Post-transplant malignant neoplasia associated with cyclosporine-based immunotherapy: prevalence, risk factors and survival in feline renal transplant recipients

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Abstract

The study objective was to compare the prevalence of malignant neoplasia in feline renal transplant recipients (n = 111) with a control population of cats that did not receive transplantation (n = 142); and to determine whether the development of post-transplant malignant neoplasia (PTMN) affects long-term survival. Twenty-five (22.5%) renal transplant recipients were diagnosed with PTMN, and of those 14 (56%) were diagnosed with lymphoma. The overall survival time in cats that developed PTMN following renal transplantation (median 646 days, IQR 433 – 1620 days) was not significantly different from the survival time in cats that did not develop PTMN (median 728 days, IQR 201 – 1942 days), although median survival after diagnosis of PTMN was only 13 days. Six control cats (4.2%) were diagnosed with malignant neoplasia. Compared to the control population, transplant cats had a 6.6 times higher odds of developing malignant neoplasia and a 6.7 times higher odds of developing lymphoma.

Introduction

The advent of cyclosporine-based immunosuppressive therapy has made renal allograft transplantation a therapeutic option for cats with end-stage renal failure.1 The benefit of cyclosporine with regards to improved graft survival has been clearly elucidated in human transplant recipients.2,3 However, deleterious effects of chronic immunosuppression in these patients have also been identified including systemic toxicity,4 increased risk of infectious disease,5–7 and development of de novo malignancies.8–14 It has been well documented that in human medicine, immunosuppressed organ allograft recipients have a three- to four-fold increased risk of developing tumours, with the risk of developing certain cancers being increased several hundred-fold.15 Interestingly, the most common malignancies seen in the general population are not the same as those seen with increased incidence in immunosuppressed human transplant recipients. Instead, there is a higher frequency of relatively rare tumours, including post-transplant lymphoma and lymphoproliferative disorders.15 The development of these de novo malignancies in human transplant patients is mainly correlated with the dose and duration of immune suppressive therapy.5,16–19 However, certain patient factors such as age and gender appear to be contributing factors, as well.5,19,20

There have been two veterinary publications describing the development of malignant neoplasia in feline renal transplant recipients...
on cyclosporine-based immunosuppressive protocols. Wooldridge et al. (2002) found a 9.5% de novo malignant neoplasia rate in the 95 feline renal transplant recipients analysed. The median survival time of these cats (14 months) was significantly shorter than for cats that died of causes other than development of malignancy (22 months). In a more recent paper, Schmiedt et al. (2009) reported a 24% incidence of malignant neoplasia in 45 cats following renal transplantation. However, the development of malignancy did not influence long-term survival in this group of cats.

The inconsistent findings of the above-mentioned reports, both with respect to the prevalence and incidence of post-transplant malignant disease as well as its effect on long-term survival in feline transplant recipients, bring to light the need for additional research on the consequences of chronic immunosuppressive therapy in this population of cats. To this end, the goal of this study was to describe the prevalence and biological behavior of post-transplant malignant neoplasia (PTMN) in cats that underwent renal allograft transplantation at the University of Pennsylvania, to identify risk factors associated with the development of malignant disease, and to determine whether development of malignancy affects long-term survival following renal transplantation.

**Materials and methods**

**Case selection**

The medical records of the Veterinary Hospital of the University of Pennsylvania were reviewed to identify all cats that underwent renal transplantation between 1998 and 2010. Cats were excluded from the study if they did not survive to discharge following the transplant procedure. Cats diagnosed with neoplasia during pre-operative screening (as described below) were defined as unacceptable renal transplantation candidates. Two cats were diagnosed with neoplasia during abdominal exploratory at the time of renal transplantation [lymphoma (1), intestinal mast cell tumor (1)] and were therefore excluded from the study.

**Pre-operative screening**

All cats were thoroughly screened prior to surgery for underlying systemic, infectious, and/or neoplastic disease. Pre-operative evaluation included clinicopathologic testing (complete blood count, serum biochemistry, thyroid hormone level, blood type and cross-match), urine testing (urinalysis, urine culture and urine protein:creatinine ratio), abdominal imaging (radiographs and ultrasound), infectious disease testing (feline leukemia virus, feline immunodeficiency virus, and toxoplasmosis immunoglobulin G and immunoglobulin M titers) and evaluation for cardiovascular disease (thoracic radiographs, echocardiogram, electrocardiogram and blood pressure).

**Immunosuppression**

In cats deemed suitable for renal transplantation, cyclosporine therapy was initiated 24–96h prior to surgery at a dose of 1–4 mg/kg PO q12h. A 12h, whole-blood, trough concentration was measured on or the day before the transplant procedure to ensure adequate blood concentration (300–500 ng/mL) and allow for dose adjustment if necessary. This level was maintained for 1–3 months after surgery and then tapered to approximately 250 ng/mL for maintenance therapy. Cyclosporine trough levels were measured once weekly post-operatively until stable levels were achieved, at which point quarter yearly level checks were recommended. Prednisolone was also administered to transplant recipients starting the morning of surgery at a dose of 0.5–1.0 mg/kg PO q12h. This dose was continued for the first 3 months post-operatively and then tapered to once daily thereafter.

**Medical records review**

Information collected from the medical records of the transplant cats included signalment, weight at the time of renal transplantation and survival beyond transplantation for cats that had died or been euthanized prior to the time of follow-up (defined as the last client contact during data collection for the study). If a cat received greater than one allograft during the study period, all outcome
measures were calculated from the initial transplant date. Median blood cyclosporine levels at 1 week, 1 month, 3 months, 6 months, and one year post-operatively were recorded for each cat. In cats diagnosed with PTMN, the time at which the diagnosis was made relative to the transplant procedure and the time between diagnosis and death were evaluated. In addition, the cytologic or histologic findings in these cats were recorded.

**Control population**

A medical records search was conducted to identify all cats diagnosed with chronic renal disease between 1998 and 2010 that did not undergo renal transplantation, and a Student’s t-test was used to create an age-matched control population from this group of cats. A minimum of two hospital visits and two months of follow-up (after diagnosis of neoplasia or after last hospital visitation) were required for inclusion into the control group. Cats were excluded from the control population if they had a confirmed diagnosis of neoplastic disease at the time of presentation to the hospital and/or if they had a positive FeLV or FIV status. For control cats diagnosed with malignant neoplasia, time to disease onset was defined as the interval between first hospital visitation and diagnosis of neoplasia.

**Statistical analysis**

Statistical analysis was performed using a commercial software package (SPSS version 19.0, for Macs, Chicago, IL). The student’s t-test was used to match the transplant population and control population for age and to evaluate age differences in malignant neoplasia development in the transplant and control populations. A Pearson’s chi-squared statistic was used to determine differences between the transplant and control populations with respect to sex, breed, overall malignant neoplasia rate and lymphoma rate. A Fisher’s exact probability test was used to determine the incidence of lymphoma in the transplant population compared to the control population.

Survival time of the transplant cats was determined using Kaplan–Meier analysis. A cox regression analysis was performed to determine the effect of PTMN on survival after renal transplantation, to determine survival differences based on the type of PTMN (lymphoma versus other) and to identify risk factors for decreased survival time. A logistic regression was used to evaluate risk factors for PTMN development.

Data are reported as median and IQR where applicable. Odds ratios were reported with 95% confidence intervals (CIs) where appropriate. Significance level for all tests was set at $P < 0.05$.

**Results**

**Transplant population**

A total of 126 cats underwent renal transplantation at the University of Pennsylvania during the 12-year study period. Of these cats, 13 (10.3%) did not survive to discharge and were excluded from the study. Two additional cats were excluded due to the presence of neoplastic disease identified at the time of transplantation. Thus, a total of 111 cats met the study inclusion criteria. Of these, 35 were spayed females, 75 were castrated males, and 1 was an intact female. An ovariohysterectomy was performed at the time of renal transplantation in the single intact female patient and therefore all female cats were spayed post-transplantation. The majority of cats (81/111, 73%) were domestic short hairs. Other predominant breeds included in the study population were Siamese (9 cats, 8.1%), domestic long hair (8 cats, 7.2%), and Abyssinian (3 cats, 2.7%). Two cats of the following breeds: Maine Coon, Persian, and Tonkinese (each breed represented 1.8% of total population), and one of each of the following breeds: Himalayan, Oriental shorthair, Ragdoll and Russian Blue were also included in the transplantation cohort. The median age and weight at the time of renal transplantation were 7.4 years (interquartile range: 5.5–9.9 years) and 3.6 kg (interquartile range: 3–4.28 kg), respectively. Twenty-three of the cats (20.7%) were still alive at the time of follow-up. The median survival time after transplantation in this group of cats was 728 days (IQR: 303–1756 days). Eighty-eight (79.3%) of the cats were deceased at the time of follow-up, with the median survival time in this group being 568 days (IQR: 200–1140 days). The only factor that significantly influenced survival time in transplant recipients was age at the time of surgery; specifically,
Table 1. Cyclosporine (CsA) measurements (ng/mL) in the transplant population.

<table>
<thead>
<tr>
<th>CsA measurements</th>
<th>n</th>
<th>Median (ng/mL)</th>
<th>Interquartile Range (ng/mL)</th>
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<tbody>
<tr>
<td>1 week</td>
<td>97</td>
<td>294</td>
<td>156–462</td>
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<tr>
<td>1 month</td>
<td>101</td>
<td>313</td>
<td>235–426</td>
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<tr>
<td>3 months</td>
<td>95</td>
<td>353</td>
<td>283–434</td>
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<tr>
<td>6 months</td>
<td>88</td>
<td>388</td>
<td>331–471</td>
</tr>
<tr>
<td>1 year</td>
<td>387</td>
<td>387</td>
<td>348–488</td>
</tr>
<tr>
<td>At PTMN diagnosis</td>
<td>25</td>
<td>383</td>
<td>282–574</td>
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cats that were younger (less than 7.8 years) at the time of transplantation had an increased survival time relative to older cats ($P = 0.018$, OR = 1.1, 95% CI = 1.015–1.180). Patient sex and weight at the time of transplantation had no effect on survival post-operatively.

Serial cyclosporine measurements in the transplant population are outlined in Table 1. Fifty-six cats (50%) had at least one recorded cyclosporine level greater than 1000 ng/mL. Neither the median cyclosporine level at each recorded time interval nor having at least one cyclosporine level greater than 1000 ng/mL was correlated with survival. Ten cats (9%) required a second transplantation during the study period. This also had no effect on survival post-operatively.

PTMN

Of the transplanted cats included in the study, 25/111 (22.5%) developed PTMN (Figure 2). Of these, 14 (56%) were diagnosed with lymphoma, making this the most commonly diagnosed post-transplant malignancy. The overall rate of post-transplant lymphoma in the transplant population was 12.6% (14/111 cats). Other malignancies represented included fibrosarcoma (2), renal carcinoma (2), adrenal cortical carcinoma (1), nasal adenocarcinoma (1), pulmonary carcinoma (1), nasal squamous cell carcinoma (1), menigioma (1), cutaneous mast cell tumor (1), splenic mast cell tumor (1), malignant melanoma (1), renal sarcoma (1) and neuroendocrine tumor (1). When analysed by univariate analysis or as part of a logistic regression model, none of the factors evaluated (age, sex, weight, cyclosporine levels or multiple allograft transplants) were associated with the development of PTMN.

In cats diagnosed with PTMN, a diagnosis was made a median of 631 days following transplantation (IQR: 379–1540 days). When comparing the onset of lymphoma (median 631 days post-transplant, IQR: 406–1540 days) to the onset of all other post-transplant malignancies (median 597 days, IQR: 167–1712 days), no significant difference was identified ($P = 0.480$). In transplant cats with PTMN, the median survival following diagnosis was 13 days (IQR: 0–87 days); cause of death was related to malignancy in all cases. In six cats (24%), a diagnosis was made at the time of necropsy [lymphoma (4), renal sarcoma (1) and renal carcinoma (1)]. The median survival time following diagnosis of lymphoma (3 days, IQR: 0–24 days) was not significantly different ($P = 0.154$) from the median survival time following diagnosis of other malignancies (37 days, IQR: 3–226 days).

The overall median survival time of cats that developed PTMN following renal transplantation was 646 days (IQR: 433–1620 days). This was not significantly different ($P = 0.617$) from the median survival time of transplanted cats that did not develop PTMN (728 days, IQR: 201–1942 days, Figure 1). Similarly, when comparing the overall median survival in transplant cats diagnosed with lymphoma (1096 days, IQR: 579–1620 days) to transplant cats diagnosed with other malignancies (495 days, IQR: 236–1681 days), no significant difference was appreciated ($P = 0.646$).

Control population

A total of 297 cats diagnosed with chronic renal disease that had not undergone renal transplantation were identified between 1998 and 2010. Of these, nine were excluded from further evaluation due to significant clinical diagnostic suspicion or histologic confirmation of neoplasia at the time of initial presentation to the veterinary hospital. An additional eight cats were excluded due to positive FIV status. Of the remaining 280 cats, the 138 oldest were excluded to provide an age-matched control population ($P > 0.05$). The control population had a total of 142 cats with a median age of 9.0 years (IQR: 6.4–11.2 years). Sixty-two (43.7%) of the control cats were spayed females and eighty (56.3%) were
neutered males; this was not significantly different from the study population ($P = 0.069$). Similar to the renal transplantation group, 105 of the control cats (73.9%) were domestic short hairs ($P = 0.862$). The median follow-up time for control cats was 363 days (IQR: 158–853 days).

Malignant neoplasia was diagnosed in six of the control cats (4.2%) during the study period, which was significantly lower than the prevalence in the transplant population ($P < 0.001$, Figure 2). Specifically, transplant cats had a 6.6 times higher odds of developing malignant neoplasia compared to the control population (95% CI: 2.6–16.7). Of the six control cats diagnosed with neoplastic disease, three were diagnosed with lymphoma. Thus, the overall incidence of lymphoma in the control population was 3/142 (2.1%). This was significantly lower than the incidence of lymphoma in the post-transplant population ($P = 0.001$), with transplant cats having a 6.7 times higher odds of developing lymphoma compared to control cats (95% CI: 1.9–23.9). Other neoplasias represented in the control population included ceruminous gland adenocarcinoma (1), intestinal adenocarcinoma (1) and meningioma (1). A necropsy was not performed in any of the control cases.

**Discussion**

Clinicians caring for transplant recipients face a dilemma with regards to how to balance the advantage of preserving graft function against the side effects of chronic immunosuppression. Of significant concern is the development of de novo neoplasia in transplant recipients receiving immunosuppressive therapy. Cyclosporine, the calcineurin inhibitor and attenuator of T-cell mediated cytotoxic allograft responses, has been implicated in the development of post-transplant neoplasia in the majority of human studies documenting this phenomenon. Numerous mechanisms have been proposed for how cyclosporine may potentiate malignancy in transplant recipients, including increasing the likelihood of de novo infection and recrudescence of oncogenic viruses, reducing immunosurveillance and neoplastic cell clearance, promoting DNA mutations and tumor angiogenesis and increasing morphological traits characteristic of malignancy in both transformed and non-transformed cells.

There have been two previous reports documenting PTMN in feline transplant recipients receiving cyclosporine-based immunosuppressive therapy, with somewhat discordant results. In the first study examining 95 feline renal transplant recipients by Wooldridge et al. (2002), a PTMN prevalence of 9.5% was documented, with 44% of cases the result of lymphoma. It should be noted that a control population of cats was not used in this study for comparison. In this study, the development of PTMN had a negative effect on survival in transplant recipients compared to those not developing PTMN. Specifically, cats that developed PTMN had a median survival time of 14 months after renal transplantation compared with a median survival time of 22 months after renal transplantation for cats that died of other disease processes. Our results showed a similar survival in renal transplant patients that did not develop malignant neoplasia (median survival of 24.3 months) to those in the Wooldridge et al. study. In contrast, median survival in our patients that developed PTMN was 21.5 months (7.5 months longer than the Wooldridge study), which did not differ significantly from those patients that did not
develop PTMN ($P > 0.05$). A more recent study involving 45 feline renal transplant recipients by Schmiedt et al. (2009) reported a 24% incidence of PTMN, with lymphoma overrepresented (34%). Importantly, the development of PTMN in that study did not result in a survival disadvantage when compared with transplanted cats that did not develop PTMN or a control population of cats. In concordance with Schmiedt et al., we report a 22.5% prevalence of de novo neoplasia in the 111 feline transplant recipients from the University of Pennsylvania evaluated in this study. We also found no significant survival difference between transplant cats diagnosed with malignancy and those that were not. Notably, although the Woolridge et al. study documented a significantly shorter survival in transplant patients that developed PTMN compared to those that did not, median survival time in cats with PTMN was 5 months beyond the median time to diagnosis of neoplasia. In contrast, our patients and those in the Schmiedt et al. study had a very limited survival following diagnosis of PTMN, 13 days and 15 days, respectively. In our study, patient deaths in the PTMN population were all related to malignancy. Therefore, it is important to consider these short survival times following a diagnosis of PTMN, despite a lack of statistical significance in survival of feline renal transplant patients that develop PTMN and those that do not. Future studies that compare the clinical course of neoplastic disease in transplant recipients to cats diagnosed with neoplasia disease that do not undergo transplantation may prove interesting; one could speculate that the short survival following PTMN diagnosis could be due to the development of more aggressive forms of neoplasia in this cat population. Studies evaluating patient- and treatment-related prognostic factors in cats that do develop PTMN would help clinicians formulate the best strategies for treating these patients, with the goal of improving long-term outcome.

Similar to Schmiedt et al., lymphoma comprised the majority of malignancies diagnosed in our study. In both studies, renal transplant recipients had approximately 6 times higher odds for developing malignancy compared with a control population of cats. None of the other non-lymphoma PTMN diagnoses occurred more than once in either study and there was not overlap between the studies with respect to non-lymphoma PTMN diagnoses. In human renal transplant patients, skin and lip carcinomas are the most common malignancies seen post-transplantation, followed by carcinomas in other locations and lymphoproliferative disorders. Interestingly, Non-Hodgkin lymphoma is the most common neoplastic disease seen in the first year following renal transplantation of human patients; the incidence then falls and remains reasonably constant thereafter at 0.06–0.08% per year. This finding suggests that the development of lymphoma after renal transplantation in humans is likely dependent on underlying tumor initiating factors present at the time of exposure to immunosuppressants.
For all other post-transplant malignancies, there is an increasing incidence with duration of follow-up. In our cat population, the onset of lymphoma was not significantly different from the onset of all other malignancies, and no patient-specific factors were associated with the development of this neoplasm. These differences between the human and feline renal transplant populations with regards to post-transplant lymphoma may reflect differences in neoplastic disease pathogenesis between the species.

*De novo* malignancy is an important cause of morbidity and mortality in human kidney transplant recipients and ranges in prevalence from 18 to 40%.16–18,20 The incidence of PTMN in human patients has been correlated with the dosage and type of immunosuppressive therapy, which has led to alterations in the drug protocols used by many transplant hospitals.16–18,32,33 In this study, there was no significant difference in cyclosporine levels between transplant cats that developed PTMN and those that did not; and the incidence of PTMN was not significantly associated with cyclosporine levels in the transplant population. Interestingly, the immunosuppressive protocol used in this study (twice daily cyclosporine dosing) was similar to that used by Wooldridge et al.,21 despite the difference in PTMN prevalence between the two cat populations. In contrast, the immunosuppressive regimen used in this study differed from the regimen used by Schmiedt et al.22 (78% of cats were treated with ketoconazole in addition to cyclosporine). Although no correlation between cyclosporine levels and risk of PTMN development was identified at the drug levels maintained in our feline transplant population, we believe that the drug likely has similar carcinogenic effects to those documented in humans on cyclosporine-based immunosuppressive regimens.23–27 Additional research is needed to elucidate the true relationship between immunosuppressive therapies and malignant neoplastic disease, as the relative carcinogenicity of various immunosuppressive agents or combinations of agents is not well understood.

Certain patient factors have also been associated with the development of PTMN in human transplant recipients, including increasing age at the time of renal transplantation and male sex.5,19,20 Although age at the time of transplantation was found to negatively impact survival time in feline renal transplant patients in our study, in contrast to humans, increasing age was not found to be associated with development of PTMN in our cat population. Furthermore, we found no association between sex of patient and development of PTMN, although it should be noted that all but one cat was neutered at the time of transplantation. Future studies are needed to identify patient-associated risk factors to predict which feline patients are at a heightened risk of developing PTMN. Not only would this influence post-operative monitoring and treatment in this subset of cats, but it would be informative in the context of pre-operative owner counseling.

Inherent limitations in this study exist due to its retrospective nature. In addition, although this is the largest study to date examining PTMN in feline renal transplant patients, the small sample size of transplant cats diagnosed with PTMN may have contributed to the lack of significant difference in several variables evaluated in this study. Another limitation and a potential source of bias in this study was the control population used, which was an age-matched group of cats that had been diagnosed with chronic renal disease. Given the nature of transplant medicine and the owners that pursue this advanced treatment option, the control group of chronic renal failure cats that received ongoing medical management may have received less aggressive diagnostic and therapeutic follow-up in the course of their treatments, which may have contributed to the lower cancer diagnosis rate. Although a potentially more appropriate reference population may have been feline patients diagnosed with chronic renal failure and maintained on dialysis, given that the degree of renal disease, diagnostics, and veterinary monitoring in these patients would be more comparable to that of our study population,13,34,35 the relatively limited number of cases receiving dialysis makes this impractical. Future studies are needed to better elucidate the underlying risk factors and prognostic indicators associated with PTMN in feline renal transplant recipients. Furthermore, critique and modification of the currently used immunosuppressive regimens in feline transplant medicine may help to reduce
the prevalence of PTMN as well as the incidence of other negative side-effects associated with chronic immunosuppressive therapy.

In conclusion, the prevalence of PTMN was high in this population of feline transplant recipients (22.5%), with lymphoma predominating (56% of all PTMN cases). The high prevalence of malignancy seen in this group of cats emphasizes the need for routine and long-term follow-up in transplant recipients as well as the continued recommendation for thorough pre-transplantation screening for occult neoplasia. No patient-specific factors were found to be associated with the development of PTMN, and PTMN had no significant effect on long-term survival in renal transplant recipients, although the median survival time after PTMN diagnosis was only 13 days. Nonetheless, owners considering renal transplantation as a treatment option for their cats should be made aware of the potential for malignancy following the transplant procedure, as this complication would necessitate additional life-long monitoring, veterinary attention, and treatment.

References
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