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

Genders, R.E.

CHAPTER 5

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

Am J Transplant. 2014;14(3):668-76

J.N. Bouwes Bavinck[1], C.A. Harwood[2], R.E. Genders[1], H.C. Wisgerhof[1], E.I. Plasmeijer[1], L. Mitchell[2], E.B. Olasz[3], D.D. Mosel[3], M.S. Pokorney[3], A.L. Serra[4], L. Feldmeyer[4], K. Baumann Conzett[4], S. Piaserico[5], A. Belloni Fortina[5], K. Jahn[6], A. Geusau[6], M.J.P. Gerritsen[7], D. Seçkin[8], T. Güleç[8], P. Cetkovská[9], J. Ricar[9], B. Imko-Walczuk[10], C.M. Proby[11], G.F.L. Hofbauer[4]

[1] Department of Dermatology, Leiden University Medical Center, Leiden, the Netherlands, [2] Department of Dermatology, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK. [3] Department of Dermatology, Medical College of Wisconsin, Milwaukee, USA. [4] Departments of Dermatology and Nephrology (A.L.S.), University Hospital Zürich, Zürich, Switzerland. [5] Dermatology Unit, Department of Medicine, University of Padua, Padua, Italy. [6] Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases (DIAID), Medical University of Vienna, Vienna, Austria. [7] Department of Dermatology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands. [8] Department of Dermatology, Başkent University Faculty of Medicine, Ankara, Turkey. [9] Department of Dermatology, University Hospital, Pilsen, Czech Republic. [10] Department of Dermatology, Pomeranian Trauma Center, Gdansk, Poland. [11] Department of Dermatology, Division of Cancer Research, Medical Research Institute, College of Medicine, Dentistry, and Nursing, Ninewells Hospital, University of Dundee, Dundee, UK

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.

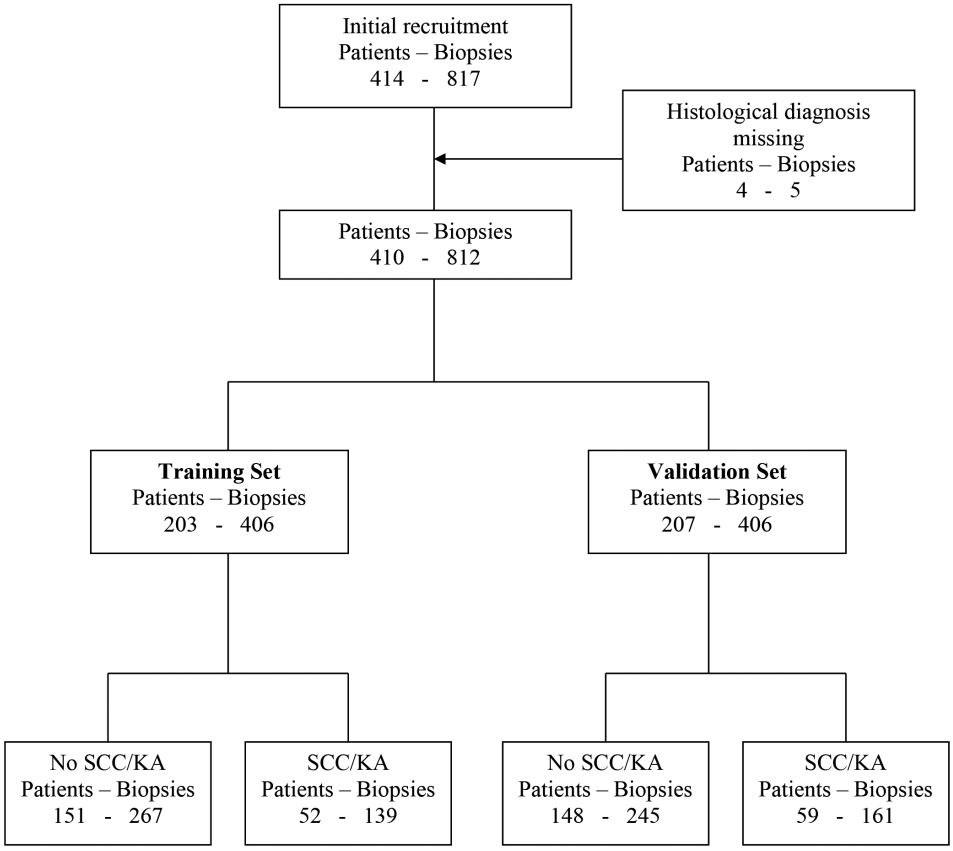

Figure 1. Study profile. SCC depicts invasive squamous cell carcinoma and KA keratoacanthoma.

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.

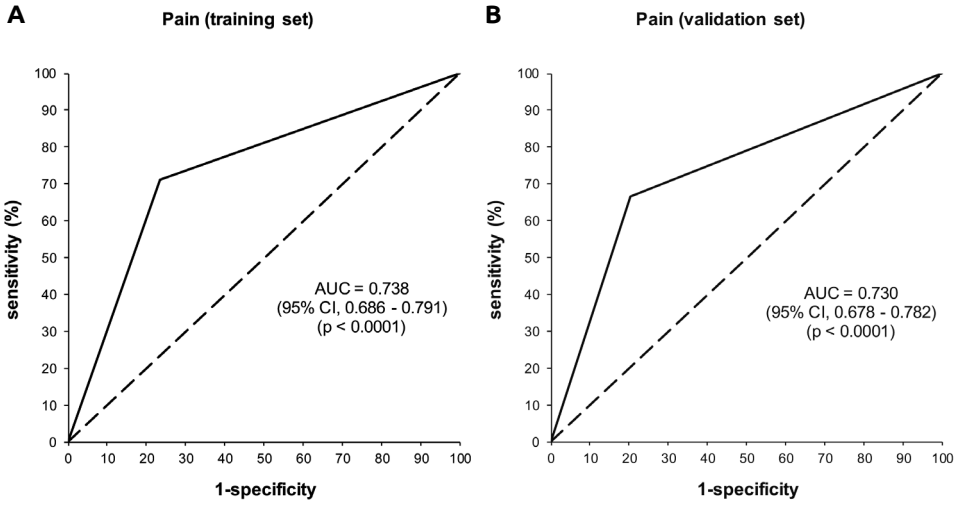

Figure 2. Receiver-operating-characteristic (ROC) curves indicating sensitivity and specificity of the self-reported symptom of pain (yes vs. no and on a visual analog scale) in the training (panel A) and validation (panel B) set to predict the histological presence of invasive squamous cell carcinoma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

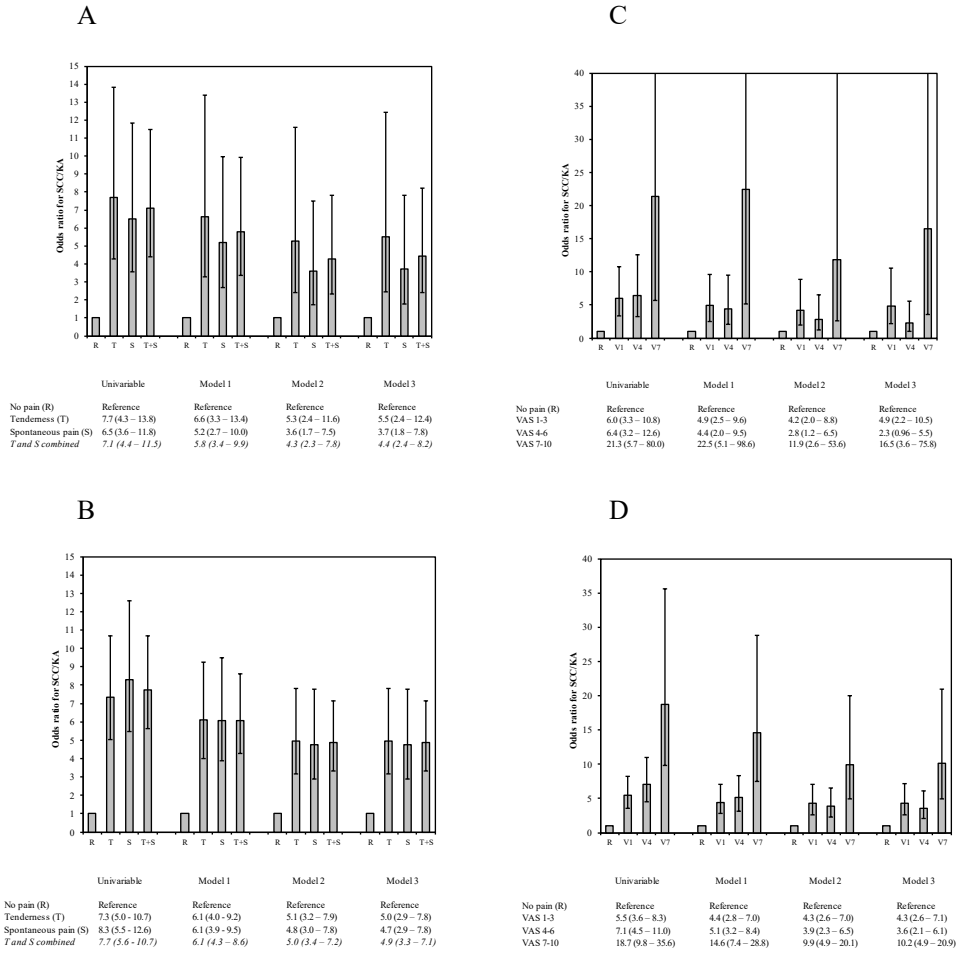

Figure 3. Univariable and multivariable adjusted odds ratios with 95% confidence intervals for squamous cell carcinoma or keratoacanthoma compared to no pain (R) according to tenderness (T), spontaneous pain (S) and the combination of tenderness and spontaneous pain (panels A and B) and according to 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.