Investigating the threshold for early renal allograft biopsy: A South African single-centre perspective

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Background. The most common clinical indication for renal biopsy in the early post-transplant period is early graft dysfunction (EGD), which may present either as delayed graft function (DGF) or acute graft dysfunction. Even though it is a valuable diagnostic tool, renal allograft biopsy is not without risk of major complications. Recent studies have suggested that, with modern immunosuppressive induction regimens and more accurate ways to determine high immunological risk transplants, early acute rejection (AR) is uncommon and routine biopsy for EGD does not result in a change in management.
Objectives. To describe the histological findings and complications of renal allograft biopsies for EGD in our setting, and to determine whether our current threshold for biopsy is appropriate.
Methods. This study was a retrospective audit that included all patients who underwent renal allograft biopsy within the first 30 days of transplantation at Groote Schuur Hospital, Cape Town, South Africa, from 1 June 2010 to 30 June 2018. The indication for biopsy was any patient who showed significant EGD, characterised by acute graft dysfunction or DGF with dialysis dependence.
Results. During the study period, 330 patients underwent renal transplantation, of whom 105 (32%) had an early biopsy and were included in the study. The median age of recipients was 39 (range 17 - 62) years, with 65% males and 35% females. The majority of donors were deceased donations after brain death (70%), with an overall median cold ischaemic time of 9 hours (interquartile range (IQR) 4 - 16). The average number of human leukocyte antigen mismatches was 5 (IQR 4 - 7). A donor-specific antibody was recorded for 18% of recipients and a panel-reactive antibody score of >30% was recorded for 21%. The median duration after transplant for biopsy was 8 (IQR 6 - 10) days. During the first month of EGD, AR was diagnosed in 42% of patients who underwent biopsies. In 21% of these patients, there was acute cellular rejection, in 16% antibody-mediated rejection, and in 5% both of these. Acute tubular necrosis was the primary finding in 32%, with acute interstitial nephritis in 8%, and acute calcineurin toxicity in 4% of cases. A significant biopsy-related complication was recorded in 3 patients: 1 small-bowel perforation repaired via laparotomy, and 2 vascular injuries successfully embolised by interventional radiology.
Conclusions. Considering the relative safety and high rate of detection of AR, a liberal approach to renal biopsy for EGD remains justifiable in our setting.

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Renal biopsy is the gold standard for providing diagnostic information after renal transplantation.[1,2] The most common clinical indications for biopsy in the first month or early post-transplant period, is early graft dysfunction (EGD), which may present as delayed graft function (DGF) or acute graft dysfunction, where a period of initial graft function is followed by acute deterioration.[2] Prompt therapy of the cause of EGD, especially when due to acute rejection (AR), may not only preserve graft function, but also improve long-term outcome.[3-5] The most common causes of EGD within the first month or early postoperative period are acute tubular necrosis (ATN), acute cellular rejection (ACR), acute antibody-mediated rejection (ABMR) and acute calcineurin-inhibitor (CNI) toxicity.[1,2,5] Although one can test for CNI levels, clinically differentiating between ATN and AR is very challenging; therefore, allograft biopsy is key to distinguishing between the two.

Although it is considered as a valuable diagnostic tool, renal allograft biopsy is not without risk of major complications, including the need for interventional radiology procedures, operative exploration and graft loss.[6] Most authors have advocated a low threshold for early post-transplant biopsy in the setting of EGD, citing its relative safety and high rate of diagnosis of pathology that requires a change in management.[7,8] Recent studies have suggested that, with modern induction immunosuppression regimens, early AR is uncommon and routine biopsy for EGD may be unnecessary, with the risks outweighing the potential benefit.[9,10]

Due to the high costs associated with transplantation and follow-up care, Africa remains underdeveloped in terms of transplantation services.[11] A resource-constrained environment introduces a number of unique variables that may predispose the renal allograft to a higher risk of ATN and AR, both of which may present similarly as EGD. Biopsies showing AR, infection or a drug reaction, lead to a specific change in the management of the recipient, whereas grafts with ATN require continuation of supportive management only. Our unit has historically maintained a low threshold for early graft biopsy.

The aim of this study was to describe the histological findings and complications of renal allograft biopsies for EGD in our setting, and to determine whether our current threshold for biopsy is appropriate.

Methods

This study was a retrospective audit that included all patients who underwent renal allograft biopsy within the first 30 days of transplantation at Groote Schuur Hospital, Cape Town, South Africa, during the study period of 1 June 2010 - 30 June 2018. The indication for biopsy was any patient who presented with significant EGD, characterised by acute graft dysfunction or DGF, with dialysis dependence by day 5. Unexplained haematuria and proteinuria were also considered indications for biopsy. Implantation biopsies (T0), as well as HIV-positive to positive transplant biopsies, were excluded, as the latter will be reported in a separate study. Where a patient had >1 biopsy, the first biopsy with a significant finding was recorded.

All biopsies were performed under local anaesthesia and sedation in the transplant unit – under ultrasound guidance. Clotting parameters were assessed prior to biopsy to exclude a significant coagulopathy. Biopsies were not performed in grafts that were ultrasonographically hydronephrotic. Patients were monitored in the ward during the night for bleeding. Biopsies were reported by a consultant pathologist and reviewed at a weekly multidisciplinary meeting, which included nephrologists familiar with transplantation care. AR was reported according to the latest Banff criteria.[12]

In terms of perioperative immunosuppression, all patients received a course of methylprednisolone post transplant: 500 mg on day 1, 250 mg on day 2 and 125 mg on day 3. In addition, 20 mg oral prednisone was commenced on day 4 with the aim to wean by 5 mg every 2 weeks to 5 mg daily. Patients with a high immunological risk received antithymocyte globulin (ATG) on induction, and were defined by having a donor-specific antibody (DSA), panel-reactive antibody (PRA) >30% or a previously rejected transplant. High-risk patients were also commenced on tacrolimus and mycophenolate mofetil (MMF) on the day of transplant. Patients with an intermediate immunological risk received basiliximab as induction, and were defined by any degree of human leukocyte antigen (HLA) mismatch, without meeting the criteria for high immunological risk. Basiliximab only became available in the second half of 2014; therefore, before this time, intermediate-risk patients received only steroids for induction. Before 2017, intermediate-risk patients were administered azathioprine and cyclosporin after transplantation. From 2017 onwards, all intermediate-risk patients were commenced on tacrolimus and MMF owing to a perceived high rejection rate in the unit at that time. Low-risk patients were HLA identical and given no induction – azathioprine and cyclosporin being initial immunosuppressives. The desensitisation protocol for patients who had a confirmed DSA with associated positive-flow cytometry crossmatch, was 3 - 5 sessions of plasma exchange (1.5 × plasma volume, alternate days) and intravenous immunoglobulin (IVIG) 100 mg/kg administered after each session.

Statistical analysis

Statistical analysis was performed with R version 3.5.2 (https://www.r-project.org/about.html). Numerical variables were assessed for normality using the Shapiro-Wilk test, and subsequently analysed by appropriate parametric and non-parametric tests. Categorical variables were analysed by Fisher's exact and χ^2 tests. Unless otherwise indicated, a two-tail test hypothesis was used with 0.05 as discriminator for rejection of the null hypothesis.

Ethical approval

Ethical approval for the study was received from the Human Research Ethics Committee, University of Cape Town (ref. no. HREC 538/2018). This approval permitted a folder review of patients who received a transplant within the relevant time period. Informed consent for folder review was waived. A number of variables were collected, including information pertaining to the transplant recipient, donor, donor-recipient immunological factors and biopsy procedure. All patients were anonymised prior to statistical analysis.

Results

During the study period, 330 patients underwent renal transplantation, of whom 105 (32%) underwent an early biopsy and were included in the study. The median age of recipients was 39 (range 17 - 62) years, with 65% males and 35% females. The majority were index transplants, with 5% being re-transplants.

Donors

The average donor age was 34 (range 14 - 67) years, with 57% males and 43% females. The majority of donors were deceased donations after brain death (70%) compared with 30% living-related donations. The overall median cold ischaemic time (CIT) was 9 (interquartile range (IQR) 4 - 16) hours.

Immunological risk

In terms of initial CNI, 56% received cyclosporin, while 44% received tacrolimus. In terms of initial antimetabolite, 58% received azathioprine and 42% MMF. The median number of HLA mismatches was 5 (IQR 4 - 7). A DSA was recorded for 18% of recipients, 37% of whom were living-related transplants and underwent desensitisation. A PRA of >30% was recorded for 21%. Immunological risk was assessed as being high for 23%, intermediate for 72% and low for 5%. The most commonly used induction agent was basiliximab (27%), whereas 21% received ATG and 52% no specific induction agent as addition to the standard course of corticosteroids.

Biopsy findings

The median duration post transplant for biopsy was 8 (IQR 6 - 10) days. The primary findings are shown in Table 1.

During the first month, AR was diagnosed in 42% of biopsies for EGD, of which 21% had ACR, 16% ABMR and 5% both. The second most common primary diagnosis was ATN (32%). Other major findings included acute interstitial nephritis (AIN) (8%) and acute CNI toxicity (4%). There was no statistical difference in the proportion of cases of AR before (37%) and after (49%) the introduction of basiliximab in 2014 (p=0.29).

Predicting acute rejection

Logistic regression analysis was performed to determine the effect of recipient, donor and immunological parameters in predicting AR. No factors were found to be statistically significant in predicting the likelihood of biopsy-proven AR in patients with EGD (Table 2).

Biopsy complications

A significant biopsy-related complication was recorded in 3 patients. The first had a small-bowel perforation, which was diagnosed on both histology and clinical evaluation the day after biopsy. The patient was taken for a laparotomy, where minimal peritoneal contamination was found and the perforation was primarily repaired, after which the patient had an uneventful recovery. The second patient presented with haematuria and a decreased haemoglobin level the day after

biopsy. An angiogram showed an arteriovenous fistula in the upper pole of the graft, which was successfully embolised. The third patient presented with an unexplained decrease in haemoglobin level the first day after biopsy. The angiogram showed an abdominal wall pseudoaneurysm along the biopsy tract, which was also successfully embolised by the interventional radiologist.

Discussion

This study describes the findings and complications of renal biopsies done in the first month post transplant for patients with EGD. During the study period, 105 of a total of 330 transplants (32%) displayed EGD in the first month and underwent allograft biopsy. This rate of EGD and subsequent biopsy are slightly higher than those in other similar units, which had a similar threshold for biopsy, ranging between 25% and 30%.[3,5,7]

Biopsy findings

The rate of early AR in patients who underwent biopsy in the first month for EGD was 42%, which is higher than the 18 - 30% found in similar studies.[3,5,7,8] Although just fewer than half of the patients in this study received tacrolimus and MMF, or a specific induction agent, this is much the same as regimens described in previous studies.[5,8] Therefore, a less aggressive immunosuppressive induction regimen does not account for the significantly higher incidence of AR.

Table 1. Primary findings on biopsy

Result	*n* (%)
ATN	34 (32)
Acute rejection	44 (42)
ACR	22 (21)
ABMR	17 (16)
ACR and ABMR	5 (5)
Acute interstitial nephritis	8 (8)
Acute CNI toxicity	4 (4)
Other drug reaction	4 (4)
Acute pyelonephritis	3 (3)
Granulomatous interstitial nephritis	3 (3)
Other	6 (6)

ATN = acute tubular necrosis; ACR = acute cellular rejection; ABMR = acute antibody-mediated rejection; CNI = calcineurin inhibitor.

We would argue that the higher rate of AR could be due to a number of factors related to our developing-world setting. First, the majority of transplants are from deceased donors, which are inherently at greater risk of AR. Second, because of the ethnic diversity of our population, the Groote Schuur transplant unit disregards HLA matching for allocation, a practice which is dissimilar to that in most other centres worldwide. The diverse genetic exposure may lead to higher rates of immunogenic antigen exposure in the recipient, and thus a higher rate of AR than in centres with similar immunosuppressive regimens.[13]

As ATN is usually the predominant finding in patients with EGD, especially with DGF, it is unsurprising that this study, which found a high rate of AR, had a correspondingly lower rate of ATN as the primary finding compared with that in other studies.[2,5,7] However, 17% of patients who had another primary finding, had ATN as a significant secondary finding; thus, ATN contributed to EGD in ~50% of cases. Numerous factors related to the local context may increase the risk of ATN and subsequent EGD in transplanted kidneys. First, the majority of transplants in our setting are from deceased donors, which not only have a higher risk of AR, but also a greater risk of ATN due to an increased CIT.[14,15] Second, the lack of a dedicated transplant theatre and a relatively small team of surgeons often result in an institutional delay from procurement to implantation, which may prolong CIT. Geographically, the unit also serves as the transplantation service for Eastern and Northern Cape provinces, which means that recipients may need to travel significant distances to reach the unit and be ready for surgery. The median CIT of transplants from deceased donors, which comprised 70% of cases, was 12 hours, which demonstrates these delays and supports the hypothesis that allografts in this setting may have a higher rate of EGD due to ATN.

Complications

The major complication rate of 3% was similar to that found in other studies.[5,6] Although the complications were not insignificant, all presented within 24 hours of biopsy, and were detected and dealt with without major detriment to the patient or graft. This demonstrates the relative safety of renal biopsy for this patient group in our setting.

Threshold for biopsy: Current and future

Our unit has historically maintained a low threshold for allograft biopsy in the setting of EGD. This approach appears justifiable when

Table 2. Multivariable logistic regression for factors predictive of biopsy-proven acute rejection

Parameter	OR (95% CI)	*p*-value
Recipient age	1.00 (0.94 - 1.06)	0.98
Recipient gender (male v. female)	0.92 (0.31 - 2.75)	0.90
Re-transplant	0.23 (0.01 - 2.97)	0.37
Antimetabolite (MMF v. azathioprine)	0.64 (0.05 - 5.72)	0.74
CNI (tacrolimus v. cyclosporin)	1.46 (0.13 - 22.33)	0.80
ATG/basiliximab induction	0.53 (0.11 - 2.42)	0.50
Donor age	1.01 (0.97 - 1.05)	0.71
Donor gender (male v. female)	1.08 (0.31 - 3.76)	0.92
Donor type (living v. deceased)	0.19 (0.03 - 1.04)	0.12
HLA mismatches	0.83 (0.62 - 1.09)	0.27
CIT	0.94 (0.85 - 1.04)	0.33
PRA >30%	0.68 (0.16 - 2.77)	0.65
DSA	1.68 (0.19 - 16.50)	0.70
High immunological risk	1.65 (0.15 - 19.16)	0.73

OR = odds ratio; CI = confidence interval; MMF = mycophenolate mofetil; CNI = calcineurin inhibitor; ATG = antithymocyte globulin; HLA = human leukocyte antigen; CIT = cold ischaemic time; PRA = panel-reactive antibody; DSA = donor-specific antibody.

considering the high rate of AR detected in this study, a finding which undeniably changes the patient's acute post-transplantation management. In our setting, there is a significant shortage of kidneys, as well as poor access to re-transplantation, with patients >60 years of age not even given consideration. Therefore, there is little margin for error, and a delay in management of a rejection episode, with a consequent poorer long-term outcome, may be devastating to the patient.

When compared with studies that argue against a liberal biopsy approach, our unit has employed a less aggressive approach to immunosuppressive induction. In the study by Ortiz *et al.*,[10] all patients received ATG, and in the study by Hatoum *et al.*,[9] every patient received either ATG or basiliximab. In this study, only 48% of patients received either ATG or basiliximab. The majority of patients who were not administered ATG or basiliximab, received a transplant before mid-2014, when basiliximab was not available for intermediate-risk patients. From 2014 onwards, all but 2 patients received either ATG or basiliximab. Despite this change in induction protocol, the rate of AR after the introduction of basiliximab remained unchanged, which suggests that a less-aggressive induction regimen is not the only cause of the high AR rate in this setting, as discussed above.

Since 2017, both tacrolimus and MMF have become more freely available and are therefore readily used for immunosuppressive induction in our unit. Furthermore, since July 2018, we have started performing a complement-dependent cytotoxicity (CDC) B-cell crossmatch in addition to the standard T-cell crossmatch. We have also introduced rituximab for the prevention of ABMR in living-donor recipients with a DSA. Despite a low threshold for biopsy in our setting remaining appropriate, this may not be the case in future. Therefore, further monitoring and reassessment of our biopsy findings in the clinical context of EGD are indicated.

Study limitations

As a retrospective study, our research has several weaknesses. The first is giving a single or primary diagnosis to a biopsy result, where the pathologist may have reported several significant findings. Where this occurred, the original biopsy report and patient records were reviewed by a consultant nephrologist familiar with renal transplantation histology, and the primary diagnosis decided as best possible. The second is the inclusion of a heterogeneous group of patients, which was done to increase the generalisability of the findings to all patients under the routine daily care of the transplant team. Furthermore, transplant recipients are by nature a very heterogeneous group, with multiple donor, recipient and perioperative factors that may affect early function and risk of rejection. Therefore, deciding which factor(s) should be used to stratify subgroups or exclude patients is problematic. In this study, no preoperative factors, including donor type and immunological risk, were found to be predictive of AR on early biopsy. However, the small number of patients in the study does mean a high likelihood of type 2 statistical error.

Conclusions

Our unit has maintained a low threshold for renal biopsy in the setting of EGD and, considering the high rate of AR detected with regard to these early biopsies, a liberal approach remains justifiable. Transplant centres with similar immunosuppression protocols and demographic and socioeconomic settings, may use these findings to justify the cost and risk of biopsy in their own practice. However, advances in immunological screening and induction immunosuppression may demand a re-evaluation of the biopsy threshold for EGD in all settings, where a more conservative approach to biopsy may become more widely acceptable.

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