"**Risk of De Novo or Secondary Cancer after Solid Organ or Allogeneic Haematopoietic Stem Cell Transplantation**"

Running title: Cancer Risk after Transplantation

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Abstract

Purpose

Solid organ (SOT) and allogeneic haematopoietic stem cell (HSCT) transplant recipients have elevated risk of de novo or secondary cancers. We explored risk factors hereof.

Methods

Among SOT and HSCT transplanted between January 2004-December 2014, standardized incidence ratio (SIR) of de novo/secondary cancer compared with the Danish population was determined and risk factors identified using Poisson regression.

Results

During a median of 3.4 (IQR 1.3-6.4) and 2.6 (0.8-5.4) person-years (PY) after SOT and HSCT, a total of 212/1656 (13%) and 75/992 (8%) persons developed cancer; SIR 3.61 (3.0-4.3) and 2.2 (1.6-3.0) resp.). SIR correlated with younger age and was highest for skin and haematological cancers for both types of transplantation. Within the cohort, cancer was associated with older age (adjusted incidence rate ratio >50 vs ≤19 years among SOT and HSCT: 9.4 (3.4-25.7) and 25.4 (5.1-126.0), resp.) and current elevated C reactive protein (CRP) (≥10 vs <10 mg/L: 2.5 (1.8-3.4) and 2.3 (1.4-3.9), resp.), but not with prior cancer nor type of immunosuppressants.

Conclusion

Rates of de novo or secondary cancers are elevated in both SOT and HSCT compared with the general population and mainly for skin and haematological cancers. Among transplant recipients, older age and current elevated CRP are risk factors.

Key words: Transplantation, Cancer, Secondary Cancer, Inflammation.

Background

Solid organ (SOT) and allogeneic haematopoietic stem cell (HSCT) recipients have elevated rates of cancer compared to the general background population (1-4) and compared to non-transplant patients with similar end-stage organ diseases (5). This seems to be driven mainly by infection-related cancers, e.g. Epstein Barr virus related lymphomas (6) or Human papilloma virus (HPV) associated squamous cell carcinomas (7), suggesting a role of post-transplant medically induced immunosuppression as a driver of this excess risk (8;9). Further, prior treatment with chemotherapy and irradiation for an underlying cancer have been associated with an excess risk of new distinct cancers, i.e. secondary cancers, among HSCT (10-12).

The varying cancer rates within the different transplant types have in part been attributed to tumorigenic effects of specific immunosuppressive drugs (13), although comprehensive studies assessing the relationship with exposure to various types of immunosuppressive medications are lacking. Previous experimental *in vivo* studies have demonstrated azathioprine to be mutagenic (14) and cyclosporine to be directly carcinogenic (15). Further, epidemiologic studies have reported excess cancer risk after exposure to azathioprine and steroids whereas mycophenolate mofetil and sirolimus may be associated with lower risk (16-19).

Chronic inflammation has been suggested as one of the hallmarks of cancer development and several components of the inflammatory pathway have been associated with an excess cancer risk (20). C-reactive protein (CRP), a down-stream inflammatory biomarker synthetized in the liver as a response to infection or tissue damage, has been suggested to reflect the extent and activity of a pro-neoplastic and pro-metastatic environment and has been associated with cancer risk in healthy and diseased populations (21-24). The role of this inflammatory biomarker has not been assessed in a transplant setting.

This study aims to assess the incidence of cancer in a large transplant cohort relative to that in the general population and secondly to explore factors associated with cancer within the transplant population.

Material and Methods

Patients

We included all SOT and allogeneic HSCT, transplanted at a large transplant hospital, in Copenhagen, Denmark between January 2004 and December 2014. This includes all liver and lung transplants performed in Denmark and all kidney, heart and stem cell transplantations performed in the eastern region of Denmark. The recipients are all registered in the MATCH cohort (25), an ongoing clinical database containing demographic and transplant details. Immunosuppressive treatment regimens have previously been published (26).

Data Sources

All data were retrieved from the Centre of Excellence for Personalized Medicine for Infectious Complications in Immune Deficiency (PERSIMUNE) data repository of electronic health records as previously described (27;28). In brief, incident, non-relapse cancer events were identified through linkage to nationwide pathology data, regional data on ICD10 codes registered in connection to an admission to hospital or the outpatient clinic, and information of chemotherapy treatment and results from FDG PET/CT imaging performed locally at our hospital. Further, fatal events were reviewed as described previously to be able to ascertain possible overlooked cancer, i.e. cancers not previously recorded (29).

Other potential predictors of cancer were also retrieved from the data repository, such as biochemical data and exposure to individual immunosuppressants. All data sources including geographic coverage and calendar periods have been described in detail previously (please see supplemental material 1 of the following reference) (28).

Data from the Danish Cancer Registry were also collected for validation and to be able to compare cancer incidence between the transplanted compared to the background population (see statistical methods).

End-Point Definition

Incident non-relapse cancers were included as primary end-point. Cancers diagnosed ≤30 days from transplantation were considered pre-transplant cancers.

Any cancer and the three most frequent categories of cancer were assessed, i.e. non-melanoma skin (referred to as skin cancer), haematological, and lung cancers.

Statistical methods

Transplant recipients were considered at risk from date of first transplantation to date of last visit in the clinic plus 60 days, death or February 21st 2017, whichever came first. Last visit was defined as the last measured biochemical or microbial tests, such as haemoglobin or CMV PCR etc., measured at our hospital. In stratified analyses of specific cancer categories, participants with a cancer of another category continued to be followed for incident cancer.

Crude incidence rates (IR) were estimated per 1000 person-years of follow-up (PYFU) for any cancer and for the three most frequent cancer types (skin cancers, haematological cancers and lung cancers) among SOT and HSCT.

Incident cancers observed in the transplant population was compared to incident cancers in the Danish population, i.e expected cancers, within the same calendar period (i.e. 2004-2014). The expected cancers were calculated by multiplying gender and age-specific rates in the Danish population with the corresponding person-year of follow-up in the transplanted population. The standardized incidence ratios (SIR) of observed and expected numbers were calculated for the largest cancer categories, i.e. any, skin, haematological and lung cancers. Ninety-five percent confidence intervals (CI) were calculated using Byars approximation (30). The incidence data for the Danish population was obtained from the

Association of the Nordic Cancer Registries (ANCR) which is based on the Danish Cancer Registry and is available on http://www-dep.iarc.fr/NORDCAN/DK/frame.asp. To reduce ascertainment bias, cancer events in the transplant population were obtained from the same source, (i.e the Danish Cancer Registry) when calculating SIRs. Furthermore, to reduce surveillance bias, cancer events within 180 days from transplant were excluded when calculating SIRs although, we acknowledge that this could lead to underestimation of certain early occurring cancers such as post-transplant lymphoproliferative disorders.

Poisson regression models were used to investigate the association between incident cancer and potential risk factors stratified by SOT and HSCT and results were presented with incidence rate ratios (IRR). The primary model was adjusted for gender, age, type and year of transplantation, number of transplantations (fitted as time-updated variable), a history of cancer prior to transplantation and Charlson comorbidity index score at time of transplantation (31).

Secondly, a potential link between specific á priori selected biomarkers and cancer was assessed in uni- and multivariate analyses. The assessed biomarkers included routinely measured laboratory parameters at baseline and during follow-up, i.e. haemoglobin, leukocytes, lymphocytes, neutrocytes, thrombocytes, creatinine, alanine transaminase (ALT), albumin, CRP, and lactate dehydrogenase (LDH). Abnormal values of CRP showed the strongest association with excess cancer rates and were further assessed in multivariate models using elevated compared to normal values (Normal values of CRP: <10 mg/L) and fitted as time-updated co-variates. Both current (the latest value within 3 months prior to or on the date of cancer diagnosis) and lagged values (i.e values from a certain time before cancer diagnosis) were assessed to identify a potential association with cancer before onset of clinical disease. Lagged values at 6, 12 and 24 months before cancer diagnosis were assessed.

Finally, the association between exposure to specific transplant immunosuppressants and excess risk of cancer was assessed in uni- and multivariate analyses. The assessed immunosuppressants included anti-thymocyte globulin, azathioprine, cyclosporine, tacrolimus, sirolimus, everolimus, steroids,

and mycophenolic acid. Exposure was defined as ever vs never exposure and fitted as time updated variable.

Several sensitivity analyses were performed. Analyses were repeated including skin cancers. Among HSCT additional risk factors were assessed, i.e. donor relation (related vs unrelated), T cell depleting treatment, total body irradiation (TBI), chronic graft versus host disease (cGvHD) (as time-updated variable), and ever vs. never experiencing relapse from the underlying cancer leading to HSCT (as time-updated variable). Death as a competing event was assessed by excluding all fatal cases among recipients where no de novo or secondary cancer developed during follow-up.

All P values are 2-sided. A P value < .05 indicates statistical significance. Statistical analyses were conducted using SAS statistical software version 9.4 (SAS institute, Cary, NC, USA).

Results

Of the 2648 recipients included in the study, 1656 were SOT (63%) and 992 were HSCT (37%) (Table 1). Most of the recipients were males (59.8%), above 50 years of age at time of transplantation (42.9%) and receiving their first transplants (96.3%). The vast majority of HSCT had a cancer diagnosis prior to transplantation (89.5%) whereas less than 10% had this event prior to SOT.

The recipients were followed for a total of 10,376 person-years (PY); a median of 3.36 (interquartile range (IQR) 1.29-6.37) years among SOT and 2.59 years (0.76-5.41) among HSCT. During this time, 287 (10.84%) recipients developed at least one distinct de novo or secondary (i.e. non-relapse) cancer after transplantation corresponding to an overall incidence rate (IR) per 1000 PY of 27.66 (95% CI 24.46-30.86). Incidence rates of any cancer among SOT and HSCT were 30.81 (26.66-34.96) and 21.46 (16.60-26.32), respectively.

The most frequent cancer type was skin cancer (N=161; 121 among SOT and 40 among HSCT) (IR per 1000 PY: 17.20 (14.13-20.26) and 11.23 (7.75-14.71), resp.), followed by haematological cancer (mainly Hodgkin and non-Hodgkin lymphoma followed by acute leukaemia) (N=51; 37 and 14, resp.) (IR

5.09 (3.40-6.77) and 4.29 (2.40-7.08)), and lung cancer (N=22; 19 and 3) (IR 2.76 (1.66-4.31) and 0.86 (0.18-2.51)).

Excess risk of cancer compared to the Danish population

To estimate SIRs, a total of 1656 and 992 recipients between 2004 and 2014 were included, representing a total of 5,477 and 2,832 PYFU, respectively. Compared to the general population, cancer rates were significantly higher for the assessed cancer types among SOT and for most types among HSCT (Figure 1A). Overall, SOT experienced a more than 3-fold higher cancer rate compared to the general population (SIR 3.61 (3.04-4.25), whereas cancer rate was twice as high among HSCT (2.18 (1.57-2.96)). The greatest difference in cancer rate was observed for skin and secondly for haematological cancer. Lung cancer, on the other hand was observed with significantly higher rates only among SOT but not among HSCT.

SIRs for any cancer were further calculated according to age at cancer diagnosis. While, the higher cancer rates in the transplant population were observed across all age groups, there was a clear inverse relationship between age at cancer diagnosis and SIR (Figure 1B). Thus, SIR was 16.43 (1.85-59.33) vs. 2.98 (2.43-3.63) in the youngest and oldest age groups among SOT and 12.14 (1.36-43.81) vs. 2.18 (1.52-3.03) in the same age groups among HSCT.

Factors associated with any cancer among SOT

Within the SOT population, the association between several baseline characteristics and cancer rate were assessed and are presented in Table 2. The strongest relationship was observed between older age at time of transplantation and cancer rate. The IRR increased with increased age and the oldest age group had an almost 10-fold higher cancer rate compared to the youngest age group (adjusted IRR (aIRR) for those aged 35-50 and those above 50 vs. those aged ≤19 years at transplant: 3.02 (1.07-8.52) and 9.40 (3.44-25.71), respectively). Further, lung recipients had a marginal increase in cancer rate compared to kidney recipients

(1.53 (1.00-2.33)), whereas none of the other assessed baseline characteristics reached statistical significance.

Recipients with current elevated CRP had a more than 2-fold higher cancer rate compared to those with normal values (2.46 (1.78-3.39)) (Table 2). However, when CRP measurements performed 6, 12, or 24 months prior to cancer diagnosis were assessed, the association between elevated levels and excess cancer rated disappeared (Figure 2A).

Univariate analyses of individual immunosuppressants suggested that recipients ever exposed to azathioprine (1.42 (1.06-1.92)) or those ever exposed to cyclosporine (1.51 (1.11-2.06)) had excess rates of any cancer compared to recipients who were never exposed to these immunosuppressants. Further, those ever exposed to tacrolimus (0.66 (0.46-0.88)) had lower rates of any cancer compared to those never exposed in univariate analyses. However, after adjustments for baseline characteristics these associations no longer reached statistical significance (Table 3A). On the other hand, older age remained strongly associated with cancer risk after adjustment for any of the immunosuppressants.

Factors associated with any cancer among HSCT

Similar to results among SOT, older age was also strongly associated with excess cancer rate after HSCT (aIRR for those aged 35-50 and those above 50 vs. those aged ≤19 years at transplant: 8.83 (1.67-46.65) and 25.37 (5.11-126.02), respectively) (Table 2). Further, those transplanted with umbilical cord blood donor cells (UCBT) compared to adult donor transplantation (aIRR 2.32 (0.87-6.23)) and those with ≥2 vs. 1 transplantation (10.24 (2.28-46.02)) were associated with excess cancer rate. The remaining assessed baseline characteristics did not influence rates of any cancer.

Also similar to results among SOT, current elevated CRP (elevated vs normal values: 2.29 (1.35- 3.89)) was associated with an excess cancer rate (Table 2), whereas CRP measured more than 6 months prior to cancer diagnosis were not associated and were with wide confidence intervals (Figure 2B).

Similar to the analyses among SOT, none of the assessed immunosuppressants significantly influenced rates of cancer after adjustment (Table 3B). Further, after adjustment for individual immunosuppressants, older age and multiple transplantations remained strongly associated with excess cancer rate.

Sensitivity analyses

Results from sensitivity analyses including skin cancers only, were generally hampered by wide confidence intervals. However, in both SOT (121 cancers, 7037 PYFU) and HSCT (40 cancers, 3562 PYFU), older age remained a strong risk factor for skin cancer (age >50 vs ≤ 50 years among SOT and HSCT: 5.10 (3.20-8.12) and 7.86 (2.94-21.00), resp.). Further, there was a non-significant trend towards higher cancer rates among HSCT with multiple transplantations (≥2 vs. 1 transplantation (7.59 (0.94-61.13)).

Further sensitivity analyses among HSCT included adjustment for donor relation (related vs unrelated), T cell depleting treatment, TBI, and cGvHD, although these results were generally hampered by wide confidence intervals. TBI was associated with excess rates of any cancer (9.58 (1.03-89.00)) whereas none of the other factors reached statistical significance.

To account for impact of death as a competing event on cancer rate, we excluded fatal cases among SOT (N=336) and HSCT (N=380) with no incident cancer event during follow-up in sensitivity analyses. These results remained similar to the main results. In addition, analyses among HSCT were further adjusted for ever vs never experiencing relapse of an underlying cancer (196 (22.1%, 95% CI 19.3-24.8%) of 888 HSCT with an underlying cancer relapsed) which also led to similar results as the main results.

Discussion

In this large transplant cohort, we found that de novo or secondary cancer rates among SOT and HSCT relative to the general population were increased several-fold for cancer of any type, and in particular for skin and haematological cancer. Within the cohort, older age and current elevated CRP were consistent risk

factors, whereas this was not observed for either a history of cancer or type of immunosuppressive medication.

Our observation that age was an important risk factor for an excess cancer risk among both SOT and HSCT recipients is consistent with the known literature (2;6;10;12;32;33). In agreement with previous studies, the excess rate of cancer in transplant recipients compared with the general population was greater in the youngest age group whereas absolute cancer risk was higher among the older transplant recipients. This suggests that while the burden of cancer in transplant recipients lies within older persons, similar to what is observed in the general population, younger persons seem to be more vulnerable to immunosuppression.

The association between elevated CRP, a downstream marker of the inflammatory pathway, and cancer has been extensively studied and has been reported in different patient populations, also in samples measured years prior to the cancer diagnosis (34;35). The association has been most consistent for risk of lung cancer (21;36), consistent with a putative role of activation of the inflammasome in lung cancer pathophysiology according to results from the CANTOS trial (37). However, studies investigating risk of other cancer types, such as breast, colorectal and prostate cancer, have been with more variable results (38-40). To our knowledge, the association between CRP and cancer has not previously been studied in a transplant setting. In the present study, we found an association between elevated CRP and excess cancer rates but only the current CRP value. Analyses using lagged values of 6 months or more before cancer diagnosis were not associated with increased rates of cancer. This could be due to confounding by other biologic processes during follow-up affecting the levels of CRP, such as liver impairment or infections after transplantation. This could also be an expression of reverse causality where the observed alterations in these biomarker levels were in fact caused by the cancer itself. Another explanation could be that cancer development in transplant recipients is less prolonged with short subclinical duration causing changes in biomarker levels only immediately prior to the cancer diagnosis. Nevertheless, our results suggest that CRP could have a role in diagnosing cancer after transplantation.

Treatment with chemotherapy and irradiation for cancer prior to transplantation have been reported to be significant risk factors for the development of secondary cancers after transplantation (10;12). Further, persons who have developed a cancer once may presumably be more genetically predisposed to developing a new cancer compared to those who have no history of cancer (41). While, we found an association between TBI and excess cancer risk after transplantation, we found no association between a history of cancer prior to transplantation and risk. One explanation could be the impact of competing risks from relapse from the previous cancer and subsequent death precluding a new cancer from occurring. However, sensitivity analyses taking relapse and death into account did not change these results. On the other hand, given that recipients transplanted for chronic non-malignant diseases also have increased risks of cancer after transplantation (5;42) the potential contribution of a history of cancer on risk of secondary cancer after transplantation may be overshadowed. Not all cancers are necessarily treated with chemotherapy or irradiation, e.g. skin cancers. Thus, another explanation for the lack of association could be that it is in fact the treatment related to cancer that increases risk of secondary cancers in our study rather than having a history of cancer per se.

An association between azathioprine use and cancer (16-18) has been shown in several previous studies, whereas reports of other commonly used immunosuppressants have been with variable results (13). We did not identify a significant contributing cancer risk associated with exposure to any of the assessed immunosuppressants including azathioprine. This could be due to inadequate statistical power or that we were unable to asses area under the curve of a specific drug exposure. However, this could also suggest that when considering cancer risk, it is the immunosuppression as such that may play the most important role, rather than contributing risk from specific immunosuppressive drugs. This was supported by findings in other immunosuppressed patient populations where HIV infected patients who were treated with interrupted or deferred antiretroviral medication were associated with higher cancer risk compared to those treated consistently and more intensively (43). Further, it is plausible that the combination of immunosuppression and specific infectious agents in part explains the higher rates of cancer in transplant

recipients, e.g. the observed relationship between HPV and squamous cell carcinoma in transplant recipients (7).

There are some limitations to be considered. Owing to the low number of distinct cancers we had to pool etiologic different cancers in larger groups and were thus not able to assess risk factors of distinct cancer entities. However, we did assess a variety of end-points in addition to those presented here, such as non-skin cancers and infection vs non-infection related cancers which showed consistent results. We were unable to explore confounding with other exposures commonly associated with cancer, such as smoking, sun exposure, and other life style factors as these data were not available to us. Further, we were not able to explore effects of the previous chemoimmunotherapy regimens used as primary treatment. Due to consideration of statistical power we were not able to explore potential confounding of other concomitant disease entities associated with inflammation, such as asthma, allergies, chronic inflammatory bowel diseases, rheumatoid disease etc., which could influence the relationship between CRP levels and cancer. We did however, include Charlson score (31) to account for underlying comorbidities prior to transplantation which did not influence our findings.

In conclusion, we observed higher rates of cancer, in particular skin and haematological cancers, in transplant recipients compared to the general population and especially in younger persons. Furthermore, we observed that older age and high levels of CRP were associated with excess cancer within the transplant cohort whereas individual immunosuppressants did not influence rates of cancer.

Additional information

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The research is conducted after approval of the National Data Protection Agency (2012-58-0004; RH-2016–47; 04433) and the Danish National Board of Health (3-3013-1060/1).

Informed consent: All relevant approval for this project was obtained from the Danish Health and Medicines Authorities according to Danish legislation on retrospective studies. For this type of study, formal consent is not required.

Data availability: The datasets generated during and/or analyzed during the current study are not publicly available as these data were used under license for the current study but are available from the corresponding author on reasonable request and subject to any regulations required for data sharing.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: This work was supported by Danish National Research Foundation [Grant number 126].

Author's contributions: NEW, AM, JDL designed the study, contributed to the analyses and interpretation of the results and drafted the manuscript. QL performed the analyses of the results and contributed to writing the manuscript. HS, CDB, FG, SSS, AR, MP, CH all contributed to the study design, interpreting the results, supervision and to writing the manuscript. All authors approved the final version and agreed to be accountable for all aspects of the work.

Acknowledgements: The authors would like to acknowledge the work of Allan Lind-Thomsen and Viktor Søgaard Rasmussen who contributed with the data management and quality assurance. Further, the authors would like to thank Pernille Iversen for all her help with obtaining legal approvals for the project from the Danish authorities.

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Figure Legends

Table 1. Characteristics at time of solid organ and haematopoietic stem cell transplants.

Figure 1A. Standardized incidence ratio of cancer after solid organ (SOT) and haematopoietic stem cell transplantation (HSCT) compared to the Danish population, according to cancer type.

Figure 1B. Standardized incidence ratio of cancer after solid organ (SOT) and haematopoietic stem cell transplantation (HSCT) compared to the Danish population, according to age at cancer diagnosis.

Table 2. Adjusted incidence rate ratios for any cancer among SOT and HSCT.

Figure 2A. Incidence rate ratios of any cancer at various time points prior to cancer diagnosis of elevated CRP compared to normal values among SOT.

Figure 2B. Incidence rate ratios of any cancer at various time points prior to cancer diagnosis of elevated CRP compared to normal values among HSCT.

Table 3A. Association between Ever vs. Never Exposure of Immunosuppressive Medications and Excess Cancer Rate among SOT.

Table 3B. Association between Ever vs. Never Exposure of Immunosuppressive Medications and Excess Cancer Rate among HSCT.

Table 1. Characteristics at time of solid organ and haematopoietic stem cell transplants.

	All		*SOT*		*HSCT*	
	N	*(%)*	*N*	*(%)*	*N*	*(%)*
Gender						
Male	1584	59.82	978	59.06	606	61.09
Female	1064	40.18	678	40.94	386	38.91
Age at transplantation						
≤19	379	14.31	142	8.57	237	23.89
20-≤34	388	14.65	261	15.76	127	12.80
35-≤50	746	28.17	507	30.62	239	24.09
>50	1135	42.86	746	45.05	389	39.21
Transplant year						
≤2006	613	23.15	382	23.07	231	23.29
2007-≤2009	663	25.04	421	25.42	242	24.40
2010-≤2012	827	31.23	524	31.64	303	30.54
>2012	545	20.58	329	19.87	216	21.77
Transplant type						
Heart	135	5.10	135	8.15	n.a	n.a
Kidney	772	29.15	772	46.62	n.a	n.a
Liver	436	16.47	436	26.33	n.a	n.a
Lung	313	11.82	313	18.90	n.a	n.a
NMAT	399	15.07	n.a	n.a	399	40.22
MACT	552	20.85	n.a	n.a	552	55.65
UCBT	41	1.55	n.a	n.a	41	4.13
Number of transplantations						
1	2551	96.34	1576	95.17	975	98.29
≥2	97	3.66	80	4.83	17	1.71
Pre-transplant cancer						
Yes	1050	39.65	162	9.78	888	89.52
No	1598	60.35	1494	90.22	104	10.48

Abbreviations: SOT, solid organ transplantation; HSCT, haematopoietic stem cell transplantation; NMAT, non-myeloablative HSCT; MACT, myeloablative HSCT; UCBT, umbilical cord blood donor HSCT.

Table 2. Adjusted incidence rate ratios for any cancer among SOT and HSCT.

Characteristics	*Incidence rate ratios (95% CI) of any cancer*	
	SOT	HSCT
Gender		
Male	Ref.	Ref.
Female	1.06 (0.78-1.46)	0.85 (0.50-1.46)
Age at transplantation, years		
≤19	Ref.	Ref.
20-≤34	1.86 (0.60-5.76)	2.90 (0.39-21.66)
35-≤50	3.27 (1.16-9.25)	9.94 (1.88-52.46)
>50	9.82 (3.58-26.93)	26.40 (5.35-130.25)
Transplant year		
≤2006	Ref.	Ref.
2007-≤09	0.60 (0.39-0.92)	0.94 (0.44-2.04)
2010-≤12	0.63 (0.42-0.94)	0.90 (0.41-2.00)
>2012	0.42 (0.24-0.74)	0.75 (0.31-1.86)
Transplant type		
Kidney	Ref.	n.a
Heart	0.81 (0.40-1.65)	n.a
Liver	0.74 (0.49-1.12)	n.a
Lung	1.68 (1.02-2.76)	n.a
Adult donor	n.a	Ref.
UCBT	n.a	2.62 (0.98-7.04)
Number of transplantations		
1	Ref.	Ref.
≥2	1.34 (0.55-3.29)	9.27 (2.05-41.96)
Pre-transplant cancer		
No	Ref.	Ref.
Yes	0.74 (0.43-1.30).	0.55 (0.15-2.11)
Charlson comorbidity index, per increased score	1.05 (0.93-1.18)	0.97 (0.77-1.22)
Current CRP		
Normal value*	Ref.	Ref.
Elevated value	2.25 (1.60-3.17)	2.17 (1.26-3.75)

Abbreviations: SOT, solid organ transplantation; HSCT, haematopoietic stem cell transplantation; ref., reference group; UCBT, umbilical cord blood donor transplantation; CRP, C reactive protein.
Model adjusted for variables in the table. * Normal values of CRP: <10 mg/L.

Table 3A. Association between Ever vs. Never Exposure of Immunosuppressive Medications and Excess Cancer Rates among SOT.

Drug *Ever exposed*	*Cancer* *N (%)*	*Non-Cancer* *N (%)*	*Univariate analysis* *Incidence rate ratios (95% CI)*	*Multivariate analysis* *Incidence rate ratios (95% CI)*
Anti-thymocyte globulin				
Yes	55 (30)	381 (28)	1.12 (0.82 – 1.53)	1.12 (0.70 – 1.79)
No	130 (70)	963 (72)	1.00	1.00
Azathioprine				
Yes	66 (36)	382 (28)	1.42 (1.05 – 1.92)	1.34 (0.90 – 2.00)
No	119 (64)	962 (72)	1.00	1.00
Cyclosporine				
Yes	125 (68)	706 (53)	1.51 (1.11 – 2.06)	1.06 (0.73 – 1.54)
No	60 (32)	638 (47)	1.00	1.00
Tacrolimus				
Yes	96 (52)	904 (67)	0.66 (0.50 – 0.88)	0.95 (0.69 – 1.31)
No	89 (48)	440 (33)	1.00	1.00
Mycophenolic acid				
Yes	152 (82)	1129 (84)	0.78 (0.54 – 1.12)	1.27 (0.79 – 2.03)
No	33 (18)	215 (16)	1.00	1.00
Steroids				
Yes	183 (99)	1291 (96)	2.56 (0.62 – 10.52)	3.06 (0.72 – 13.10)
No	2 (1)	53 (4)	1.00	1.00
Everolimus				
Yes	26 (14)	219 (16)	1.03 (0.68 – 1.56)	0.99 (0.64 – 1.53)
No	159 (86)	1125 (84)	1.00	1.00
Sirolimus				
Yes	7 (4)	20 (1)	1.47 (0.72 – 2.98)	1.30 (0.64 – 2.60)
No	178 (96)	1324 (99)	1.00	1.00

Multivariate analyses adjusted for age, gender, transplant type, number of transplantations, Charlson score, calendar year, pre-transplant cancer.

Prescription data was available from 2009 and onwards, and thus the number of cases and controls does not add up to the total number of SOTs included in this study.

Table 3B. Association between Ever vs. Never Exposure of Immunosuppressive Medications and Excess Cancer Rates among HSCT.

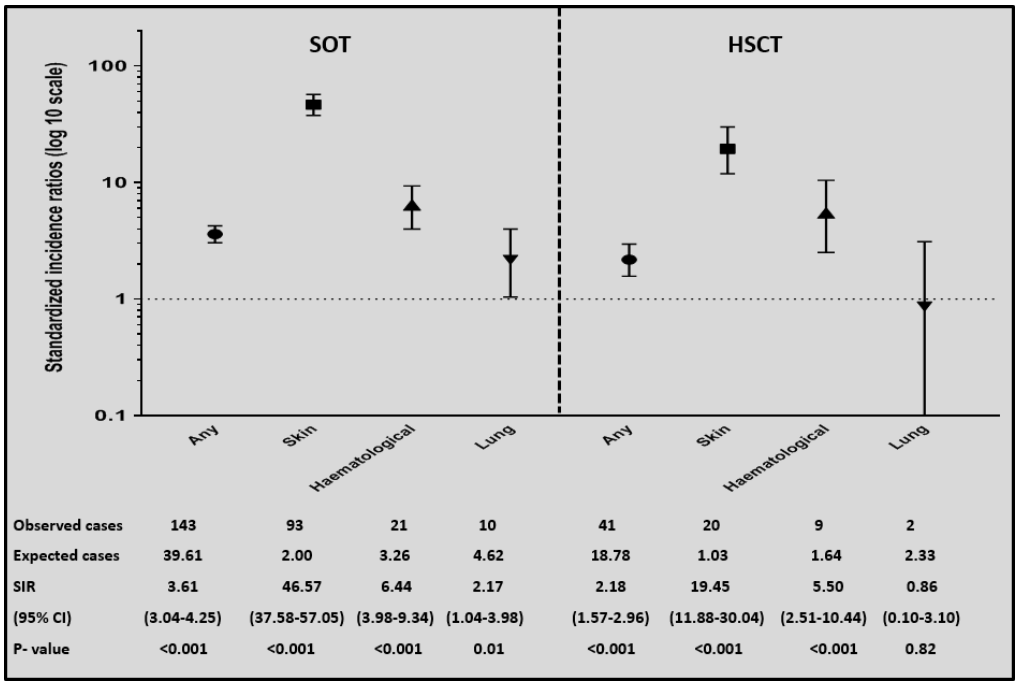
Drug *Ever exposed*	*Cancer* *N (%)*	*Non-Cancer* *N (%)*	*Univariate analysis* *Incidence rate ratios (95% CI)*	*Multivariate analysis* *Incidence rate ratios (95% CI)*
Anti-thymocyte globulin				
Yes	5 (7)	81 (9)	0.84 (0.33-2.14)	1.42 (0.60-3.34)
No	67 (93)	772 (91)	1.00	1.00
Azathioprine				
Yes	1 (1)	5 (1)	2.23 (0.39-12.72)	2.93 (0.83-10.37)
No	71 (99)	848 (99)	1.00	1.00
Cyclosporine				
Yes	37 (51)	594 (70)	0.44 (0.28-0.70)	0.87 (0.48-1.59)
No	35 (49)	259 (30)	1.00	1.00
Tacrolimus				
Yes	48 (67)	405 (47)	2.43 (1.49-3.96)	1.17 (0.62-2.23)
No	24 (33)	448 (53)	1.00	1.00
Mycophenolic acid				
Yes	58 (81)	475 (56)	3.61 (2.02-6.46)	1.08 (0.56-2.07)
No	14 (19)	378 (44)	1.00	1.00
Steroids				
Yes	63 (88)	735 (86)	1.28 (0.64-2.58)	1.50 (0.76-2.98)
No	9 (13)	118 (14)	1.00	1.00
Sirolimus				
Yes	22 (31)	185 (22)	1.64 (1.00-2.69)	1.15 (0.69-1.92)
No	50 (69)	668 (78)	1.00	1.00

Multivariate analyses adjusted for age, gender, transplant type, number of transplantations, Charlson score, calendar year, pre-transplant cancer.

No HSCTs were ever exposed to Everolimus and this immunosuppressant was thus not included in this table.

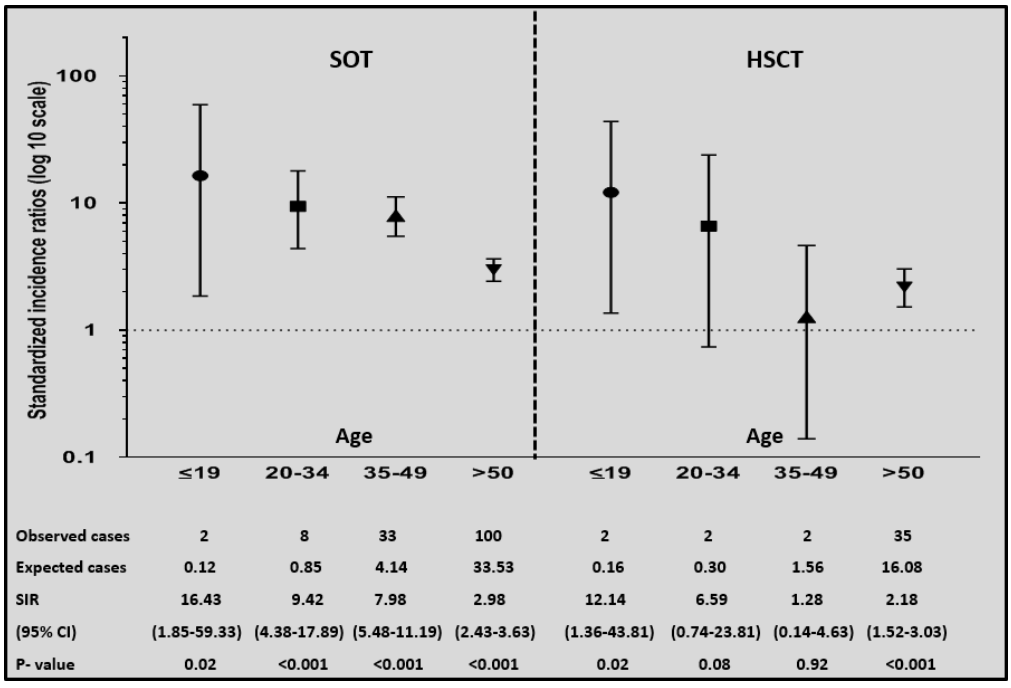
Prescription data was available from 2009 and onwards, and thus the number of cases and controls does not add up to the total number of HSCTs included in this study.

Figure 1A. Standardized incidence ratio of cancer after solid organ (SOT) and haematopoietic stem cell transplantation (HSCT) compared to the Danish population, according to cancer type.



Observed cases	143	93	21	10	41	20	9	2
Expected cases	39.61	2.00	3.26	4.62	18.78	1.03	1.64	2.33
SIR	3.61	46.57	6.44	2.17	2.18	19.45	5.50	0.86
(95% CI)	(3.04-4.25)	(37.58-57.05)	(3.98-9.34)	(1.04-3.98)	(1.57-2.96)	(11.88-30.04)	(2.51-10.44)	(0.10-3.10)
P- value	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	<0.001	0.82

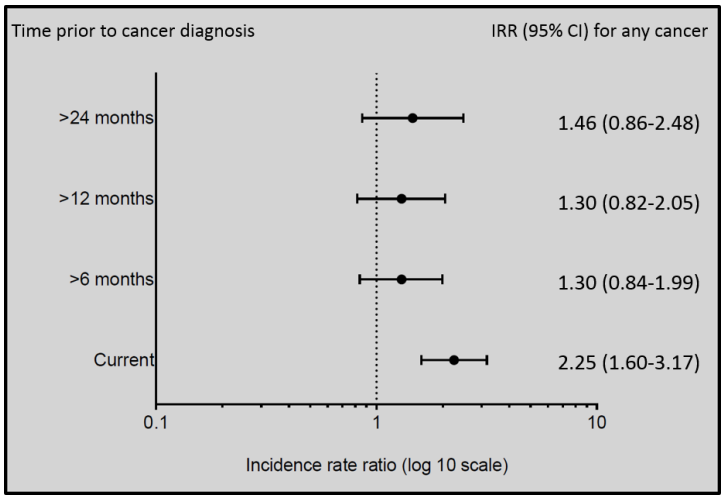
Figure 1B. Standardized incidence ratio of cancer after solid organ (SOT) and haematopoietic stem cell transplantation (HSCT) compared to the Danish population, according to age at cancer diagnosis.



Observed cases	2	8	33	100	2	2	2	35
Expected cases	0.12	0.85	4.14	33.53	0.16	0.30	1.56	16.08
SIR	16.43	9.42	7.98	2.98	12.14	6.59	1.28	2.18
(95% CI)	(1.85-59.33)	(4.38-17.89)	(5.48-11.19)	(2.43-3.63)	(1.36-43.81)	(0.74-23.81)	(0.14-4.63)	(1.52-3.03)
P- value	0.02	<0.001	<0.001	<0.001	0.02	0.08	0.92	<0.001

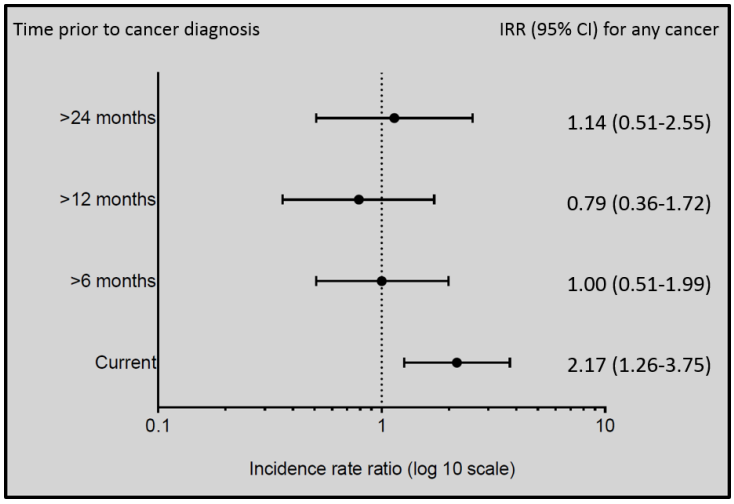
Abbreviation: SOT, Solid organ transplantation; HSCT, haematopoietic stem cell transplantation; SIR, standardized incidence ratio; CI, confidence interval.

Figure 2A. Incidence rate ratios of any cancer at various time points prior to cancer diagnosis of elevated CRP compared to normal values among SOT.



Abbreviations: C reactive protein, CRP.
Models adjusted for gender, age, transplant year, transplant type, number of transplantations, pre-transplant cancer, Charlson score.

Figure 2B. Incidence rate ratios of any cancer at various time points prior to cancer diagnosis of elevated CRP compared to normal values among HSCT.



Abbreviations: C reactive protein, CRP.
Models adjusted for gender, age, transplant year, transplant type, number of transplantations, pre-transplant cancer, Charlson score.