Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma

Åsa Ingvar1, Karin Ekström Smedby2, Bernt Lindelöf3, Pia Fernberg1, Rino Bellocco1,4, Gunnar Tufveson2, Petter Höglund5 and Johanna Adami2

1Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Box 281, SE-171 77, 2Department of Medicine, Clinical Epidemiology Unit, Karolinska University Hospital, 3Department of Medicine, Dermatology and Venerology unit, Karolinska University Hospital, SE-171 76 Stockholm, Sweden, 4Department of Statistics, University of Milano-Bicocca, U7, 201 26 Milan, Italy, 5Department of Transplantation and Liver Surgery, Uppsala University Hospital, SE-751 85 Uppsala and 6Department of Microbiology, Tumour and Cell Biology, Karolinska Institutet, 171 77 Stockholm, Sweden

Correspondence and offprint requests to: Åsa Ingvar; E-mail: asa.odenbro@ki.se

Abstract

Background. The risk of cutaneous squamous cell carcinoma (CSCC) is found to be substantially increased after organ transplantation. The association with specific immunosuppressive regimens has been previously investigated, but results are not concordant. We aimed to clarify the relationship between separate immunosuppressive drugs, drug load, timing and risk of post-transplant CSCC.

Methods. A population-based nested case-control study was performed in the Swedish organ transplantation cohort (n = 5931). All patients who developed CSCC during the follow-up (1970–97) were eligible as cases (n = 207). Controls (n = 189) were randomly selected from the cohort and individually matched to the cases on follow-up time, age at and calendar period of transplantation. Exposure information was collected through extensive and standardized review of medical records.

Results. The median time to CSCC was 6.7 years. Post-transplant azathioprine (Aza) treatment considerably increased the risk of CSCC during all time periods analysed, and the risk augmented with increasing dose and duration. Patients who after the entire follow-up period had received a high accumulated dose of Aza had an 8.8-fold increased risk of CSCC in multivariate analysis (P < 0.0001), compared to patients never treated with Aza. Additionally, a high accumulated dose of corticosteroids during the same period conferred a 3.9-fold elevated risk of CSCC (P = 0.09), compared to the lowest accumulated dose of corticosteroids. Cyclosporine treatment was not associated with the risk of CSCC post-transplantation.

Conclusions. This study provides evidence that Aza treatment, but not cyclosporine treatment, is strongly associated with post-transplant CSCC risk. The results suggest that the risk of CSCC after organ transplantation is not only an effect of the immunosuppressive load per se.

Keywords: case-control study; cutaneous squamous cell carcinoma; immunosuppressive treatment; organ transplantation

Introduction

With improved survival after organ transplantation, the long-term complications have become evident, such as the increased risk of malignancies. Cutaneous squamous cell carcinoma (CSCC) is the most common malignancy in the post-transplantation period and is responsible for ~50% of the cancers arising de novo [1–3]. It has been shown repeatedly that the risk of CSCC increases with time after the transplantation. At 10 and 20 years following transplantation, cumulative incidences of CSCC of ~7% and 20%, respectively, have been reported in Europe [2]. The corresponding incidences in Australia are considerably higher, at 52% and 82%, reflecting the additional impact of sun exposure in this setting [4]. Compared to the general population, in which sun exposure is also a well-known risk factor for CSCC, studies have detected an up to 250-fold increased risk in organ transplant patients [2,5–7].

The markedly raised risk of CSCC in organ transplant recipients has often been attributed to the immunosuppression achieved by the increasingly effective pharmacological drugs. A role of specific drugs in skin carcinogenesis has been suggested in prior studies [4,8–12]. Nevertheless, the association is poorly elucidated, and previous results are not concordant. Most previous studies have crudely investigated the association with different immunosuppressive regimens, rather than with actual doses administered [7,8,13–17]. Several studies were also hampered by low statistical power to detect a difference between treatment regimens [10,14,16–20], and some studies did not clearly distinguish drugs included in treatment groups under comparison [3,8,15,16]. Although most previous studies have

© The Author 2009 Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please e-mail: journals.permissions@oxfordjournals.org
not identified differences in the risk of CSCC related to immuno-
suppressive regimen [3,13–15], some have found an increased risk in patients receiving triple treatment with aza-
thioprine (AzA)+cyclosporine (CsA)+corticosteroids (Cs) compared to double treatment with AzA+Cs [7,8,16]. Con-
sequently, several studies have concluded that an overall drug load confers the increased risk rather than a specific agent [7,13,14,18]. However, a few previous studies have presented evidence of a drug-dependent increased risk in both treatment with AzA [8–10] and with CsA [8,10].

In order to further clarify the relationship between the risk of CSCC and separate immuno-suppressive drugs, drug load and other transplant characteristics, we performed a population-based case-control study, nested in the large Swedish cohort of close to 6000 organ transplant individuals.

Methods

Swedish organ transplantation cohort

This study was performed within the Swedish organ transplantation cohort previously described by Adami et al. [2]. In short, the cohort consisted of 5931 patients, with no history of cancer, who underwent solid organ transplantation in Sweden from 1970 to 1997 and were registered in the Swedish National Patient Register. In this register, up to eight discharge diagnoses and 10 surgical codes are recorded for each patient discharge, and the geographical coverage gradually increased from 60% in 1969 to close to 100% of Sweden from 1987 onwards [21]. An evaluation of the validity of the register showed that 98% of the records had correct surgical codes [22]. In Sweden, organ transplantations are only performed in four public University hospitals (Karolinska, Malmö/Lund, Sahlgrenska, Uppsala)—therefore, the national registration of organ transplantations is population based. However, since two of the hospitals did not register or the National Patient Register before 1972, while the other two reported already from 1970, a few early organ transplantations (~50) were not included in the study [5].

Identification of CSCC cases and controls

The cohort of organ transplant patients was linked to the Swedish Can-
cer Register by employing the individually unique National registration number. In Sweden, both the diagnosing physician and the pathologist are required by law to report to the Cancer Registry which ensures recording of >98% of all incident tumours with a histological verification of 97% [21]. The seventh edition of International Classification of Diseases (ICD-7) code 191 was used for identifying incident nonmelanoma skin cancer (NMSC). Since basal cell carcinomas were not registered in the Cancer Register until after the termination of this study (starting in 2003), almost all NMSC in the register have been shown to represent CSCC [23]. All individuals in the cohort of transplant patients who developed CSCC (n = 242) as a first malignancy during the follow-up were eligible as cases. Controls were randomly chosen from the Swedish organ transplantation cohort and individually matched to the cases (1:1) by age (±5 years) and calendar period of transplantation (±5 years). The controls were also re-
quired to be alive and free from cancer at the time of the corresponding case CSCC diagnosis. Nineteen patients (3.9%) were excluded since they had an unregistered prior transplantation or because they died before the end of the follow-up, and two patients (0.4%) developed a cancer that was not registered in the Swedish Cancer Register. From all living cases and controls (n = 233), we requested a written, informed consent, which all but 14 patients (6%) approved. Of the 449 remaining patients, 53 patients (12%) were lost to the follow-up since their medical records could not be located. After these exclusions, the study consisted of 207 cases (88% of eligible cases) and 189 controls (84% of eligible controls).

Ethical guidelines

This study was approved by the Regional Ethics Review Board, Stockholm, Sweden (01-006).

Data collection

An extensive form was created for retrieving information in a standardized manner from the patient medical records. Data collection was carried out by trained personnel that were blinded for case/control status. Information recorded for all transplantations included age and sex of the recipient, type of organ transplanted, cause of organ failure, human leucocyte anti-
gens (HLA) and rejection episodes. Mismatch of HLA was defined as an antigen present in the donor but absent in the recipient. Additionally, we recorded the daily doses, administration forms (oral/intravenous) and all dose changes with dates of treatment initiation and termination of the follow-

ing drugs: AzA, CsA, CsA microemulsion, Cs (prednisolone, methyl-
prednisolone, hydrocortisone, betamethasone, cortisone), tacrolimus, mycophenolate motetil, sirolimus, cyclophosphamide, anti-thymocyte globulin (ATG), anti-lymphocyte globulin (ALG) and muromonab-CD3 (OKT-3).

Statistical methods

We combined generically similar drugs and converted different admin-
istration forms to reflect equal bioavailability. All Cs medications were standardized to the corresponding prednisolone dose. CsA microemulsion was standardized to CsA and orally administered drugs were transformed to correspond to 100% bioavailability (algorithms listed in Appendix 1) [24].

Drug doses were categorized according to the tertiles of the distribution among controls with never treatment as reference (Cs was divided in quartiles). To reflect a weighted total dose load of AzA+CsA+Cs treatment (restricted to patients who received all three drugs), we assigned reference values to the accumulated dose categories of each drug (reference = 0, low = 1, intermediate = 2, high = 3) and summed them up. Odds ratios and 95% confidence intervals (CI) were computed using conditional logistic regression models and were used as estimates of relative risk (RR). By using the conditional logistic regression model, adjustment was achieved for potential confounding by the matching factors age and calendar period at transplantation in intervals. We also checked for residual confounding by these factors through separate addition of age and calendar year of transplantation as continuous variables to the model, but RR estimates remained essentially unchanged and therefore the results of the original model are presented. Multivariate model building was based on purposeful selection criteria [25], relying on both statistical and biological relevance of factors considered. Potential risk factors and confounders were entered in a multivariate model and their effects were tested with likelihood ratio tests. Thereafter, all exposure variables were added, one at a time, to the multivariate conditional logistic regression model and tested accordingly for significance. The final model consisted of sex of the recipient and total accumulated dose of AzA, CsA and Cs. Analyses of combinations of drugs or drug doses in different treatment phases were not adjusted for the total accumulated dose of the same drug. Effect modification, on a multiplica-
tive scale, between two variables of AzA, CsA, Cs, sex and age was tested by including an interaction term in the model. Statistical analyses were carried out in Stata (StataCorp. 2003, Stata Statistical Software: Release 9.0. College Station, TX, USA).

Some cases and controls (n = 71) had lost their originally matched partner due to loss to follow-up or technical errors. These subjects were joined together in new pairs or entered into other risk sets if they fulfilled the original matching criteria (n = 18). In order to use the information from additional subjects without a matched partner, we applied slightly revised matching criteria (±10 years for age and calendar time and <60 days difference in the follow-up time) allowing another 34 participants to enter into existing risk sets. Analyses were performed both with and without the additional 52 participants and the results changed only marginally; therefore, the analyses including these subjects are reported.

Results

Selected characteristics of the 207 CSCC cases and 189 controls are displayed in Table 1. The median age at first transplantation (51 years) and the median follow-up time (6.6 years) were similar among cases and controls. Eighty-four percent had a single transplantation, and the absolute majority (95%) received a kidney graft. Study
participants who developed CSCC during the first 8 years post-transplantation were older than patients who developed CSCC after 8 years or more (median age 56 years and 41 years, respectively).

Tables 2 and 3 present crude RRs of CSCC in relation to transplant characteristics and immunosuppressive treatment. All patients were treated with Cs, in combination with either Aza or CsA or in triple treatment combination (Aza+CsA+Cs); only about 1% of the study participants were treated with either of tacrolimus, mycophenolate mofetil or sirolimus. Both triple treatment and ever use of Aza were associated with a statistically significant 5-fold increase in the risk of CSCC, compared with CsA+Cs and never use of Aza, respectively. Patients with a high accumulated dose combination of Aza, CsA and Cs were at a 4.6-fold increased risk compared with patients receiving a low dose combination (weighted dose score 8–9 versus 2–3). There was no significant association with either ever use or increasing accumulated dose of ATG and/or ALG. Male recipients were associated with a borderline-

significant increased risk of CSCC ($P = 0.06$). One mismatched HLA-B antigen and more than two rejection episodes increased the risk of CSCC almost 2-fold, but there was no significant trend with increasing number of mismatches or rejections.

Treatment with Aza was associated with an increase in the risk of CSCC during all time periods analysed, and the risk was enhanced by higher dose (Table 3). After the entire follow-up period, the risk of CSCC in relation to the accumulated dose of Aza was close to a 3-fold increase in the low-dose group, and more than 6-fold increase in the intermediate and high-dose groups, compared to never treatment with Aza. With a maximum treatment of 1 year, a high accumulated dose was associated with an elevated risk of 5.1 (95% CI, 1.9–14.0), compared to never treatment with Aza. After a treatment during up to five years, this risk rose to 6.9 (95% CI, 2.5–19.1). Similarly, a high mean dose of Aza during actual treatment periods increased the risk 5.7-fold compared to never treatment. Patients with a high accumulated dose of Cs were at a more than 4-fold increased risk of CSCC after the entire follow-up period, compared to those with a very low accumulated dose of Cs (reference category). An increasing dose of Cs was associated with an increased risk of CSCC, but the low and intermediate dose groups rarely reached statistical significance. A high mean dose of Cs during all treatment periods and during the second and third year post-transplantation (maintenance therapy) increased the risk of CSCC almost 2-fold. The accumulated and mean doses of CsA were consistently not associated with CSCC risk in the crude analyses.

Tables 4 and 5 present the RRs of CSCC computed in multivariate models. Associations with ever treatment with CsA and Aza were more pronounced in the multivariate analysis, whereas the risk of CSCC by treatment regimen, weighted dose combinations of Aza+CsA+Cs and treatment with ATG and/or ALG remained unchanged.

Adjusted risk estimates of CSCC by accumulated and mean dose of Cs were generally unchanged from the crude analyses (Table 5). A high accumulated dose of Cs increased the risk of CSCC 2.5-fold (95% CI, 1.0–6.1) after 3-year treatment duration and 3.9-fold (95% CI, 1.2–12.3) after the entire follow-up period, compared to a very low accumulated dose of Cs (reference category). In contrast, the risk of CSCC in association with Aza was more pronounced upon multivariate adjustment. Patients with a high accumulated dose of Aza were at a 5.4-fold increased risk of CSCC after 1 year of follow-up, and after 3 and 5 years, the risk had risen to 7.5- and 8.9-fold increased, respectively, compared to never treatment with Aza (Table 5, Figure 1). A significant trend in the risk of CSCC with increasing dose of Aza was present for all time periods analysed. Risk estimates of CSCC by accumulated dose of CsA at 1, 3 and 5 years were not associated with the risk of CSCC. However, the adjusted risk of CSCC was 2-fold non-significantly increased in patients with either low or high accumulated dose of CsA after the entire follow-up period, compared to never use. There was no trend in the risk of CSCC with increasing dose of CsA. No effect modification between Aza, CsA, Cs, age and sex could be detected in this study. However, interaction analyses were generally low-powered and were therefore difficult to evaluate.
Table 2. Crude relative risks (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to sex of the recipient, HLA-mismatches in the first transplantation, total number of acute rejection episodes and post-transplant immunosuppressive treatment

|                                | No. of cases (%) | No. of controls (%) | RR (95% CI) | P-value$^\S$ |
|--------------------------------|------------------|--------------------|-------------|-------------|
| **Sex of recipient**           |                  |                    |             |             |
| Female                         | 56 (27)          | 70 (37)            | Ref         |             |
| Male                           | 151 (73)         | 119 (63)           | 1.6 (1.0–2.4) | 0.06        |
| **HLA mismatches**             |                  |                    |             |             |
| **HLA-A**                      |                  |                    |             |             |
| 0                              | 47 (27)          | 49 (30)            | Ref         |             |
| 1                              | 97 (55)          | 79 (48)            | 1.1 (0.6–2.0) | 0.86        |
| 2–3                            | 33 (18)          | 36 (22)            | 0.9 (0.5–1.9) | 0.86        |
| **HLA-B**                      |                  |                    |             |             |
| 0                              | 27 (15)          | 37 (23)            | Ref         |             |
| 1                              | 96 (54)          | 74 (45)            | 1.9 (1.0–3.7) | 0.16        |
| 2                              | 55 (31)          | 53 (32)            | 1.5 (0.7–3.1) | 0.16        |
| **HLA-DR**                     |                  |                    |             |             |
| 0                              | 31 (23)          | 35 (25)            | Ref         |             |
| 1                              | 79 (56)          | 67 (49)            | 1.0 (0.5–2.1) | 0.71        |
| 2–3                            | 32 (23)          | 36 (26)            | 0.8 (0.3–1.8) | 0.71        |
| **Acute rejection episodes**   |                  |                    |             |             |
| 0                              | 70 (34)          | 72 (38)            | Ref         |             |
| 1–2                            | 96 (46)          | 95 (50)            | 1.0 (0.6–1.6) | 0.11        |
| ≥3                             | 41 (20)          | 22 (12)            | 1.9 (1.0–3.6) | 0.44        |
| **ATG and/or ALG treatment**   |                  |                    |             |             |
| Never                          | 151 (73)         | 145 (77)           | Ref         |             |
| Ever                           | 56 (27)          | 44 (23)            | 1.3 (0.8–2.2) | 0.22        |
| **Weighted dose combination of ATG and/or ALG** |                  |                    |             |             |
| Never use                      | 151 (73)         | 145 (77)           | Ref         |             |
| Low                            | 26 (13)          | 22 (12)            | 1.2 (0.7–2.4) | 0.44        |
| High                           | 30 (14)          | 22 (12)            | 1.5 (0.8–2.8) | 0.44        |
| **OKT-3 treatment**            |                  |                    |             |             |
| Never                          | 201 (97)         | 183 (97)           | Ref         |             |
| Ever                           | 6 (3)            | 6 (3)              | 0.6 (0.1–2.5) | 0.46        |
| **Aza treatment**              |                  |                    |             |             |
| Never                          | 9 (4)            | 29 (15)            | Ref         |             |
| Ever                           | 198 (96)         | 160 (85)           | 5.2 (2.0–13.6) | 0.0001     |
| **CsA treatment**              |                  |                    |             |             |
| Never                          | 48 (23)          | 40 (21)            | Ref         |             |
| Ever                           | 159 (77)         | 149 (79)           | 1.0 (0.5–1.8) | 0.94        |
| **Immunosuppressive regimen**  |                  |                    |             |             |
| CsA + Cs                       | 9 (4)            | 28 (15)            | Ref         |             |
| Aza + Cs                       | 48 (23)          | 39 (21)            | 4.1 (1.4–12.2) | 0.0009     |
| Aza + CsA + Cs                 | 150 (72)         | 121 (64)           | 5.3 (2.0–14.4) | 0.0009     |
| **Weighted dose combinations of Aza+CsA+Cs$^b,c$** |                  |                    |             |             |
| ≤3                             | 27 (18)          | 33 (27)            | Ref         |             |
| 4–5                            | 47 (31)          | 42 (34)            | 1.5 (0.6–3.8) | 0.30        |
| 6–7                            | 45 (30)          | 32 (26)            | 3.0 (0.9–10.2) | 0.20        |
| 8–9                            | 31 (21)          | 15 (12)            | 4.6 (1.1–19.9) | 0.20        |

Aza = azathioprine, CsA = cyclosporine, Cs = corticosteroids. ATG = anti-thymocyte globulin, ALG = anti-lymphocyte globulin, OKT-3 = muromonab-CD3 Ab.

Controls were matched to cases by age (±5 years) and calendar period of transplantation (±5 years) and were further required to be alive and free from cancer at the time of the case CSCC diagnosis.

a Relative risks estimated by odds ratios and 95% confidence intervals (CI) using univariate conditional logistic regression models.

b Restricted to persons who received all three drugs.

c The weighted dose combination score was created by adding the assigned values to the accumulated dose categories of each drug, range 2(low)–9(high).

§ Statistical significance (P-value) was estimated with likelihood ratio tests.

Discussion

In this large population-based nested case-control study, with detailed recording of administered immunosuppressive drugs during the post-transplantation period, we found evidence that treatment with Aza is strongly associated with the risk of post-transplant CSCC. We observed highly significant trends of increasing risk of CSCC with increasing accumulated and mean dose of Aza, and the risk was more pronounced with longer treatment duration. Furthermore, we observed an association between a high accumulated dose of Cs after longer treatment durations (at least three years) and the risk of CSCC. In contrast, CsA was not associated with the risk of CSCC post-transplantation.

The risk of CSCC in association with immunosuppressive drug treatment regimens has been investigated in several previous observational studies, but analyses of actual administered doses of these drugs are rare. Our findings
Table 3. Crude relative risks (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to accumulated dose (after 6 months, 1, 3, 5 years and after the entire follow-up period) and to mean dose (during treatment periods and during the second and third year) of azathioprine (Aza), cyclosporine (CsA) and corticosteroids (Cs).

| Accumulated dose | Azathioprine treatment | Cyclosporine treatment | Corticosteroid treatment |
|------------------|------------------------|------------------------|-------------------------|
|                   | No. of cases/controls | RR (95% CI) | P-value | No. of cases/controls | RR (95% CI) | P-value | No. of cases/controls | RR (95% CI) | P-value |
| Entire follow-up period | 9/29 | 1-Ref | 48/40 | 1-Ref | 36/47 | 1-Ref |
| Low               | 41/54 | 2.9 (1.0–8.5) | 50/47 | 1.0 (0.5–2.2) | 52/47 | 1.7 (0.8–3.5) |
| Intermediate      | 80/52 | 6.3 (2.2–17.7) | 54/52 | 0.9 (0.4–1.9) | 48/47 | 2.1 (0.9–5.2) |
| High              | 77/54 | 6.6 (2.3–19.2) | 55/50 | 1.0 (0.5–1.9) | 71/48 | 4.4 (1.6–12.2) | 0.03 |
| 0–6 months        | | | | | | | | |
| Low               | 48/51 | 4.4 (1.5–13.0) | 45/43 | 0.8 (0.3–2.0) | 42/44 | 1.6 (0.7–3.6) |
| Intermediate      | 69/51 | 6.1 (2.1–17.3) | 46/43 | 0.9 (0.4–2.3) | 51/48 | 1.7 (0.8–3.9) |
| High              | 68/51 | 4.6 (1.7–12.5) | 33/44 | 0.7 (0.3–1.6) | 77/48 | 2.5 (1.1–5.4) | 0.11 |
| 0–1 year          | | | | | | | | |
| Low               | 50/51 | 4.1 (1.4–11.9) | 41/44 | 0.7 (0.3–1.7) | 46/45 | 1.7 (0.8–3.9) |
| Intermediate      | 67/53 | 5.1 (1.8–14.5) | 53/44 | 0.9 (0.4–2.3) | 55/47 | 1.8 (0.8–4.0) |
| High              | 72/51 | 5.1 (1.9–14.0) | 33/44 | 0.7 (0.3–1.5) | 70/48 | 2.4 (1.1–5.2) | 0.18 |
| 0–3 years         | | | | | | | | |
| Low               | 48/53 | 3.2 (1.1–9.2) | 35/44 | 0.6 (0.2–1.4) | 38/47 | 1.4 (0.6–3.0) |
| Intermediate      | 53/53 | 4.2 (1.5–12.2) | 57/46 | 0.8 (0.3–2.0) | 63/47 | 2.2 (1.0–5.0) |
| High              | 92/53 | 6.3 (2.3–17.2) | 40/46 | 0.8 (0.3–1.8) | 70/48 | 2.6 (1.2–5.9) | 0.05 |
| 0–5 years         | | | | | | | | |
| Low               | 45/53 | 3.0 (1.0–8.7) | 39/45 | 0.7 (0.3–1.7) | 44/47 | 1.6 (0.8–3.8) |
| Intermediate      | 56/53 | 3.9 (1.4–11.4) | 47/48 | 0.6 (0.3–1.5) | 48/47 | 1.9 (0.8–4.2) |
| High              | 94/54 | 6.9 (2.5–19.1) | 50/47 | 0.9 (0.4–2.0) | 79/48 | 3.2 (1.4–7.2) | 0.03 |
| Mean dose         | | | | | | | | |
| Reference         | 9/29 | Ref | 48/40 | Ref | 43/48 | Ref |
| During treatment  | | | | | | | | |
| Low               | 53/53 | 3.9 (1.4–11.2) | 45/49 | 0.8 (0.4–1.7) | 34/46 | 0.9 (0.4–1.6) |
| Intermediate      | 71/54 | 5.6 (2.0–15.5) | 58/50 | 1.1 (0.5–2.2) | 66/47 | 1.9 (1.0–3.5) |
| High              | 74/53 | 5.7 (2.1–15.8) | 56/50 | 1.0 (0.5–2.1) | 64/48 | 1.9 (1.0–3.5) | 0.02 |
| During second and third year | | | | | | | | |
| Low               | 41/46 | 3.5 (1.1–11.4) | 33/41 | 0.7 (0.3–2.0) | 27/37 | 0.8 (0.4–1.7) |
| Intermediate      | 37/32 | 5.3 (1.6–17.5) | 60/41 | 1.3 (0.5–3.2) | 41/38 | 1.4 (0.7–2.8) |
| High              | 100/63 | 6.9 (2.3–21.1) | 33/40 | 0.9 (0.4–2.1) | 87/56 | 1.8 (1.0–3.1) | 0.08 |

Controls were matched to cases by age (±5 years) and calendar period of transplantation (±5 years) and were further required to be alive and free from cancer at the time of the case CSCC diagnosis.

§ Relative risks estimated by odds ratios and 95% confidence intervals using univariate conditional logistic regression models.

b Reference category = never use of Aza and CsA and very low accumulated/mean dose of Cs.

c Excluding intravenously administered drugs and periods of no treatment.

d Statistical significance (P-value) was estimated with likelihood ratio tests.

with regard to Aza are supported by a few previous reports. In one cohort study, including 361 renal transplant recipients [4], there was a 2.4-fold increased risk of CSCC, in patients ever treated with Aza. Similarly, Hiesse et al. found that a treatment regimen without Aza was related to a RR of NMSC of 0.21 (P = 0.03), compared to a treatment regimen including Aza, in a retrospective cohort study with 1710 renal transplant recipients [8]. Additionally, two prospective studies, one cohort study with 5172 immuno-suppressed non-transplant patients and one randomized controlled trial including 231 renal transplant recipients, detected an association between a high mean dose of Aza and the risk of skin carcinoma (P < 0.01 and P < 0.03, respectively) [9,10]. In contrast, no relationship between administered doses of Aza, CsA or Cs and the risk of NMSC could be detected in three other, smaller studies [18–20].

The majority of previous studies investigating immuno-suppressive regimens have not detected an association with the risk of NMSC [3,13–15,17]. We found that a treatment regimen with Aza+Cs or triple treatment increased the risk of CSCC compared to treatment with CsA+Cs. In line with our results, Ramsay et al. [4] found a reduced risk in CsA+Cs-treated patients compared to those who received triple treatment (RR 0.4; 95% CI, 0.2–1.0). While several studies have not found a difference between patients treated with CsA+Cs compared with Aza+Cs [7,13–15,17], three cohort studies, two large and one smaller, found that triple treatment (Aza+CsA+Cs) may confer an increased risk and/or earlier occurrence of NMSC compared to treatment with Aza+Cs [7,8,16]. Based on the latter findings, investigators have concluded that a heavy drug load, rather than specific drugs, confers the elevated risk of CSCC. To investigate this, we created a variable for increasing drug dose burden in persons receiving triple treatment. In line with the heavy drug load hypothesis, we found that a high dose load elevated the risk of CSCC compared to a low...
Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma

Table 4. Relative riska,b (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to sex of the recipient, HLA-B-mismatches in the first transplantation, total number of acute rejection episodes and post-transplant immunosuppressive treatment

| Sex of recipient | RR (95% CI) | P-value∞ |
|------------------|-------------|-----------|
| Female           | 1.8 (1.1–3.1) | 0.02      |
| Male             | 0.8 (0.4–1.3)  | 0.29      |

| No. of HLA-B mismatches | Acute rejection episodes | ATG + ALG treatment | Aza treatment | CsA treatment |
|-------------------------|--------------------------|---------------------|---------------|--------------|
| 0                       | 1.5 (0.7–3.2)            | Ref                 | Ref           | Never        |
| 1                       | 1.4 (0.6–3.2)            |                     | Ever          | 1.4 (0.8–2.4) |
| ≥2                      | 1.4 (0.6–3.0)            |                     |               |              |

| Immunosuppressive regimen | Weighted dose combinations of CsA + Cs | Aza + Cs | Aza + CsA + Cs |
|---------------------------|---------------------------------------|---------|----------------|
| CsA + Cs                  | Ref                                   | 4.6 (1.5–13.8) | 5.7 (2.0–15.8) |
| Aza + Cs                  |                                       |         |                |
| Aza + CsA + Cs            |                                       |         |                |

| Weighted dose load | Ref |
|--------------------|-----|
| ≤3                 | 1.5 (0.6–3.8) |
| 4–5                | 1.5 (0.6–3.8) |
| 6–7                | 1.5 (0.6–3.8) |

Aza = azathiprine, CsA = cyclosporine, Cs = corticosteroids, ATG = anti-thymocyte globulin. Controls were matched to cases by age (±5 years) and calendar period of transplantation (±5 years) and were further required to be alive and free from cancer at the time of the case CSCC diagnosis.

aRelative risks estimated by odds ratios and 95% confidence intervals using multivariate conditional logistic regression models. Estimates adjusted for sex of the recipient, accumulated dose of Aza, CsA and Cs after the entire follow-up period. Analyses of combinations of drugs were not adjusted for the total accumulated dose of the same drug.

bRestricted to persons who received all three drugs. The weighted dose combination score was created by adding assigned values to the accumulated dose categories of each drug, range 2 (low)–9 (high).

For each of the following outcomes, Aza, CsA and Cs contributed to a statistically significant increase in risk. There were no statistically significant differences in the magnitude of the risk between the different treatment groups.

The observed indication that Cs treatment is associated with an increased risk of CSCC is supported by two relatively large previous studies in non-transplant populations [26,27]. However, this has not been described in organ transplant patients before, but previous studies were hampered by small size [18,20].

There are at least three basic mechanisms through which immunosuppressive drugs could cause CSCC—by an impaired immune surveillance, by a direct carcinogenic effect and by increasing the susceptibility to other carcinogenic agents. Additional to their immunosuppressive capacity, it has been shown that CsA is directly carcinogenic and that Aza is mutagenic [28,29]. Moreover, it has become clear that Aza enhances the effect of sun-emitted UV radiation (UVR) on skin cancer risk. Kelly et al. showed that Aza had a strong inducing and promoting effect on tumours in hairless mice exposed to UVR, whereas CsA and Cs had a moderate and no effect, respectively [11]. Furthermore, O’Donovan et al. found that Aza photosensitizes the skin to UVR by changing the absorption interval of DNA when the metabolite 6-thioguanine is incorporated [12]. The absorption of UVR then causes the formation of reactive oxygen species that previously have been linked to DNA damage and cutaneous malignancies [12].

Fig. 1. Adjusted relative risks of cutaneous squamous cell carcinoma (CSCC) in relation to accumulated dose of azathioprine (Aza) 1, 3 and 5 years after the first organ transplantation.
### Table 5. Relative risks\(^a\(^b\) (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to accumulated dose (after 6 months, 1, 3, 5 years and after the entire follow-up period) and to mean dose (during treatment periods and during the second year and third year) of azathioprine (Aza), cyclosporine (CsA) and corticosteroids (Cs) post-transplantation

|                      | Azathioprine treatment | Cyclosporine treatment | Corticosteroid treatment |
|----------------------|------------------------|------------------------|-------------------------|
|                      | RR (95% CI)            | P-value\(^∞\)          | RR (95% CI)            | P-value\(^∞\)          | RR (95% CI)            | P-value\(^∞\)          |
| **Accumulated dose** |                        |                        |                         |                         |                        |                         |
| Reference\(^c\)      | 1-Ref                  | 1-Ref                  | 1-Ref                  |
| **Entire follow-up period** |                        |                        |                         |                         |                        |                         |
| Low                  | 3.1 (1.0–9.7)          | 1.9 (0.8–4.7)          | 1.9 (0.8–4.3)          |
| Intermediate        | 8.2 (2.7–25.1)         | 1.4 (0.6–3.4)          | 0.35                   | 1.9 (0.7–5.2)          | 0.09                   |
| High                 | 8.8 (2.6–29.9)         | 1.9 (0.8–4.5)          | 0.31\(^f\)            | 3.9 (1.2–12.3)         | 0.03\(^f\)            |
| **0–1 year**         |                        |                        |                         |                         |                        |                         |
| Low                  | 4.5 (1.4–14.5)         | 1.1 (0.4–3.0)          | 2.1 (0.8–5.4)          |
| Intermediate        | 6.0 (1.9–18.8)         | 1.3 (0.4–4.0)          | 0.77                   | 1.7 (0.7–4.2)          | 0.25                   |
| High                 | 5.4 (1.8–16.2)         | 0.9 (0.3–2.6)          | 0.92\(^g\)            | 2.3 (0.9–5.6)          | 0.14\(^g\)            |
| **0–3 years**        |                        |                        |                         |                         |                        |                         |
| Low                  | 3.6 (1.2–11.1)         | 0.9 (0.3–2.7)          | 1.5 (0.6–3.7)          |
| Intermediate        | 5.0 (1.6–16.0)         | 1.5 (0.5–4.6)          | 0.50                   | 2.1 (0.9–5.2)          | 0.18                   |
| High                 | 7.5 (2.4–22.9)         | 1.1 (0.4–3.1)          | 0.84\(^h\)            | 2.5 (1.0–6.1)          | 0.05\(^h\)            |
| **0–5 years**        |                        |                        |                         |                         |                        |                         |
| Low                  | 3.4 (1.1–10.6)         | 1.2 (0.4–3.3)          | 1.8 (0.8–4.1)          |
| Intermediate        | 5.4 (1.7–17.0)         | 0.9 (0.3–2.5)          | 0.78                   | 2.0 (0.8–4.8)          | 0.17                   |
| High                 | 8.9 (2.9–27.8)         | 1.2 (0.4–3.3)          | 0.91\(^i\)            | 2.8 (1.1–7.2)          | 0.04\(^i\)            |
| **Mean dose**        |                        |                        |                         |                         |                        |                         |
| Reference\(^c\)      | 1-Ref                  | 1-Ref                  | 1-Ref                  |
| **During treatment** |                        |                        |                         |                         |                        |                         |
| Low                  | 4.1 (1.3–12.8)         | 1.6 (0.7–3.9)          | 0.9 (0.4–1.8)          |
| Intermediate        | 6.6 (2.1–20.9)         | 2.2 (0.9–5.2)          | 0.35                   | 1.5 (0.8–3.1)          | 0.21                   |
| High                 | 6.4 (2.1–19.6)         | 1.7 (0.8–3.9)          | 0.24\(^i\)            | 1.7 (0.9–3.4)          | 0.08\(^i\)            |
| **During second and third year** |                        |                        |                         |                         |                        |                         |
| Low                  | 4.3 (1.1–15.9)         | 1.5 (0.4–5.2)          | 0.7 (0.3–1.7)          |
| Intermediate        | 6.7 (1.8–25.3)         | 2.8 (0.8–9.2)          | 0.19                   | 1.5 (0.7–3.3)          | 0.18                   |
| High                 | 8.7 (2.5–30.3)         | 1.6 (0.5–4.8)          | 0.71\(^i\)            | 1.6 (0.9–3.1)          | 0.05\(^i\)            |

Controls were matched to cases by age (±5 years) and calendar period of transplantation (±5 years) and were further required to be alive and free from cancer at the time of the case CSCC diagnosis;\(^a\)Relative risks estimated by odds ratios and 95% confidence intervals in a multivariate conditional logistic regression model;\(^b\)Estimates adjusted for sex of the recipient, accumulated dose of Aza, CsA and Cs after the entire follow-up period. Analyses of drug doses in different treatment phases were not adjusted for the total accumulated dose of the same drug;\(^c\)Reference category = never use of Aza and CsA and very low accumulated/mean dose of Cs;\(^d\)Excluding intravenously administered drugs and periods of no treatment with the drug;\(^∞\)Statistical significance (P-value) was estimated with likelihood ratio tests;\(^f\)P-value for linear trend in risk of CSCC.

Aza and CsA. Nevertheless, Aza and CsA are still used as anti-rejection treatment in transplant patients as well as in treatment of many other inflammatory and autoimmune disorders. Lastly, we have not been able to adjust for body weight or individual variations in bioavailability.

In summary, we found evidence of a strong association between the risk of CSCC and treatment with Aza. We also observed an increased risk with Cs treatment, specifically with a high accumulated dose after longer treatment periods. We conclude that there may be important differences in the risk of post-transplant CSCC conferred by specific drugs and by interaction with environmental factors, rather than an effect of the immunosuppressive drug load per se.

**Acknowledgements.** We thank the Swedish Cancer Society, the Swedish Society of Medicine, Martin Rinds and Edvard Welanders foundations who have funded this project.

**Conflict of interest statement.** None declared.

### Appendix 1. Algorithms for combining generically similar immunosuppressive drugs

| Generic name of drug administered orally | Conversion factor used | Product comparable to |
|------------------------------------------|------------------------|----------------------|
| Betamethasone                            | 8.33                   | Prednisolone         |
| Methylprednisolone                       | 1.25                   | Prednisolone         |
| Hydrocortisone                           | 0.25                   | Prednisolone         |
| Cortisone                                | 0.20                   | Prednisolone         |
| Prednisone                               | 1.00                   | Intravenously administered methylprednisolone (100% bioavailable) |
| Prednisolone                             | 0.80                   | Cyclosporine (100% bioavailable) |
| Cyclosporine microemulsion               | 0.38                   | Intravenously administered cyclosporine (100% bioavailable) |
| Azathioprine                             | 0.47                   | Intravenously administered azathioprine (100% bioavailable) |
References

1. Birkeland SA, Storm HH, Lamm LU et al. Cancer risk after renal transplantation in the Nordic countries, 1966–1986. Int J Cancer 1995; 60: 183
2. Adami J, Gabel H, Lindelof B et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. Br J Cancer 2003; 89: 1221
3. Gruber SA, Gillingham K, Sothern RB et al. De novo cancer in cyclosporine-treated and non-cyclosporine-treated adult primary renal allograft recipients. Clin Transplant 2004; 18: 388
4. Ramsay HM, Fryer AA, Hawley CM et al. Factors associated with nonmelanoma skin cancer following renal transplantation in Queens-land, Australia. J Am Acad Dermatol 2003; 49: 397
5. Lindelof B, Sigurgeirsson B, Gabel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol 2000; 143: 513
6. Hartevelt MM, Bavinck JN, Kootte AM et al. Incidence of skin cancer after renal transplantation in The Netherlands. Transplantation 1990; 49: 506
7. Jensen P, Hansen S, Moller B et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol 1999; 40(Pt 1): 177
8. Hiesse C, Rieu P, Kriaa F et al. Malignancy after renal transplantation: analysis of incidence and risk factors in 1700 patients followed during a 25-year period. Transplant Proc 1997; 29: 831
9. Kinlen LJ, Sheil AG, Peto J et al. Collaborative United Kingdom-Scotland registry. The diagnostic data are better than their reputation. Br J Cancer 1999; 79:590
10. Ramsay HM, Fryer AA, Reece S et al. Clinical risk factors associated with nonmelanoma skin cancer in renal transplant recipients. Am J Kidney Dis 2000; 36: 167
11. National Board of Health and Welfare, Centre for Epidemiology. In-patient diseases in Sweden 1987–2006. Cancer incidence in Sweden 2006, 2007.
12. Nilsson AC, Spetz CL, Carsjo K et al. Reliability of the hospital registry. The diagnostic data are better than their reputation. Lakartidningen 1994; 91: 598
13. Wassberg C, Thorn M, Johansson AM et al. Increasing incidence rates of squamous cell carcinoma of the skin in Sweden. Acta Derm Venereol 2001; 81: 268
14. Ardizzone S, Bianchi Porro G. Comparative tolerability of therapies for ulcerative colitis. Drug Saf 2002; 25: 561
15. Hosmer D, Lemeshow S. Model building strategies. Applied Logistic Regression. Amherst: Wiley, 1989, Chapter 4.
16. Karagas MR, Cushing GL Jr., Greenberg ER et al. Non-melanoma skin cancers and glucocorticoid therapy. Br J Cancer 2001; 85: 683
17. Caraglione A, Cattolica E, Tecchio E et al. Skin cancers and non-hodgkin lymphoma among users of systemic glucocorticoids: a population-based cohort study. J Natl Cancer Inst 2004; 96: 709
18. Yuan B, Wang Y. Mutagenic and cytotoxic properties of 6-thioguanine, S6-methylthioguanine, and guanine-S6-sulfonic acid. J Biol Chem 2008; 283: 23665
19. Hojo M, Morimoto T, Maluccio M et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999; 397: 530
20. Lindelof B, Granath F, Dal H et al. Sun habits in kidney transplant recipients with skin cancer: a case-control study of possible causative factors. Acta Derm Venereol 2003; 83: 189

Received for publication: 28.4.09; Accepted in revised form: 29.7.09