.
14. Carucci JA, Martinez JC, Zeitouni NC, Christenson L, Coldiron B, Zweibel S, Otley CC. In-transit metastasis from primary cutaneous squamous cell carcinoma in organ transplant recipients and nonimmunosuppressed patients: clinical characteristics, management, and outcome in a series of 21 patients. DermatolSurg. 2004;30(4 Pt 2):651-5.
15. Veness MJ, Quinn DI, Ong CS, Keogh AM, Macdonald PS, Cooper SG, Morgan GW. Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience. Cancer. 1999;85(8):1758-64.
16. Dinehart SM, Chu DZ, Maners AW, Pollack SV. Immunosuppression in patients with metastatic squamous cell carcinoma from the skin. JDermatolSurgOncol. 1990;16(3):271-4.
17. Khurana VG, Mentis DH, O'Brien CJ, Hurst TL, Stevens GN, Packham NA. Parotid and neck metastases from cutaneous squamous cell carcinoma of the head and neck. AmJSurg. 1995;170(5):446-50.
18. Veness MJ, Palme CE, Morgan GJ. High-risk cutaneous squamous cell carcinoma of the head and neck: results from 266 treated patients with metastatic lymph node disease. Cancer. 2006;106(11):2389-96.
19. Cherpelis BS, Marcusen C, Lang PG. Prognostic factors for metastasis in squamous cell carcinoma of the skin. DermatolSurg. 2002;28(3):268-73.
20. Martorell-Calatayud A, Sanmartin JO, Cruz MJ, Guillen BC. Cutaneous squamous cell carcinoma: defining the high-risk variant. Actas Dermosifiliogr. 2013;104(5):367-79.
21. Veness MJ. Defining patients with high-risk cutaneous squamous cell carcinoma. AustralasJDermatol. 2006;47(1):28-33.

22. Farasat S, Yu SS, Neel VA, Nehal KS, Lardaro T, Mihm MC,... Liegeois NJ. A new American Joint Committee on Cancer staging system for cutaneous squamous cell carcinoma: creation and rationale for inclusion of tumor (T) characteristics. JAmAcadDermatol. 2011;64(6):1051-9.
23. Cutaneous squamous cell carcinoma and other cutaneous carcinomas. In: Edge SB, Byrd DR, Compton CC, editors. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; 2010. p. 301-14.
24. In: Sobin L, Gospodarowicz M, Wittekind C, editors. UICC International Union Against Cancer TNM Classification of Malignant Tumors. 7th ed. West Sussex, United Kingdom: Wiley-Blackwell; 2009.
25. Karia PS, Jambusaria-Pahlajani A, Harrington DP, Murphy GF, Qureshi AA, Schmults CD. Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. JClinOncol. 2014;32(4):327-34.
26. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH, Meijer GA. Pathology databanking and biobanking in the Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol. 2007;29(1):19-24.
27. Nuno-Gonzalez A, Vicente-Martin FJ, Pinedo-Moraleda F, Lopez-Estebaranz JL. High-Risk Cutaneous Squamous Cell Carcinoma. Actas Dermosifiliogr. 2012.
28. Breuninger H, Schaumburg-Lever G, Holzschuh J, Horny HP. Desmoplastic squamous cell carcinoma of skin and vermilion surface: a highly malignant subtype of skin cancer. Cancer. 1997;79(5):915-9.
29. Johnson TM, Rowe DE, Nelson BR, Swanson NA. Squamous cell carcinoma of the skin (excluding lip and oral mucosa). JAmAcadDermatol. 1992;26(3 Pt 2):467-84.
30. Cooper JZ, Brown MD. Special concern about squamous cell carcinoma of the scalp in organ transplant recipients. ArchDermatol. 2006;142(6):755-8.
31. Weinberg AS, Ogle CA, Shim EK. Metastatic cutaneous squamous cell carcinoma: an update. DermatolSurg. 2007;33(8):885-99.
32. Haisma MS, Plaat BE, Bijl HP, Roodenburg JL, Diercks GF, Romeijn TR, Terra JB. Multivariate analysis of potential risk factors for lymph node metastasis in patients with cutaneous squamous cell carcinoma of the head and neck. J Am Acad Dermatol. 2016;75(4):722-30.
33. Veness MJ, Porceddu S, Palme CE, Morgan GJ. Cutaneous head and neck squamous cell carcinoma metastatic to parotid and cervical lymph nodes. Head Neck. 2007;29(7):621-31.
34. Kuijken I, Bouwes Bavinck JN. Skin cancer risk associated with immunosuppressive therapy in organ transplant recipients: epidemiology and proposed mechanisms. BioDrugs. 2000;14(5):319-29.
35. Coghill AE, Johnson LG, Berg D, Resler AJ, Leca N, Madeleine MM. Immunosuppressive Medications and Squamous Cell Skin Carcinoma: Nested Case-Control Study Within the Skin Cancer after Organ Transplant (SCOT) Cohort. AmJTransplant. 2016;16(2):565-73.
36. Euvrard S, Kanitakis J, Decullier E, Butnaru AC, Lefrancois N, Boissonnat P,... Chapuis F. Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. Transplantation. 2006;81(8):1093-100.
37. Rashtak S, Dierkhising RA, Kremers WK, Peters SG, Cassivi SD, Otley CC. Incidence and risk factors for skin cancer following lung transplantation. JAmAcadDermatol. 2015;72(1):92-8.
38. Breuninger H, Black B, Rassner G. Microstaging of squamous cell carcinomas. AmJClinPathol. 1990;94(5):624-7.
39. Friedman HI, Cooper PH, Wanebo HJ. Prognostic and therapeutic use of microstaging of cutaneous squamous cell carcinoma of the trunk and extremities. Cancer. 1985;56(5):1099-105.
40. Clayman GL, Lee JJ, Holsinger FC, Zhou X, Duvic M, El-Naggar AK,... Lippman SM. Mortality risk from squamous cell skin cancer. JClinOncol. 2005;23(4):759-65.
41. Griffiths RW, Feeley K, Suvarna SK. Audit of clinical and histological prognostic factors in primary invasive squamous cell carcinoma of the skin: assessment in a minimum 5 year follow-up study after conventional excisional surgery. BrJPlastSurg. 2002;55 (4):287-92.

42. Hanlon A. Metastatic Squamous Cell Carcinoma: Epidemiology and Available Systemic Therapies. Curr Derm Rep. 2013;2: 118-24.
43. Mullen JT, Feng L, Xing Y, Mansfield PF, Gershenwald JE, Lee JE,... Cormier JN. Invasive squamous cell carcinoma of the skin: defining a high-risk group. AnnSurgOncol. 2006;13(7):902-9.
44. Stevenson ML, Kim R, Meehan SA, Pavlick AC, Carucci JA. Metastatic Cutaneous Squamous Cell Carcinoma: The Importance of T2 Stratification and Hematologic Malignancy in Prognostication. Dermatol Surg. 2016;42(8):932-5.
45. Levine DE, Karia PS, Schmults CD. Outcomes of Patients With Multiple Cutaneous Squamous Cell Carcinomas: A 10-Year Single-Institution Cohort Study. JAMA Dermatol. 2015;151(11):1220-5.



CHAPTER 8

Summary and discussion

Introduction

The risk of cutaneous squamous cell carcinoma (cSCC) is highly increased in organ transplant recipients (OTR) and these tumors carry a major health burden for these patients. This thesis dealt with several aspects of cSCC in OTR. The chapters 2 and 3 review the role of HPV in cSCC and study HPV as a prognostic factor in the aetiology of cSCC. Subsequently, diagnostic factors were studied. In chapter 4 we intended to identify p16 as a possible histopathological marker for cSCC and in chapter 5 we studied the clinical implication of pain as a symptom to differentiate cSCC from other keratinocyte lesions. In chapter 6 we reviewed the literature on metastasis risk in immunocompetent versus OTR population. We studied in chapter 7 in our own population the risk for metastasis, and subsequently risk factors, of cSCC in OTR compared to immunocompetent patients.

The role of HPV

Most studies investigating HPV seroresponses in association with keratinocyte carcinoma (KC) development concerned cross-sectional and case-control studies in the immunocompetent and OTR population with conflicting results.[1-10]

Case-control studies have the disadvantage that a temporal relationship between the exposure and outcome cannot be established. Cohort studies are better equipped for this type of investigation and, therefore, we designed a cohort study with a prolonged follow-up period up to 22 years. HPV serology before and just after the organ transplantation was determined and the OTR were followed until the development of a first KC or by the death of the patient or the end of the follow-up period. The hazard ratio (HR) to develop KC in OTR who were tested Beta-PV seropositive around the time of transplantation compared with Beta-PV-seronegative OTR was 2.9 (95% confidence interval (CI) 1.3–6.4). The HR for cSCC was 2.9 (95% CI 0.99–8.5) and for basal cell carcinoma (BCC) it was 3.1 (95% CI 1.2–8.0). These data were largely confirmed in a recent study which prospectively evaluated the association of Beta-PV in eyebrow hair and seroresponses with KC in OTR.[11] The presence of 5 or more different Beta-PV types in eyebrow hair was associated with a HR of 1.7 (95% CI 1.1- 2.6). No significant association was seen between serum antibodies and cSCC, but a trend of a positive association could be observed. This study did not show an association between Beta-PV and BCC.[11]

A similar association between Beta-PV and cSCC has been established in

immunocompetent individuals. A recently published systematic review included 14 studies with a total of 3112 adult immunocompetent study participants with cSCC and 6020 controls to clarify this association and to evaluate Beta-PV-HPV involvement in cSCC development.[12] The overall association between Beta-PV and cSCC was significant with an adjusted pooled OR of 1.42 (95% CI 1.18-1.72). Subgroup analysis in studies using only serology for HPV detection showed a significant association between Beta-PV with an increased risk of cSCC development with a pooled OR of 1.4 (95% CI 1.2-1.8).
Animal studies also support the role of Beta-PV in the development of cSCC. An elegant study with HPV positive and negative Mastomys coucha (a multimammate rodent that functions as a model which reflects the human situation in many aspects) showed that after UV-exposure the papillomavirus positive animals developed cSCC much more rapidly than the papillomavirus negative animals.[13]

Diagnostic factors

cSCC can be difficult to diagnose in some cases , both clinically and histopathologically. Especially the differentiation from other keratinocyte lesions can be challenging. Since OTR develop numerous types of keratinocyte lesions and the malignant lesions need to be identified as early as possible a search for new diagnostic cSCC markers is useful.

The role of p16 immunostaining

We investigated p16 immunohistochemical staining patterns in order to differentiate between different types of keratinocyte neoplasia; actinic keratosis (AK), Bowen' s disease (BD) , cSCC and benign keratotic lesions in OTR. We found a gradual increase in P16 immunostaining patterns from AK grade 1 to cSCC, as also described in a previous study.[14] The most prominent staining pattern, throughout the whole epidermis, was seen in BD. Furthermore, BD did not follow the gradual staining pattern as observed in AK and cSCC. This might support the opinion that BD could be regarded as a separate entity, instead of part of the classical spectrum of keratinocyte dysplasia from AK to BD to cSCC.[15] It is known that BD does not have to be preceded by an AK and it has clinically different features compared to AK and cSCC.[16-20] However, this is highly speculative and further scientific evidence is needed to confirm this hypothesis.[21] Unfortunately, we did not find distinct patterns of p16 immunostaining that could be used in differentiating cSCC from AK or BD.

Pain as a diagnostic factor

We have found that pain is a powerful warning signal for cSCC in OTR. Especially in patients with numerous skin lesions, pain can be helpful in differentiating cSCC from benign keratinocyte lesions.

Pain is shown to be associated with the degree of inflammation, presence of neutrophils and eosinophils in the inflammatory infiltrate, ulceration, perineural invasion, depth of invasion, and largest diameter length of skin lesion.[22] In collaboration with the University hospital of Zurich, we have found that in OTR, Prostaglandin E2, Tumor Necrosis Factor α, and Pro-opiomelanocortin are potential mediators of pain in cSCC.[23] These mediators are involved in the inflammation process and pain sensation of the skin. The results suggest a role of these mediators in the local tumor microenvironment in mediating pain in cSCC. In clinical practice, pain is a helpful indicator in differentiating cSCC from benign keratinocyte lesions in OTR. However, the exact microenvironment of cytokines involved in pain by cSCC has to be elucidated in more detail.

Metastasis of cSCC in OTR

Incidence

We systematically reviewed the literature and also studied the incidence of cSCC metastases in OTR and the immunocompetent population in our own institute. Our systematic review in chapter 6 showed a higher overall risk of cSCC metastasis in OTR compared to the immunocompetent population. Metastasis risks per single cSCC were substantially lower in both groups. The pooled metastasis risk estimate for OTR was respectively 7.3% (95% CI 6.2-8.4) for cSCC on total body, and 11.0% (95% CI 7.7-14.8) for cSCC of the head and neck area. For the immunocompetent population reported risk estimate analysis showed a pooled metastatic risk of 3.1% (95% CI 2.8-3.4) in total body cSCC and of 8.5% (95% CI 7.3-9.8) in cSCC of the head and neck area. Pooled risk estimate per single cSCC in OTR was 1.3% (95% CI 1.0-1.7) in total body cSCC and 4.0% (95% CI 2.7-5.5) in cSCC of the head and neck area.

In the immunocompetent population these pooled risk estimates were respectively 2.4% (95% CI 2.1-2.6) and 6.7% (95% CI 5.7-7.8). A very recent national population-based study in England which was not included in our systematic review identified a cumulative metastasis incidence of cSCC of 1.1% in women and 2.4% in men with a primary cSCC and immunosuppressed patients carried a twofold higher risk for metastasis.[24]

The metastasis risk of cSCC in our institution over a 10 year period in a cohort of 134 OTR and 459 immunocompetent patients was reported in chapter 7. In all, 23 patients (3.9%; 95% CI 2.6-5.8) developed a metastasis from a cSCC diagnosed in the study period, eight (6.0%; 95% CI 3.1-11.3) in OTR and 15 (3.3%; 95% CI 2.0-5.3) in immunocompetent patients. The metastasis incidence rate ratio for OTR was 1.6 (95% CI 0.67–3.81). Patients with multiple tumors, and especially OTR in our specialized clinic, may be under frequent and strict surveillance, so that new tumors will be detected at an earlier stage. This could partly explain the relatively low number of metastases that we found in our own institution.

General risk factors

Current tumor features that are considered as high risk are increased tumor size, recurrent tumor, poor differentiation grade, perineural, lymphatic, or vascular involvement and depth >6 mm or invasion beyond subcutaneous fat.[25] A case series showed that aggressive cSCC in OTR have a median depth of 6.2mm and local recurrence was present in 45% of cases. Also perineural invasion (39%), poor differentiation (41%) and location in the head and neck area (79%) were present in those cSCC.[26] In the OTR who were studied in our own institution, head and neck location, older age at transplantation and older age at diagnosis of first cSCC were associated with metastasis (Chapter 7). Most of the cSCC that metastasized were still smaller than 2 cm. In the immunocompetent patients in our institution tumor size and tumor depth were associated with metastasis (Chapter 7).

Immunosuppression as a risk factor

Results regarding immunosuppression as a risk factor are contradictory. Immunocompromised status has been considered as a high risk host factor by some studies,[25] but another study that investigated the risk of nodal metastasis in head and neck cSCC, did not find immunosuppression to be a significant factor for the development of LN metastasis.[27] A recent systematic review and meta-analysis (17248 patients with 23421 cSCC) found immunosuppression to be a significant risk factor and tumor depth was associated with the highest risk of local recurrence and metastasis of cSCC.[28] Immunosuppressed patients have demonstrated in one report a higher risk of local recurrence and regional recurrence compared to immunocompetent patients (10.5%).[29] In immunosuppressed patients with cSCC of the head and neck, these cSCC more frequently present with high-risk pathologic

features and inferior outcomes.[30] Immunosuppression is also correlated with decreased disease-specific survival, either by metastasis or because of tumor growth by local infiltration.[31-34]

Future perspectives

Because the incidence of KC is still rising, it is evident that prevention and early detection is of upmost importance in the management of these cancers. Vaccination against papillomavirus was effective in preventing cSCC in the Mastomys coucha.[35] Prophylactic vaccination potentially can also be helpful in preventing the development of cSCC in OTR,[36] but so far no studies were performed in humans to investigate this possibility.

Also the challenge in daily practice is to identify cSCC, especially high-risk cSCC, and to detect a metastasis as early as possible. There is currently no role for p16 immunostaining to be used routinely in the diagnosis of KC. Studying possible markers, which can clearly differentiate between benign, pre-malignant and malignant keratinocyte lesions, can be helpful in the future to make adequate decision for the best treatment. In clinical practice, pain is a helpful indicator in differentiating cSCC from benign lesions. However, the exact microenvironment of cytokines involved in pain by cSCC has to be elucidated in more detail. Early detection and treatment of cSCC prevents it to grow deeper and therefore lowers the risk for metastasis. Early intervention for metastases can confer to a survival advantage and radiologic imaging in patients with high-risk cSCC may influence management.[37] Ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) are currently the most frequently used methods for the detection of metastasis. Today there is no consensus in the use of radiologic imaging in the management of cSCC as to which of these methods should be used at what time points and how frequently.[37,38] Sentinel lymph node biopsy can be used as an adjuvant method in detecting metastasis. However it has to be established which patients benefit the most from this procedure, especially in high risk populations like OTR. [39]

The most commonly used tool to identify high-risk cSCC is the T stage of the TNM (tumor, nodes, metastasis) classification. However, current staging systems (7th and 8th edition of the AJCC staging system, the staging system used by Breuninger et al and the Brigham and Women's Hospital (BWH) staging system) poorly to moderately distinguish between patients who developed metastasis and those who did not.[40] A

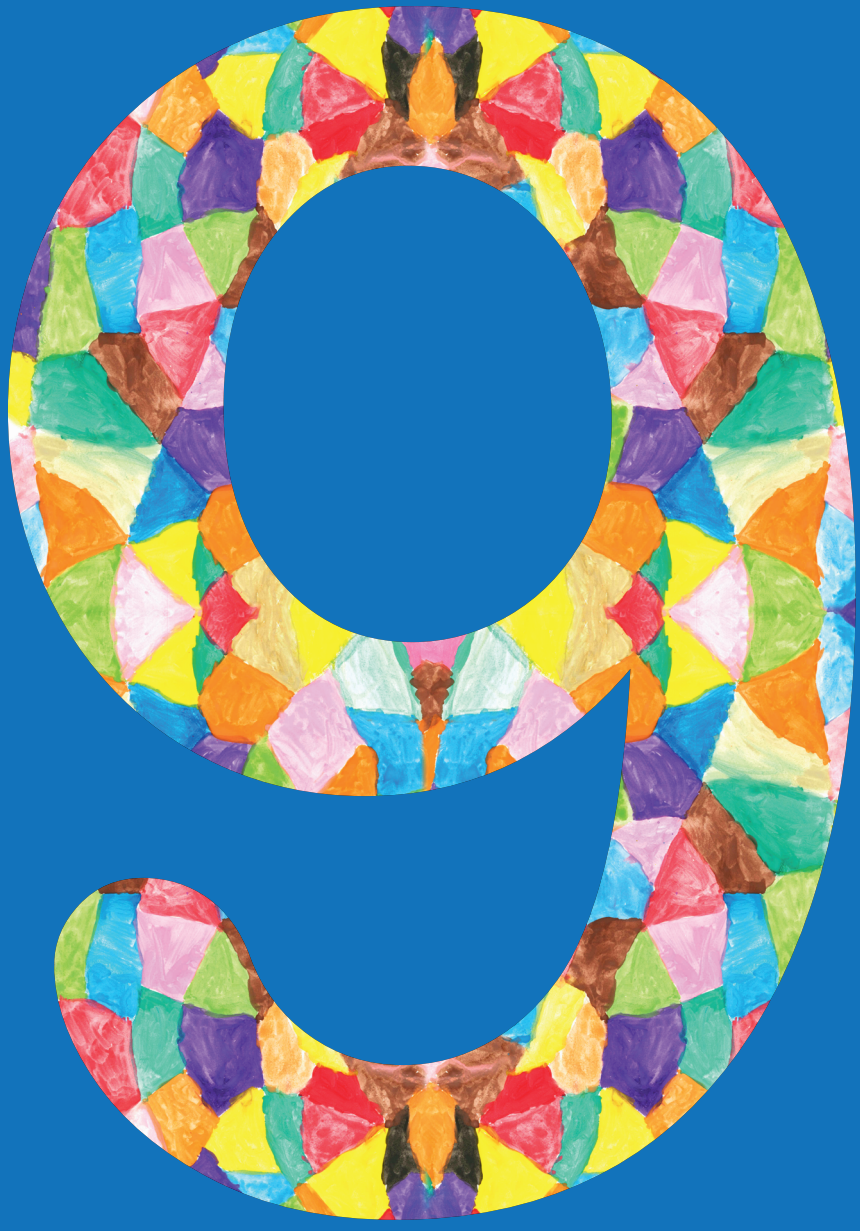
new evidence-based risk stratification system into low, intermediate, and high risk cSCC has been proposed recently, to better allow risk stratification of cSCC based on tumor characteristics and other risk factors, including immunosuppression.[41]
Due to the ongoing insight in risk factors for cSCC, evidence based guidelines for the care of cSCC will be periodically updated with the most recent published data. The improvements in staging systems leads to more precise risk stratification and identification of aggressive cSCC, allowing for an accurate framework for management based on these risk levels.[42-44] Overall, due to higher risk of immunosuppression, it is most important to keep in mind that close and regular inspection of the skin with adequate and rapid diagnosis and treatment of cSCC is important to prevent metastasis in OTR.[45]

References

1. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? DermatolSurg. 2013;39(1 Pt 1):1-23.
2. Andersson K, Michael KM, Luostarinen T, Waterboer T, Gislefoss R, Hakulinen T,... Dillner J. Prospective study of human papillomavirus seropositivity and risk of nonmelanoma skin cancer. AmJEpidemiol. 2012;175(7):685-95.
3. Plasmeijer EI, Pandeya N, O'Rourke P, Pawlita M, Waterboer T, Feltkamp MC,... Neale RE. The Association between cutaneous squamous cell carcinoma and Betapapillomavirus seropositivity: a cohort study. Cancer EpidemiolBiomarkers Prev. 2011;20(6):1171-7.
4. Casabonne D, Michael KM, Waterboer T, Pawlita M, Forslund O, Burk RD,... Newton R. A prospective pilot study of antibodies against human papillomaviruses and cutaneous squamous cell carcinoma nested in the Oxford component of the European Prospective Investigation into Cancer and Nutrition. IntJCancer. 2007;121(8):1862-8.
5. Paradisi A, Waterboer T, Sampogna F, Tabolli S, Simoni S, Pawlita M, Abeni D. Seropositivity for human papillomavirus and incidence of subsequent squamous cell and basal cell carcinomas of the skin in patients with a previous nonmelanoma skin cancer. BrJDermatol. 2011;165(4):782-91.
6. Iannacone MR, Gheit T, Waterboer T, Giuliano AR, Messina JL, Fenske NA,... Rollison DE. Case-control study of cutaneous human papillomaviruses in squamous cell carcinoma of the skin. Cancer Epidemiol-Biomarkers Prev. 2012;21(8):1303-13.
7. Waterboer T, Abeni D, Sampogna F, Rother A, Masini C, Sehr P,... Pawlita M. Serological association of Beta and Gamma human papillomaviruses with squamous cell carcinoma of the skin. BrJDermatol. 2008;159(2):457-9.
8. Bouwes Bavinck JN, Neale RE, Abeni D, Euvrard S, Green AC, Harwood CA,... Feltkamp MC. Multicenter study of the association between Betapapillomavirus infection and cutaneous squamous cell carcinoma. Cancer Res. 2010;70(23):9777-86.
9. Harwood CA, Toland AE, Proby CM, Euvrard S, Hofbauer GFL, Tommasino M,... KeraCon C. The pathogenesis of cutaneous squamous cell carcinoma in organ transplant recipients. Br J Dermatol. 2017;177(5):1217-24.
10. Quint KD, Genders RE, de Koning MN, Borgogna C, Gariglio M, Bouwes Bavinck JN,... Feltkamp MC. Human Beta-papillomavirus infection and keratinocyte carcinomas. J Pathol. 2015;235(2):342-54.
11. Bouwes Bavinck JN, Feltkamp MCW, Green AC, Fiocco M, Euvrard S, Harwood CA,... group E-H-U-C. Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study. Am J Transplant. 2018;18(5):1220-30.
12. Chahoud J, Semaan A, Chen Y, Cao M, Rieber AG, Rady P, Tyring SK. Association Between Beta-Genus Human Papillomavirus and Cutaneous Squamous Cell Carcinoma in Immunocompetent Individuals-A Meta-analysis. JAMA Dermatol. 2016;152(12):1354-64.
13. Hasche D, Stephan S, Braspenning-Wesch I, Mikulec J, Niebler M, Grone HJ,... Vinzon SE. The interplay of UV and cutaneous papillomavirus infection in skin cancer development. PLoS Pathog. 2017;13(11):e1006723.
14. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. JClinInvest. 2012;122(2):464-72.
15. Majores M, Bierhoff E. [Actinic keratosis, Bowen's disease, keratoacanthoma and squamous cell carcinoma of the skin]. Pathologe. 2015;36(1):16-29.
16. Gupta G, Madan V, Lear JT. Squamous Cell Carcinoma and its Precursors In: Griffiths C, Barker J, Bleiker T, Chalmers R, Creamer D, editors. Rook's Textbook of Dermatology. 3rd ed. New Jersey: Wiley; 2016. p. 142.1-.42.
17. O'Toole EA, Pontén F, Lundeberg J, Asplund A. Pathogenesis of SCC. In: Bolognia JL, Schaffer JV, Cerroni L, editors. Dermatology. 3rd ed. Philadelphia: Elsevier Saunders; 2012. p. 1769-76.
18. Soyer HP, Rigel DS, Wurm EMT. Actinic keratosis, Squamous cell carcinoma in situ,

Squamous cell carcinoma. In: Bolognia JL, Schaffer JV, Cerroni L, editors. Dermatology. 3rd ed. Philadelphia: Elsevier Saunders; 2012. p. 1777-83.
19. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification--part two. JCutanPathol. 2006;33(4):261-79.
20. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part one. JCutanPathol. 2006;33(3):191-206.
21. Jenni D, Hofbauer GF. Keratinocyte cancer and its precursors in organ transplant patients. CurrProblDermatol. 2015;46:49-57.
22. Yosipovitch G, Mills KC, Nattkemper LA, Feneran A, Tey HL, Lowenthal BM,... D'Agostino RB, Jr. Association of pain and itch with depth of invasion and inflammatory cell constitution in skin cancer: results of a large clinicopathologic study. JAMA Dermatol. 2014;150(11):1160-6.
23. Frauenfelder SR, Freiberger SN, Bouwes Bavinck JN, Quint KD, Genders R, Serra AL, Hofbauer GF. Prostaglandin E2, Tumor Necrosis Factor Alpha, and Pro-opiomelanocortin Genes as Potential Mediators of Cancer Pain in Cutaneous Squamous Cell Carcinoma of Organ Transplant Recipients. JAMA Dermatol. 2017;153(3):350-2.
24. Venables ZC, Autier P, Nijsten T, Wong KF, Langan SM, Rous B,... Leigh IM. Nationwide Incidence of Metastatic Cutaneous Squamous Cell Carcinoma in England. JAMA Dermatol. 2018.
25. Nehal KS, Bichakjian CK. Update on Keratinocyte Carcinomas. N Engl J Med. 2018;379(4):363-74.
26. Lanz J, Bouwes Bavinck JN, Westhuis M, Quint KD, Harwood CA, Nasir S,... Hofbauer GFL. Aggressive Squamous Cell Carcinoma in Organ Transplant Recipients. JAMA Dermatol. 2018.
27. Haisma MS, Plaat BE, Bijl HP, Roodenburg JL, Diercks GF, Romeijn TR, Terra JB. Multivariate analysis of potential risk factors for lymph node metastasis in patients with cutaneous squamous cell carcinoma of the head and neck. J Am Acad Dermatol. 2016;75(4):722-30.
28. Thompson AK, Kelley BF, Prokop LJ, Murad MH, Baum CL. Risk Factors for Cutaneous Squamous Cell Carcinoma Recurrence, Metastasis, and Disease-Specific Death: A Systematic Review and Meta-analysis. JAMA Dermatol. 2016;152(4):419-28.
29. Rose AM, Nicoll KJ, Moinie A, Jordan DJ, Evans AT, Proby CM, Naasan A. Patients with low-risk cutaneous squamous cell carcinoma do not require extended out-patient follow-up. J Plast Reconstr Aesthet Surg. 2017;70(6):852-5.
30. Manyam BV, Garsa AA, Chin RI, Reddy CA, Gastman B, Thorstad W,... Koyfman SA. A multi-institutional comparison of outcomes of immunosuppressed and immunocompetent patients treated with surgery and radiation therapy for cutaneous squamous cell carcinoma of the head and neck. Cancer. 2017;123(11):2054-60.
31. Eigentler TK, Leiter U, Hafner HM, Garbe C, Rocken M, Breuninger H. Survival of Patients with Cutaneous Squamous Cell Carcinoma: Results of a Prospective Cohort Study. The Journal of investigative dermatology. 2017;137(11):2309-15.
32. Schmidt SA, Hamilton-Dutoit SJ, Farkas DK, Steiniche T, Sorensen HT. Human papillomavirus and the incidence of nonmelanoma and melanoma skin cancer using cervical conization as a surrogate marker: a nationwide population-based Danish cohort study. Ann Epidemiol. 2015;25(4):293-6 e2.
33. Garrett GL, Lowenstein SE, Singer JP, He SY, Arron ST. Trends of skin cancer mortality after transplantation in the United States: 1987 to 2013. J Am Acad Dermatol. 2016;75(1):106-12.
34. McDowell LJ, Tan TJ, Bressel M, Estall V, Kleid S, Corry J, Johnston ML. Outcomes of cutaneous squamous cell carcinoma of the head and neck with parotid metastases. J Med Imaging Radiat Oncol. 2016;60(5):668-76.
35. Vinzon SE, Rosl F. HPV vaccination for prevention of skin cancer. Hum Vaccin Immunother. 2015;11(2):353-7.
36. Pfister H. Association between Betapapillomavirus seropositivity and keratinocyte carcinoma-prospects for prophylactic vaccination? The Journal of investigative dermatology. 2015;135(5):1211-3.
37. Ruiz ES, Karia PS, Morgan FC, Schmults CD. The positive impact of radiologic imaging

on high-stage cutaneous squamous cell carcinoma management. J Am Acad Dermatol. 2017;76(2):217-25.
38. Cousen P, Chow K, Colver G. What is the role of ultrasound evaluation of lymph nodes in patients with high-grade squamous cell carcinoma of the head and neck? BrJ Dermatol. 2010;162(5):1146-7.
39. Genders RE, Bouwes Bavinck JN. Sentinel lymph node biopsy for cutaneous squamous cell carcinoma: valuable or not valuable? Br J Dermatol. 2015;172(4):854-5.
40. Roscher I, Falk RS, Vos L, Clausen OPF, Helsing P, Gjersvik P, Robsahm TE. Validating 4 Staging Systems for Cutaneous Squamous Cell Carcinoma Using Population-Based Data: A Nested Case-Control Study. JAMA Dermatol. 2018;154(4):428-34.
41. Baum CL, Wright AC, Martinez JC, Arpey CJ, Brewer JD, Roenigk RK, Otley CC. A new evidence-based risk stratification system for cutaneous squamous cell carcinoma into low, intermediate, and high risk groups with implications for management. J Am Acad Dermatol. 2018;78(1):141-7.
42. Bean DJ, Rees RS, O'Leary JP, Lynch JB. Carcinoma of the hand: a 20-year experience. South MedJ. 1984;77(8):998-1000.
43. Que SKT, Zwald FO, Schmults CD. Cutaneous squamous cell carcinoma: Management of advanced and high-stage tumors. J Am Acad Dermatol. 2018;78(2):249-61.
44. Work G, Invited R, Kim JYS, Kozlow JH, Mittal B, Moyer J,... Rodgers P. Guidelines of care for the management of cutaneous squamous cell carcinoma. J Am Acad Dermatol. 2018;78(3):560-78.
45. Acuna SA, Huang JW, Scott AL, Micic S, Daly C, Brezden-Masley C,... Baxter NN. Cancer Screening Recommendations for Solid Organ Transplant Recipients: A Systematic Review of Clinical Practice Guidelines. Am J Transplant. 2017;17(1):103-14.



CHAPTER 9

Nederlandse samenvatting
List of abbreviations
List of publications
Curriculum Vitae
Dankwoord

Nederlandse samenvatting

Het plaveiselcelcarcinoom (PCC) van de huid is een huidkanker die ontstaat in de keratinocyten (hoorn vormende cellen), die de opperhuid vormen. Het is de op één na meest voorkomende vorm van huidkanker na het basaalcelcarcinoom (BCC). Een op de 15 Nederlanders krijgt een PCC. In 2018 waren dat bijna 15.000 personen.
Orgaantransplantatie patiënten hebben een orgaan van een donor ontvangen en krijgen immuun onderdrukkende medicijnen om afstoting hiervan te voorkomen. Echter door het gebruik van deze medicijnen is het risico op het ontstaan van PCC fors verhoogd in vergelijking met de normale populatie. De afdeling dermatologie in het Leids Universitair Medisch Centrum is al meer dan 25 jaar gespecialiseerd in de behandeling van huidkanker bij orgaantransplantatie patiënten..
Dit proefschrift behandelt verschillende aspecten van PCC in orgaantransplantatie patiënten.
Meerdere factoren geven een verhoogd risico op de ontwikkeling van PCC. De belangrijkste is langdurige zonblootstelling. Daarnaast spelen andere factoren een rol, zoals mannelijk geslacht, oudere leeftijd, roken, chronische zweren, virusinfectie en immuunsysteem onderdrukkende medicatie.
Er zijn aanwijzingen dat infectie met een HPV (humaan papillomavirus) van het bèta type een rol speelt bij de ontwikkeling van het PCC. Bij een actieve virale infectie kan door UV-licht veroorzaakte DNA schade minder goed gerepareerd worden, waardoor de huidcellen kankercellen kunnen worden. Ook wordt de door UV geïnduceerde gereguleerde celdood (apoptose) geremd. De wetenschappelijke literatuur is niet eenduidig over deze rol van HPV, waardoor er binnen de wetenschap een groep “believers” en “non-believers” aanwezig is. **Hoofdstuk 2** geeft een overzicht van HPV en de epidemiologie van HPV-infectie gerelateerd aan huidkanker, met de nadruk op HPV als risicofactor voor PCC in orgaantransplantatie patiënten. **Hoofdstuk 3** beschrijft een retrospectief vervolgonderzoek om vast te stellen of er een verband bestaat tussen de aanwezigheid van antilichamen tegen HPV aangetoond in het bloed rondom de transplantatie en de ontwikkeling van huidkanker in de jaren erna (maximaal 22 jaar). Orgaantransplantatie patiënten die rondom de transplantatie een positieve bloedtest (serologie) voor HPV van het Beta type (Beta-PV) toonden, hadden een bijna 3 keer verhoogd risico op het krijgen van huidkanker. Dit gold zowel voor het PCC als het BCC. Het risico om PCC en BCC te ontwikkelen in orgaantransplantatie patiënten, was in de groep die HPV seropositief was getest

rond het tijdstip van transplantatie 2,9 keer zo groot als in orgaantransplantatie patiënten die HPV-seronegatief waren. Voor beide tumoren afzonderlijk was het risico op het ontstaan op PCC 2,9 keer zo groot en voor BCC 3,1 keer zo groot.
Het PCC kan ontstaan uit voorlopers van huidkanker zoals actinische keratose en de ziekte van Bowen (BD; carcinoma in situ). De klassieke presentatie van een PCC is die van een huidkleurige tot rode, goed afgrensbare tumor met centraal een wondje of verhoorning. Het PCC komt meestal voor op aan zon blootgestelde huid, zoals de hoofdhuid, oren, lippen, de handruggen en de onderbenen. De omliggende huid vertoont vaak duidelijke zonneschade. Soms lijkt een PCC echter op een voorloper, meer als een vlakke rode verhoornde plek. De orgaantransplantatie patiënten hebben meestal een erg onrustige huid met veel verhoornde en wratachtige plekken, waardoor het onderscheid met PCC nog moeilijker te maken is. Om de diagnose PCC vast te stellen wordt er weefsel onder de microscoop bekeken. Echter soms kan het lastig zijn om onderscheid te maken tussen een PCC of een voorloper hiervan.
Het kan soms een uitdaging zijn om onderscheid te maken tussen de verschillende keratinocyt afwijkingen. Omdat orgaantransplantatie patiënten talrijke types van keratinocyt afwijkingen ontwikkelen (zoals wratten, goedaardige tumoren van de opperhuid en voorlopers van PCC) en de kwaadaardige laesies zo vroeg mogelijk moeten worden geïdentificeerd, is er behoefte aan additionele diagnostische markers waarmee er onderscheid gemaakt kan worden tussen PCC en andere keratinocyt afwijkingen.

In **hoofdstuk 4** hebben we p16 immunohistochemische kleuringspatronen onderzocht in verschillende goedaardige en kwaadaardige huidlaesies. Hiervoor werden 59 actinische keratoses, 51 biopten van de ziekte van Bowen, 63 PCC en 16 goedaardige tumoren van 31 orgaantransplantatie patiënten en 25 controle huidstukjes van eczeem- en psoriasispatiënten onderzocht.
P16 is een tumorsuppressor eiwit. Er zijn aanwijzingen dat UV-straling en immuunsuppressie, die beide zijn geassocieerd met verhoogd risico op PCC, leiden tot verhoogde expressie van p16. Eerdere studies met behulp van kleuring voor p16 in huidkanker hebben wisselende resultaten opgeleverd en er zijn maar weinig rapporten over huidkanker in orgaantransplantatie patiënten. We vonden een geleidelijke toename van p16 kleuring van actinische keratose tot PCC. Het meest prominente kleuringspatroon, door de gehele epidermis, werd waargenomen in de ziekte van Bowen (ook wel plaveiselcelcarcinoom in situ genoemd). Helaas hebben

we geen onderscheidende patronen van p16 kleuring gevonden die kunnen worden gebruikt voor het onderscheid tussen PCC en actinische keratosen.
Om in de spreekkamer mogelijk al onderscheid te maken tussen goedaardige en kwaadaardige huidafwijkingen hebben we ook klinische factoren onderzocht. In **hoofdstuk 5** wordt een studie beschreven, waaraan verschillende centra uit Europa hebben meegedaan. In dit onderzoek werd gekeken welke waarschuwingssignalen bruikbaar zijn om PCC te onderscheiden van andere afwijkingen voor vroege diagnose en behandeling. Hiervoor werden 812 laesies in 410 orgaantransplantatie patiënten onderzocht, verspreid over 10 centra in Europa en de Verenigde Staten.
We hebben geconstateerd dat pijn een krachtig waarschuwingssignaal is voor PCC in orgaantransplantatie patiënten. Vooral bij patiënten met talrijke huidlaesies kan pijn behulpzaam zijn bij het differentiëren van PCC van goedaardige keratinocyt laesies.
Het PCC kan metastasen (uitzaaiingen) geven, meestal naar de lymfeklieren maar ook naar andere organen zoals de longen. De kans op metastase van het PCC is variabel. De kans hierop is ongeveer 1-10%, wat kan oplopen tot wel 30 tot 40 % in PCC met een hoog risico. Risicofactoren zijn grote tumoren, terugkerende tumoren, diepe ingroei in andere onderliggende weefsels, locatie op de lip of het oor, slechte differentiatiegraad en groei in vaten en zenuwen.
Het is echter niet geheel duidelijk hoe groot de kans op metastase is van een PCC bij orgaantransplantatie patiënten. In de literatuur wordt vaak vermeld dat PCC in orgaantransplantatie patiënten vaak een agressiever gedrag lijkt te vertonen, vaker metastaseert en een slechtere uitkomst heeft vergeleken met immuuncompetente patiënten (patiënten zonder immuunsuppressie).
In **hoofdstuk 6** hebben we de literatuur over het risico op metastasen in de normale versus orgaantransplantatie patiënten -populatie onderzocht. Dit systematische review vond voor PCC patiënten een risico op metastasering van 7,3% in orgaantransplantatie patiënten en een risico van 3,1% in de immuuncompetente patiënten. Voor het risico van PCC in het hoofdhals gebied was dit respectievelijk 11,0% en 8,5%. Het risico per SCC lag in de groep van orgaantransplantatie patiënten iets lager dan in die van de immuuncompetente patiënten, omdat orgaantransplantatie patiënten vaak meer PCC ontwikkelen.
Deze resultaten zijn conform een grote recent gepubliceerde studie in Engeland waar patiënten met immuunsuppressie een tweevoudig hoger risico op metastasering hadden, waarbij het algemene risico wel lager lag (1.1% voor vrouwen en 2.4% bij mannen).

We onderzochten ook het risico op metastase bij onze eigen patiënten. Van alle PCC in een periode van 10 jaar werd onderzocht of deze tot metastase had geleid. De resultaten werden opgesplitst in twee groepen: 134 orgaantransplantatie patiënten en 459 patiënten immuuncompetente patiënten. Er waren 8 orgaantransplantatie patiënten en 15 immuuncompetente patiënten die een metastase ontwikkelden. Het risico voor orgaantransplantatie patiënten op het krijgen van een metastase was 1,6 keer zo hoog (dus met 60% verhoogd). Het lage aantal metastasen in de orgaantransplantatie patiënten groep is mogelijk te danken aan het feit dat deze patiënten regelmatig voor controle worden gezien op een gespecialiseerde poli en nieuwe tumoren zodoende in een vroeg stadium behandeld kunnen worden. Risicofactoren die bij orgaantransplantatie patiënten werden gevonden voor het ontwikkelen van een metastase was lokalisatie van het PCC in het hoofdhals gebied, oudere leeftijd bij transplantatie en oudere leeftijd bij het krijgen van een eerste PCC. Opvallend genoeg waren de tumoren die metastaseerden in de meeste gevallen kleiner dan 2 cm. Een tumor groter dan 2 cm is namelijk een bekende risicofactor. Zo vonden wij ook in onze immuuncompetente patiënten dat tumor grootte en tumordiepte de belangrijkste risicofactoren waren voor het krijgen van een metastase.

Ons onderzoek is de eerste studie die een duidelijk onderscheid maakt tussen orgaantransplantatie patiënten en immuuncompetente patiënten. Het sluit aan bij resultaten van een grote literatuurstudie met meta-analyse waarbij werd gevonden dat immuunsuppressie een risicofactor is voor metastasering.

Omdat het ontstaan van huidkanker nog steeds toeneemt, is het duidelijk dat preventie en vroege opsporing en behandeling van huidkankers belangrijk is. In de toekomst is het misschien mogelijk dat vaccinatie tegen Beta-PV kan helpen bij het voorkomen van de ontwikkeling van PCC in orgaantransplantatie patiënten, maar tot nu toe zijn er geen studies bij mensen uitgevoerd om deze mogelijkheid te onderzoeken.

De uitdaging in de dagelijkse praktijk is het identificeren van hoog risico PCC en het zo snel mogelijk detecteren van een metastase, zowel in populaties met een laag als met een hoog risico.

Bestudering van mogelijke markers, die een duidelijk onderscheid kunnen maken tussen goedaardige, voorlopers van en kwaadaardige plaveiselceltumoren, kan in de toekomst behulpzaam zijn bij het nemen van de juiste beslissing voor de beste behandeling. In de klinische praktijk is pijn een nuttige indicator om PCC te onderscheiden van goedaardige laesies. Vroege detectie en behandeling van PCC

voorkomt dat het dieper gaat groeien en verlaagt daarom het risico op uitzaaiingen. Vroegtijdige interventie voor metastasen kan een overlevingsvoordeel opleveren. Recent is een nieuw, risico-stratificatiesysteem voor laag-, middelgroot- en hoog risico PCC voorgesteld om risicostratificatie van PCC beter mogelijk te maken. Dit systeem is niet alleen gebaseerd op tumoreigenschappen, maar bevat ook andere risicofactoren, waaronder immuunsuppressie. Deze verbeteringen leiden tot een preciezere risicostratificatie en identificatie van agressieve PCC. Over het algemeen is het, vanwege het hogere risico van immuunsuppressie, het belangrijkst om in gedachten te houden dat nauwgezette en regelmatige controle van de huid met adequate en snelle diagnose en behandeling van PCC het belangrijkste is om metastase in orgaantransplantatie patiënten te voorkomen.

List of abbreviations

AJCC	American Joint Committee on Cancer
AK	actinic keratosis
BCC	basal cell carcinoma
BD	Bowen's disease
BKL	benign keratotic lesions
CIN	cervical intraepithelial neoplasia
(c)SCC	(cutaneous) squamous cell carcinoma
CT	computed tomography
ELISA	enzyme-linked immunoassay
ESR	European standardised rates
EV	epidermodysplasia verruciformis
FISH	fluorescent DNA in situ hybridization
HPV	human papillomavirus
IARC	International Agency for Research on Cancer
IC	immunocompetent
IR(R)	incidence rate (ratio)
IS	immunosuppression/immunosuppressed
ITSCC	International immunosuppression & Transplant Skin Cancer Collaborative
KA	keratoacanthoma
KC	keratinocyte carcinoma
KIN	keratinocytic intraepidermal neoplasia
MeTIMP	methyl-thioinosine monophosphate
MOOSE	Meta-analysis Of Observational Studies in Epidemiology
MMF	Mycophenolate mofetil
MRI	magnetic resonance imaging
mTOR	mammalian target of rapamycin
ORF	open reading frame
OTR	organ transplant recipient
PET	positron emission tomography
Rb	retinoblastoma gene
ROC	Receiver-operating characteristic
RT	radiotherapy

SCOPE	Skin Care in Organ Transplant Patients Europe
TGF	transforming growth factor
TNM	Tumor Nodes Metastasis
UV	ultraviolet
UICC	International Union Against Cancer
VAS	visual analogue scale
VLP	virus like particle

List of publications

Marsidi N, **Genders RE**. Tunneled transposition flap for conchal defect. J Am Acad Dermatol. 2019;21. pii: S0190-9622(19)30451-7.

Genders RE, Weijns ME, Dekkers OM, Plasmeijer EI. Metastasis of cutaneoussquamous cell carcinoma in organ transplant recipients and the immunocompetent population: is there a difference? a systematic review and meta-analysis. J Eur Acad Dermatol Venereol. 2019;33(5):828-841.

van Kester MS, Goeman JJ, **Genders RE**. Tissue-sparing properties of Mohs micrographic surgery for infiltrative basal cell carcinoma. J Am Acad Dermatol. 2019;80(6):1700-1703.

Halk AB, Habbema L, **Genders RE**, Hanke CW. Safety Studies in the Field of Liposuction: A Systematic Review. Dermatol Surg. 2019;45(2):171-182.

van Loenen D, **Genders RE**. Surgical Pearl: Use of surgical scrub sponge for auricular dressing. J Am Acad Dermatol. 2018;24. pii: S0190-9622(18)33108-6.

Lanz J, Bouwes Bavinck JN, Westhuis M, Quint KD, Harwood CA, Nasir S, Van-de-Velde V, Proby CM, Ferrándiz C, **Genders RE**, Del Marmol V, Forchetti G, Hafner J, Vital DG, Hofbauer GFL. Aggressive Squamous Cell Carcinoma in Organ Transplant Recipients. JAMA Dermatol. 2019;155(1):66-71.

Haenen CCP, Buurma AAJ, **Genders RE**, Quint KD. Squamous cell carcinoma arising in hypertrophic lichen planus. BMJ Case Rep. 2018;2018. pii: bcr-2017-224044.

van Kester MS, **Genders RE**, Quint KD. Lymphangitis carcinomatosa of a cutaneous squamous cell carcinoma. BMJ Case Rep. 2018. pii: bcr-2017-222923.

Genders RE, Osinga JAJ, Tromp EE, O'Rourke P, Bouwes Bavinck JN, Plasmeijer EI. Metastasis Risk of Cutaneous Squamous Cell Carcinoma in Organ Transplant Recipients and Immunocompetent Patients. Acta Derm Venereol. 2018;98(6):551-555. doi: 10.2340/00015555-2901.

Bouwes Bavinck JN, Feltkamp MCW, Green AC, Fiocco M, Euvrard S, Harwood CA, Nasir S, Thomson J, Proby CM, Naldi L, Diphoorn JCD, Venturuzzo A, Tessari G, Nindl I, Sampogna F, Abeni D, Neale RE, Goeman JJ, Quint KD, Halk AB, Sneek C, **Genders RE**, de Koning MNC, Quint WGV, Wieland U, Weissenborn S, Waterboer T, Pawlita M, Pfister H; EPI-HPV-UV-CA group. Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study. Am J Transplant. 2018;18(5):1220-1230.

van Kester MS, **Genders RE**. Novel uses of bilateral advancement flaps for tip defects on the nose: Part I. J Am Acad Dermatol. 2018;78(4):e91-e92.

Genders RE, van Kester MS. Novel uses of bilateral advancement flaps for tip defects on the nose: Part II. J Am Acad Dermatol. 2018;78(4):e93-e94.

van der Kolk T, Dillingh MR, Rijneveld R, Klaassen ES, de Koning MNC,Kouwenhoven STP, **Genders RE**, Bouwes Bavinck JN, Feiss G, Rissmann R, Burggraaf J. Topical ionic contra-viral therapy comprised of digoxin and furosemide as a potential novel treatment approach for common warts. J Eur Acad Dermatol Venereol. 2017; 31(12):2088-2090

Kouwenhoven ST, van Kester MS, **Genders RE**. The wingman flap: Bilateral closure of a supra-tip defect of the nose. J Am Acad Dermatol. 2017;76(2):e57-e58.

Frauenfelder SR, Freiberger SN, Bouwes Bavinck JN, Quint KD, **Genders R**, Serra AL, Hofbauer GF. Prostaglandin E2, Tumor Necrosis Factor α, and Pro-opiomelanocortin Genes as Potential Mediators of Cancer Pain in Cutaneous Squamous Cell Carcinoma of Organ Transplant Recipients. JAMA Dermatol. 2017;153(3):350-352.

Genders RE, Beck S, Bouwes Bavinck JN, van den Munckhof HA, Kouwenhoven ST, de Koning MN, de Gruijl FR, Jenkins D, Willemze R, Quint KD. p16 Immunostaining in keratinocytic neoplasia in organ transplant recipients: Bowen's disease shows a characteristic pattern. J Cutan Pathol. 2017 Jan;44(1):28-33. doi: 10.1111/cup.12840.

Tromp EE, Kouwenhoven ST, Quint KD, Gmelig Meijling KA, **Genders RE** Unilateral scrotal angiomas: An expression of underlying varicocele.. Scand J Urol. 2016;50(1): 88-9.

Genders RE, Kuizinga MC, Teune TM, van der Kruijk M, van Rengen A. Does biopsy accurately assess basal cell carcinoma (BCC) subtype? J Am Acad Dermatol. 2016;74(4):758-60.

Mutsaers ER, **Genders R,** van Es N, Kukutsch N. Dermoscopy of glomus tumor: More white than pink. J Am Acad Dermatol. 2016;75(1):e17-8.

Genders R. 'Dokter, ik zweet te veel': hyperhidrose in de praktijk Huisarts Wet 2015;58(9):492-5.

Genders RE, Bouwes Bavinck JN. Sentinel lymph node biopsy for cutaneous squamous cell carcinoma: valuable or not valuable? Br J Dermatol. 2015;172(4):854-5.

Genders RE, Mazlom H, Michel A, Plasmeijer EI, Quint KD, Pawlita M, van der Meijden E, Waterboer T, de Fijter H, Claas FH, Wolterbeek R, Feltkamp MC, Bouwes Bavinck JN. The presence of betapapillomavirus antibodies around transplantation predicts the development of keratinocyte carcinoma in organ transplant recipients: a cohort study. J Invest Dermatol. 2015;135(5):1275-82.

Gmelig Meijling KA, van Rengen AM, van den Eerenbeemt AMM, **Genders RE**. Mohs-chirurgie, Mohs' chirurgie of mohschirurgie? NTvDV 2014;24(08):483

Genders RE, Vos LE. Pseudochromhidrosis bij axillaire open comedonen. NTvDV,2014;24(06):419.

Quint KD, **Genders RE**, de Koning MN, Borgogna C, Gariglio M, Bouwes Bavinck JN, Doorbar J, Feltkamp MC. Human Beta-Papillomavirus Infection and Keratinocyte Carcinomas. J Pathol. 2015;235(2):342-54.

Houtman CJ, **Genders RE**, von dem Borne PA, Vermeer MH. [Skin disorders associated with monoclonal gammopathies]. Ned Tijdschr Geneeskd. 2014;158:A6717.

Borgogna C, Landini MM, Lanfredini S, Doorbar J, Bouwes Bavinck JN, Quint KD, de Koning MN, **Genders RE**, Gariglio M. Characterization of skin lesions induced by skin-tropic α- and β-papillomaviruses in a patient with epidermodysplasia verruciformis. Br J Dermatol. 2014;171(6):1550-4.

Genders RE, Spitaels D, Jansen CL, van den Akker TW, Quint KD. A misleading urethral smear with polymorphonuclear leucocytes and intracellular diplococci; case report of urethritis caused by Neisseria meningitidis. J Med Microbiol. 2013;62(Pt 12):1905-6.

Bouwes Bavinck JN, Harwood CA, **Genders RE**, Wisgerhof HC, Plasmeijer EI, Mitchell L, Olasz EB, Mosel DD, Pokorney MS, Serra AL, Feldmeyer L, Baumann Conzett K, Piaserico S, Belloni Fortina A, Jahn K, Geusau A, Gerritsen MJ, Seckin D, Gulec AT, Cetkovska P, Ricar J, Imko-Walczuk B, Proby CM, Hofbauer GF. Pain identifies squamous cell carcinoma in organ transplant recipients: the SCOPE-ITSCC PAIN study. Bouwes Am J Transplant. 2014;14(3):668-76.

Genders RE, Quint KD, van Leeuwen RL. A woman with a "bruise" on the face. Ned Tijdschr Geneeskd. 2013;157(4):A5456.

Franken SM, **Genders RE,** de Gruijl FR, Rustemeyer T, Pavel S. Skin hardening effect in patients with polymorphic light eruption: comparison of UVB hardening in hospital with a novel home UV-hardening device. J Eur Acad Dermatol Venereol. 2013;27(1):67-72.

Genders RE, Hamminga EA, Kukutsch NA. Securing the subcuticular running suture. Dermatol Surg. 2012;38(10):1722-4.

Quint KD, **Genders RE**, Vermeer MH. A delayed granulomatous reaction to a cosmetic tattoo of the eyebrows: a report of total regression after intralesional corticosteroid injections. Dermatol Surg. 2012;38(6):951-3.

RE Genders, PPGM Kouwenberg, RP Bleichrodt. Abdominal wall repair with human acellular dermal autograft (HADA): case report and review of the literature. Surgical Techniques Development; 2011; 2(1)

Genders RE, Vermeer MH, van Doorn R. Erythematous and purpuric plaques of the face and scalp in Sweet syndrome. Br J Haematol. 2011;154(2):159

Genders RE, Bonsing BA, van der Molen AJ, Lavrijsen APM. A red and blue multinodular tumor of the umbilicus. Image of the month-quiz case. Arch Surg. 2011; 146(3):361

Genders RE, Hamminga EA, Kukutsch NA. Autoimflammatoire aandoeningen; het Blau syndroom en Familiaire mediterrane koorts. NTvDV, 2010; 20(5):251-257

Genders RE, Basdew VR, Pavel S. Panniculitis bij alfa-1-antitrypsine deficiëntie. NTvDV, 2010; 20(6):318-319

Genders RE, Meer SV, Kouwenberg PP. Dupuytren's disease in a Chinese patient. J Hand Surg Eur Vol. 2008;33(4):533-4

Genders R, Meester HH, Hekker TA, Ritt MJ. Mepitel®: re-application after rinsing? J Hospital Infection, 2006; 64(4):40-8

Bakker J, **Genders R**, Mali W, Leenen L. Sonography as the primary screening method in evaluating blunt abdominal trauma. J Clinical Ultrasound, 2005; 33(4):155-63.

Curriculum Vitae

Roel Genders is geboren op 28 januari 1980 in Eindhoven. Na het behalen van zijn VWO diploma aan het Lorentz Lyceum in Eindhoven, begon hij dat zelfde jaar met de studie geneeskunde aan de Universiteit Utrecht. Naast zijn studie heeft hij altijd een of meerdere bijbanen gehad, waarvan die bij het trialbureau neurologie van het UMCU de eerste was waar hij voor het eerst in aanraking kwam met wetenschappelijk onderzoek. Gedurende zijn wetenschapsstage op de afdeling radiologie van het UMCU deed hij onderzoek naar de diagnostische waarde van echografie bij stomp buiktrauma. Na het behalen van zijn artsexamen in december 2004 werkte hij als AGNIO in een aantal perifere ziekenhuizen. In september 2008 startte hij met zijn opleiding tot dermatoloog in het LUMC (opleider prof. Dr. R Willemze). In de laatste jaren van zijn opleiding startte hij met zijn onderzoek betreffende plaveiselcelcarcinomen bij transplantatie patiënten (Dr. J.N. Bouwes Bavinck), waar de basis werd gelegd voor dit proefschrift. Na het afronden van zijn opleiding tot dermatoloog in 2013 ging hij werken in de Roosevelt kliniek en bleef hij werkzaam in het LUMC. Zijn promotiettraject ging toen officieel van start (promotor prof. Dr. R. Willemze, copromotoren Dr. J.N. Bouwes Bavinck en Dr. K.D. Quint) wat uiteindelijk heeft geleid tot dit proefschrift. Na voltooiing van zijn promotie zal hij wetenschappelijk onderzoek blijven doen op het gebied van de dermatochirurgie en de dermato-oncologie.

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