Cyclophosphamide Exposure Pretransplant Is Associated With Complications in the First Year After Kidney Transplant

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Abstract

Objectives: **Some patients needing a kidney transplant have used cyclophosphamide before the transplant. Long-term bone marrow damage associated with cyclophosphamide could manifest with myelotoxic complications after transplant in the context of the immunosuppressant, but evidence for this has not been published.**

Materials and Methods: **We performed a retrospective, single-center analysis of renal transplant recipients with prior cyclophosphamide exposure and compared posttransplant short-term outcomes to a random control group (clinical outcomes identified by searching automated electronic databases).**

Results: **Sixteen recipients had taken cyclophosphamide before the transplant and were compared with a control group of 32 patients. Hospitalization rates were equal, and although there were 3 times more hospitalizations secondary to an infective course in the cyclophosphamide group, this did not achieve significance (0.63 vs 0.22; *P* = .147). There was no difference in rates of bacteriuria, *cytomegalovirus*, or *Polyomavirus*. The cyclophosphamide group was at significantly greater risk of needing a blood transfusion immediately after the transplant (average number of units of blood per patient, 0.44 vs 0.19; *P* = .038). Also, they were 3 times more likely to require anemia treatments 1 year after the transplant (average number of anemia treatment medications, 0.75 vs 0.25; *P* = .014). Full blood count parameters, graft function, and graft and patient survival at 1 year posttransplant were equal.**

Conclusions: **Evidence suggests that pretransplant administration of cyclophosphamide is associated with adverse short-term outcomes posttransplant. Further analyses are warranted to investigate these preliminary findings to determine whether myelosuppressive immunosuppressant should be modified in the context of prior cyclophosphamide exposure.**

Key words: *Cytotoxic therapy, Infection, Malignancy, Posttransplant Anemia, Glomerulonephritis*

Introduction

The incidence of infection and malignancy after a transplant are increasing and challenging the position of cardiovascular disease as the leading cause of death with a functioning graft.[1] Additionally, posttransplant anemia is highly prevalent and is associated with morbidity and mortality.[2] Although many causative factors have been documented for these complications, pretransplant exposure to the myelotoxic alkylating agent, cyclophosphamide, is often overlooked. This is despite the fact that biopsy-proven glomerulonephritis is becoming the leading cause of end-stage renal disease in the United Kingdom.[3] It is used for a group of diagnoses in which myelotoxic cyclophosphamide therapy may be indicated for persons who subsequently survive to reach end-stage renal disease. With transplant being the renal replacement therapy of choice in most patients with end-stage kidney disease, a select group of patients with pretransplant cyclophosphamide exposure is likely to subsequently receive a kidney transplant.

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Experimental and Clinical Transplantation (2011) 4: 236-240

There is no evidence that a history of cyclophosphamide use before a transplant means that the patient will have myelotoxic complications after transplant; this represents a significant paucity in transplant literature. We hypothesized the combination of pretransplant exposure to cyclophosphamide and immunosuppressants posttransplant would manifest with increased myelosuppressive complications. Therefore, we conducted a retrospective analysis of our recent renal transplant recipients to investigate this further during the early postrenal transplant period.

Materials and Methods

We performed a retrospective analysis of all renal transplants performed between the years of 2004 and 2008 at the Queen Elizabeth Hospital Birmingham (QEHB). Prior to the study, the protocol was approved by our local institutional ethics committee, and conforms with the ethical guidelines of the 1975 Helsinki Declaration. We included all renal transplant recipients under long-term follow-up at the QEHB with 1 year of follow-up, who demonstrated evidence of prior cyclophosphamide use. We did this by reviewing and identifying all documented causes of end-stage renal failure among recipients that could be associated with cyclophosphamide use (ie, glomerulonephritis, Goodpasture disease, antineutrophil cytoplasmic antibody positive vasculitis, lupus nephritis, IgA nephropathy, and unknown). We then looked for evidence of pretransplant cyclophosphamide use by reviewing individual case notes and clinic letters verifying use. Evidence of cyclophosphamide use was corroborated with pharmacy databases. This group is identified as the "CYCLO" group.

For each individual with evidence of pretransplant cyclophosphamide use, we randomly selected the recipient immediately before and after, chronologically, as a control patient (in a 2:1 cohort analysis). We called this group "CONTROL." Regarding immunosuppressants, before 2007, target trough levels for tacrolimus were 5 to 10 ng/mL and for cyclosporine, they were 100 to 200 ng/mL. Azathioprine was commenced at a dosage of 2 mg/kg daily; however, azathioprine had been supplanted on the unit immunosuppressive protocol in recent years with mycophenolate mofetil, starting at a dosage of 2 g daily. Regarding corticosteroids, everyone received intravenous methylprednisolone (500 mg) intraoperatively, followed by oral prednisolone at 20 mg daily. This was weaned down over 20 weeks to 5 mg if no signs of rejection occurred and if the allograft was a suitable match. Since 2007, our unit has adopted the low-dose tacrolimus regimen as standard immunosuppressant in accordance with the SYMPHONY trial.[4] Recipients deemed a high-risk for *cytomegalovirus* infection (donor+/recipient-) received 100 days of valganciclovir prophylaxis. Surveillance for bacteriuria was conducted preemptively with samples sent at each clinic visit. Other infections (eg, *Polyomavirus*) were investigated based on clinical suspicion. Biopsies were not protocol-based and were initiated by clinical decision in the context of transplant dysfunction with no obvious course.

All clinical data for analysis was retrospectively extracted from automated electronic databases, which collate clinical and biochemical information in real time, and corroborated with the clinical notes. Parameters investigated within or at 1-year posttransplant, unless otherwise stated, included total number of hospital admissions, hospital admission secondary to an infective course (or presumed infective), number of biopsies, blood transfusions during the first week after transplant, use of anemia agents (EPO, vitamin B_{12} injections, iron replacement), *cytomegalovirus* or *Polyomavirus* infections, full blood count parameters (hemoglobin, white blood cell count, platelet count), graft function (by estimating glomerular filtration rate using the modification of diet in renal disease formula), and death-censored graft and patient survival.

Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 18.0, SSPS Inc, Chicago, IL, USA). Normality of data was assessed using the Kolmogorov-Smirnov tests. Comparison of data between groups was made using unpaired *t* tests and the Mann-Whitney *U* test for parametric and nonparametric data. Categorical data was analyzed using the Pearson product moment correlation analysis or Spearman's rank correlation coefficient as appropriate. Values for $P < .05$ were considered statistically significant.

Results

Patient demographics

The details and demographics are shown in Table 1.

Table 1. Patient demographics for study participants.

Demographic	Cyclophosphamide group	Control group
Number of recipients	16	32
Age	43 ± 14	44 ± 15
Sex (% male)	37.5%	56.3%
Cause of renal failure		
ANCA + vasculitis	5	1
Goodpasture	4	0
SLE	3	1
MPGN	3	2
FSGS	1	2
IgA nephropathy	0	7
APKD	0	4
Diabetes mellitus	0	3
Hypertension	0	1
TB	0	1
Dysplastic kidneys	0	3
Reflux nephropathy	0	1
Unknown	0	6
Ethnicity		
White	81%	72%
Black	13%	19%
Indo-Asian	6%	9%

Abbreviations: ANCA, antineutrophil cytoplasmic antibody; APKD, adult polycystic kidney disease; FSGS, focal segmental glomerulosclerosis; MPGN, membranoproliferative glomerulonephritis; SLE, systemic lupus erythematosus; TB, tuberculosis.

Hospital admissions

There was no overall difference between number of hospital admissions in the first year after transplant between the CYCLO and CONTROL groups (admissions per patient, 2.2 vs 1.6; $P = .355$). The average number of days spent in the hospital at the time of transplant was no different between the CYCLO and CONTROL groups (days in hospital, 10.3 vs 10.9; $P = .886$). There were 3 times more hospital admissions secondary to an infective episode in the CYCLO group than there were in the CONTROL group, but this was not significant (infective admission per patient, 0.63 vs 0.22; $P = .147$).

Infections and malignancy

Although there appeared to be a clinically significant doubling in the number of urinary tract infections between the CYCLO and CONTROL groups, this was not significant (episodes per patient, 0.31 vs 0.16; $P = .386$). Neither was there any significant difference in the incidence of either *cytomegalovirus* or *Polyomavirus* viremia between the 2 groups in the first year after transplant. No evidence of malignancy was observed in any patient in the first year after transplant.

Anemia

There was no difference in pretransplant hemoglobin between groups. We did demonstrate a significantly greater risk of requiring blood transfusion immediately after the transplant during the in-patient stay in the CYCLO group compared with the CONTROL group (average number of units of blood per patient, 0.44 vs 0.19; $P = .038$). A review of the operative notes did not identify any significant intraoperative bleeding to account for this difference between the groups. The CYCLO group was 3 times more likely to require any combination of anemia treatments (iron replacements, folic acid, or EPO therapy) at 1 year after transplant compared with the CONTROL group (average number of anemia treatment medications, 0.75 vs 0.25; $P = .014$).

Full blood count parameters

At 1 year after the transplant, no significant difference was observed between the CYCLO and CONTROL groups regarding hemoglobin (12.3 g/L vs 12.9 g/L; $P = .845$), white blood cells (6.6×10^9/L vs 7.3×10^9/L; $P = .541$), or platelets (243×10^9/L vs 228×10^9/L; $P = .790$).

Immunosuppressants

Details regarding the immunosuppressant agents used are shown in Table 2. No difference in outcomes was observed when comparing tacrolimus to cyclosporine as the primary immunosuppressant. Similar results occurred when we compared mycophenolate mofetil to azathioprine, although the only 2 cases of *cytomegalovirus* in the CYCLO group occurred in patients on azathioprine ($P = .070$).

Graft function and patient/graft survival

There was no difference in the number of renal allograft biopsies performed per patient for investigating graft dysfunction (CYCLO vs CONTROL, 0.75 vs 0.47; $P = .200$) or graft function

Table 2. Transplant and immunosuppressant comparison.

Parameter	Cyclophosphamide group	Control group
Transplant source		
Live	31%	25%
Donation after brain death	63%	56%
Donation after cardiac death	6%	19%
Immunosuppressant		
Tacrolimus	63%	53%
Cyclosporine	37%	47%
Mycophenolate mofetil	56%	66%
Azathioprine	38%	34%
No secondary immunosuppressant (azathioprine or mycophenolate mofetil)	6%	0%
Corticosteroids	100%	97%
Sirolimus	0%	0%

(CYCLO vs CONTROL, 55.3 mL/min vs 58.6 mL/min; $P = .458$).

Additionally, no significant difference was observed in patient and graft survival between the groups. One-year patient survival between the CYCLO and CONTROL groups was 100% and 97%, with 1 death in the CONTROL group owing to septicemia. Death-censored graft survival was 88% and 97% in the CYCLO and CONTROL groups. Causes of graft loss in the CYCLO group were renal vein thrombosis and recurrent focal segmental glomerulonephritis, while the sole death-censored cause of graft loss in the CONTROL group was due to renal artery thrombosis.

Discussion

We believe the scarcity of data on this topic merits close analyses of the limited data generated by our analysis. Comparing outcomes between patients who had cyclophosphamide exposure pretransplant with those without cyclophosphamide exposure pretransplant showed no difference in the rates of hospitalization posttransplant, although there was a trend toward more hospital admissions secondary to an infectious episode. Despite the small numbers, we identified a statistically higher incidence of blood transfusions during the hospital admission at time of transplant in patients, and a significantly higher likelihood of them requiring pharmacological treatments for anemia 1 year after transplant in the recipient group with prior cyclophosphamide exposure compared with those who did not have prior cyclophosphamide exposure. There was no difference in graft function, death-censored graft survival, and patient survival.

Cyclophosphamide is one of the most commonly used alkylating agents in clinical medicine. The main effect of cyclophosphamide is due to its metabolite, phosphoramide mustard, formed in cells containing low levels of aldehyde dehydrogenase. Cyclophosphamide has relatively little typical chemotherapy toxicity on bone marrow stem cells, as they contain high levels aldehyde dehydrogenase. This protects bone marrow stem cells against the toxic effects of phosphoramide mustard and acrolein by converting aldophosphamide to carboxy - phosphamide, which does not give rise to the toxic metabolites.[5] Therefore, it appears likely that cyclophosphamide-induced damage to bone marrow occurs from DNA damage—phosphoramide mustard forms DNA cross-links between (interstrand cross-linkages) and within (intrastrand cross-linkages) DNA strands, at guanine N-7 positions, leading to cell death.[6] This theory is consistent with findings from Takeshita and Connor[7] who previously demonstrated the persistence of cyclophosphamide-induced chromosomal damage to bone marrow in murine models. They conducted cytogenic studies, used sister chromatid exchange analysis, and demonstrated persistent DNA damage in viable cells over successive posttreatment cycles. The persistence of bone marrow suppression also may be related to cyclophosphamide-induced damage to bone marrow stromal cells, which regulate the maturation and proliferation of pluripotent hemopoietic stem cells.[8]

In the context of transplant, long-term effects of cyclophosphamide-induced myelotoxicity potentially could be aggravated by an immunosuppressant, especially with the use of antiproliferative agents (such as mycophenolic acid, azathioprine, or mTOR inhibitors), which themselves are associated with bone marrow suppression. Our study did not identify a link between these immunosuppressants and the risk of myelotoxic complications posttransplant; however, it is likely secondary to our small data set. In the long-term, risk of infection and malignancy posttransplant may be accelerated with cyclophosphamide, although there is a paucity of data in the transplant literature providing evidence for this assumption, and long-term data is lacking from our study.

Clarifying posttransplant risks associated with pretransplant use of cyclophosphamide is important owing to the significant burden of morbidity and mortality posttransplant. Death with a functioning graft remains the leading cause of kidney allograft loss, with infection and malignancy responsible for at least 29% of deaths in a recent single-center analysis (with approximately a third of causes unknown).[9] Regarding infection, late introduction of full-dose mycophenolate mofetil in renal transplant recipients has been shown to be associated with increased incidence of infections, which is inversely correlated with graft function.[10] Patients with prior exposure of cyclophosphamide may be at greater risk of infections, although there is no evidence for this assertion. However, findings from Fiorante and associates,[11] where glomerulonephritis, as a cause of end-stage renal disease, was an independent risk factor for asymptomatic bacteriuria within 3 years of the transplant, support this assertion. This association has not been previously described, and the authors speculate that the amount and

duration of immunosuppressant, before and after transplant, may be a culprit. Our work, where patients with a cyclophosphamide history demonstrated double the incidence of bacteriuria compared with other renal transplant recipients, supports this hypothesis. Additionally, prevalence of posttransplant anemia has been estimated at between 30% to 72% (depending on whether it is defined as early or late) and is associated with developing cardiovascular disease and adverse patient/allograft survival (although the literature appears conflicting with regard to these outcomes).[12] It is of note that the most recent review article on causes of posttransplant anaemia[12] does not mention pretransplant use of cyclophosphamide.

There are limitations to this work that must be appreciated for accurate interpretation. This was a retrospective analysis and therefore has methodological limitations associated with it. Our database of patients for the last 5 years generated only 16 patients with definite evidence of cyclophosphamide use. This small number of patients limited the power of any statistical analysis of clinical and biochemical endpoints when comparing the groups. It also prohibits any clear conclusions for any subanalyses as it reduces the statistical power even more. However, it is unlikely that a single-center analysis would provide sufficient numbers for a well-powered analysis, and a pooling of data will likely be necessary to analyze these putative associations further. It remains possible that the difference in clinical outcomes between the groups was related to the underlying inflammatory conditions necessitating cyclophosphamide rather than to the actual cyclophosphamide administration. For example, patients requiring cyclophosphamide are more likely to have underlying conditions, such as focal segmental glomerulosclerosis or dense deposit disease, that frequently recur after transplant and can cause graft loss: 1 of the 2 graft losses in the cyclophosphamide group in our analysis was due to recurrent focal segmental glomerulosclerosis. Regarding cyclophosphamide, we were unable to clarify the complete dosage of cyclophosphamide administered to patients. This information would be useful for any future analyses identifying putative links between increasing dosage and level of complications. Finally, the relatively short follow-up of our study prohibits assessing long-term risk of infection and malignancy associated with cyclophosphamide use.

This limited study lends credibility to anecdotal evidence that transplant recipients with pretransplant exposure to cyclophosphamide have clinical manifestations of bone marrow suppression in the short-term after a kidney transplant. Whether such exposure translates into long-term complications with infection and malignancy remains uncertain. From this preliminary work, we believe larger registry analyses are warranted to explore this likely association further and to provide such long-term data. The importance of resolving this issue rests in the potential individualization of immunosuppressant that could be initiated, for example, with rationalization of myelosuppressive antiproliferative agents, to attenuate adverse short-term and potentially fatal long-term medical complications.

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