

Pathological Spectrum of Graft Nephrectomies: A Study of 55 Cases

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ABSTRACT

Background. The purpose of this paper was to define the pathological spectrum of graft nephrectomies performed at the Singapore General Hospital, in order to lend insight into the possible causes of graft failure. In particular, the utility of the Banff 97 classification to characterise the histologic alterations was evaluated.

Methods. All graft nephrectomy specimens received by the Department of Pathology, Singapore General Hospital between January 1991 and April 2002 were reviewed pathologically and the Banff 97 classification was applied. Histologic findings were correlated with the duration between transplantation and graft nephrectomy.

Results. A total of 55 graft nephrectomies was evaluated during the study period. The most common pathology was rejection, which was found in 45 (81.8%) grafts, with underlying chronic rejection observed in 30 (72.7%) of these. A significant association was found between the Banff grade of chronic rejection and graft nephrectomies performed after one year post-transplant ($p < 0.001$). Individual parameters of acute rejection were correlated with one another, as were those for chronic rejection ($p < 0.01$). Other pathology found included vascular thromboses, IgA nephropathy, focal and segmental glomerulosclerosis, membranous glomerulonephritis, cytomegalovirus infection, abscess, and multifocal renal cell carcinoma.

Conclusion. Chronic rejection was the major finding in this graft nephrectomy series, suggesting that it is the main cause of graft failure. The Banff 97 classification could be applied readily to histologic alterations. Optimum handling and sampling of graft nephrectomy specimens can yield potentially useful information.

Keywords: acute rejection, Banff 97, chronic rejection, cytomegalovirus, glomerulonephritis, nephrectomy, renal allograft, renal cell carcinoma

INTRODUCTION

Renal graft and patient survival have improved dramatically over the last 2 decades. Studies have reported graft half-life of 19.9 years and pure graft half-life, excluding death, at 24.8 years.¹ Among the failed grafts, many are left in situ as nephrectomies are not without morbidity and mortality. Techniques to reduce the need for nephrectomies include gradual withdrawal of immunosuppression and percutaneous embolisation of the allograft.^{2,3} Nephrectomies are only performed when clinical signs and symptoms appear, such as fever, local pain or tenderness, and haematuria. The necessity for allograft nephrectomy has been reported to be lower in grafts failing after more than 6 months post-transplantation.⁴

Graft nephrectomies present an excellent opportunity for pathologists to study the spectrum of rejection, as well as other disease processes leading to graft failure, because they allow unlimited sampling as compared to renal biopsies. Arcuate arteries and larger calibred vessels are also more readily studied in nephrectomy specimens.

The primary aim of this study was to document the pathological spectrum of graft nephrectomies evaluated at the Department of Pathology, Singapore General Hospital. In particular, histological changes of rejection were categorised according to the Banff 97 classification system;⁵ and these alterations were correlated with the duration between transplantation

Table 1. Correlation between the Banff type of acute rejection and the duration between transplant and graft nephrectomy.

Type of acute rejection	Duration between transplant to nephrectomy	
	≤ 12 months	> 12 months
Banff IA	1 (4.8%)	0 (0%)
Banff IB	0 (0%)	0 (0%)
Banff IIA	6 (28.6%)	1 (4.2%)
Banff IIB	6 (28.6%)	8 (33.3%)
Banff III	8 (38.1%)	15 (62.5%)
Total	21 (100%)	24 (100%)

Table 2. Correlation between the Banff grade of chronic rejection and the duration between transplant and graft nephrectomy.

Grade of chronic nephropathy	Duration between transplant to nephrectomy	
	≤ 12 months	> 12 months
Banff I	6 (33.3%)	2 (8.3%)
Banff II	2 (11.1%)	7 (29.2%)
Banff III	1 (5.6%)	15 (62.5%)
No chronic nephropathy	9 (50.0%)	0 (0%)
Total	18 (100%)*	24 (100%)

* cases with extensive infarct precluding assessment of chronic rejection were omitted.

and graft nephrectomy. Vascular events, recurrent and de novo glomerulonephritides, infections, drug related changes and neoplasms were other pathological features that were specifically documented.

METHODS

The files of the Department of Pathology, Singapore General Hospital, were searched for graft nephrectomies evaluated between January 1991 and April 2002. Histological slides were retrieved and microscopically reviewed. Periodic acid-Schiff and Silver-Masson stained sections were mostly available for study, together with haematoxylin and eosin stained slides. Additional histochemical and immunostains were performed as deemed necessary.

Immunohistochemistry for IgA was performed on 1 allograft nephrectomy; while 2 cases with microscopic viral inclusions were subjected to immunohistochemical confirmation of cytomegalovirus (CMV). Briefly, 5 μ m thick sections were brought to room temperature, deparaffinised and taken to water. For CMV immunostaining, sections were microwaved in 600ml of Dako pH6 pretreatment buffer for 25 min at 800W, followed by standing for

10 min to cool at room temperature; while antigen retrieval for IgA immunostaining was achieved by digestion in 0.1% protease at 37°C. The slides were then washed in running tap water, after which 3% hydrogen peroxide to quench endogenous peroxidase was applied for 10 min. After rinsing in tris buffered saline (TBS), the sections were incubated in 1/150 CMV (Dako M0854) antibody and 1/40 000 IgA (Dako A0092) antibody respectively for 60 min at room temperature. The sections were then washed with TBS, followed by incubation with linking biotinylated (secondary) antibody for 30 min at room temperature, then washed in TBS again. The next step was incubation with enzyme labelled peroxidase-conjugated streptavidin (tertiary antibody) for 30 min at room temperature. After washing in TBS, the sections were developed with 3'3-diaminobenzidine for 7 min at room temperature. Subsequently, sections for CMV immunostaining were washed in running water; while that for IgA immunostaining was washed in Type 1 water. The slides were then counterstained with haematoxylin for 2½ minutes, differentiated with 1 dip in 1% acid alcohol, "blued" in running tap water, dehydrated, cleared and mounted in depex.

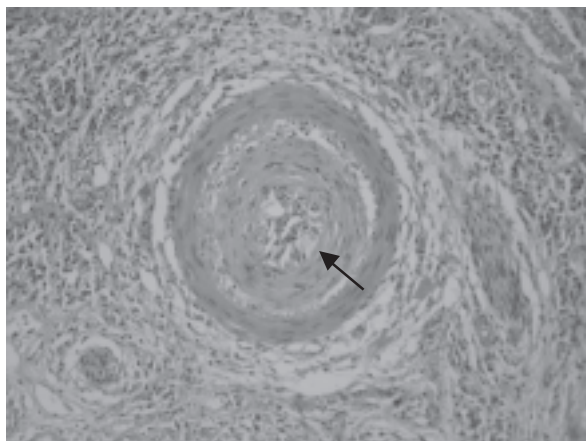


Fig. 1. Chronic rejection with an artery showing “vessel in vessel” phenomenon with smooth muscle proliferation in the expanded intima. Foam cells are present among lymphocytic infiltrate, arrowed (H&E, original magnification $\times 400$).

There was no record of immunofluorescence or electron microscopic examination in any case, as the explanted grafts were submitted to the pathology laboratory already fixed in buffered formalin, without having had tissue appropriately harvested for these modalities of assessment. Cases were classified and scored using the Banff 97 working classification of renal allograft pathology.⁵ Patient data, clinical indication for graft nephrectomy, and the duration between transplantation and graft nephrectomy were ascertained from the accession forms. Correlation between histological changes, as well as with the duration between transplantation and graft nephrectomy, was analysed using the statistical software SPSS version 10. A statistically significant result was defined as $p < 0.05$.

RESULTS

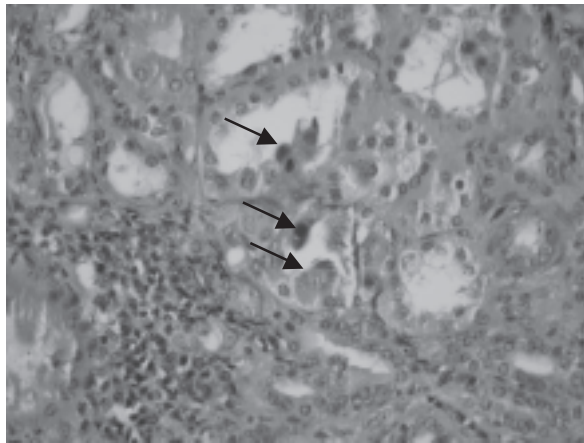
There was a total of 55 graft nephrectomies performed during this study period. The mean patient (recipient) age was 42.3 years (range 24 to 58 years). There were 33 males and 22 females. The ethnic distribution was 41 Chinese, 7 Malays and 7 Indians. The majority (75%) of the grafts were from cadaveric sources, while 17% and 8% of the grafts were from living unrelated and living related donors, respectively. The mean, median, and range of duration from transplantation to graft nephrectomies were 38.9 months, 12 months, and 1 day to 132 months, respectively. The underlying recipient renal disease that led to the need for transplantation was not identified. Similarly, the precise duration between onset of irreversible graft failure and nephrectomies was mostly not known. The reason for nephrectomy in many of the cases was specified as “rejection” without further qualification on the request form.

The most common microscopic pathology found was allograft rejection, occurring in 45 (81.8%) grafts. Acute rejection without evidence of chronic rejection was observed in 15 (27.3%) cases. Among these, 13 cases (86.7%) were nephrectomies performed within 2 months after transplantation. One case performed 4 months post-transplant showed donor-related arterial nephrosclerosis in addition to acute rejection alterations; while the other case performed 1 year after transplant disclosed extensive infarction precluding accurate assessment for chronic rejection. The remaining 30 (54.5%) cases with allograft rejection revealed both acute and chronic rejection.

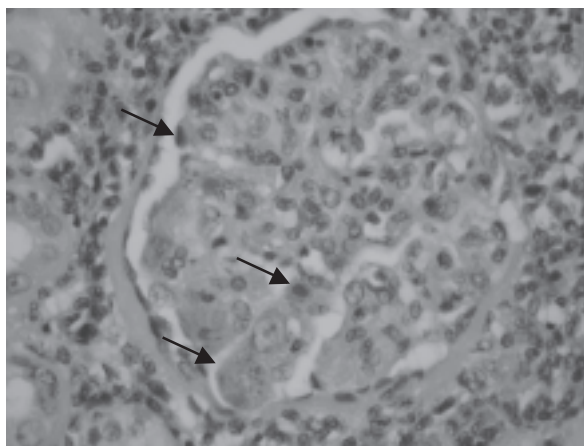
Among these 45 grafts with allograft rejection, 21 nephrectomies were performed within 12 months post-transplant and 24 were performed after more than 12 months. Table 1 shows the Banff grade of acute rejection compared against the duration from transplantation to graft nephrectomy. There was no statistically significant difference in the degree of acute rejection between these 2 groups ($p = 0.078$). Table 2 shows the Banff grade of chronic allograft nephropathy compared to the post-transplant period prior to nephrectomy. There was a significantly higher grade of chronic allograft nephropathy in the nephrectomies performed more than 12 months post-transplant ($p < 0.001$). Specific vascular changes of chronic rejection, such as foam cells in the fibrotic intima and formation of a second neointima, were easily seen in most cases, albeit focally (Fig. 1).

The parameters for acute rejection (g, i, t, v) were significantly correlated with each other ($p < 0.01$). Similarly, the parameters for chronic rejection (cg, ci, ct, cv) were also significantly associated with each other ($p < 0.01$). However, between the acute and chronic indices, there was either weaker or no correlation. Presence of more than 5 to 10% eosinophils, neutrophils or plasma cells in the inflammatory infiltrate (whereby an asterisk is added to the ‘i’ score) was correlated with severe ‘i’ score ($p < 0.05$). Similarly, the presence of interstitial haemorrhage and/or infarction (an asterisk is added to the ‘v’ score) was associated with a more severe ‘v’ score. There were no significant correlations between the recipients’ age and severity of acute or chronic changes. Likewise, no relationship was obtained between recipients’ sex or source of allografts and severity of acute or chronic changes. No specific changes that could be attributed to cyclosporine nephrotoxicity were noted.

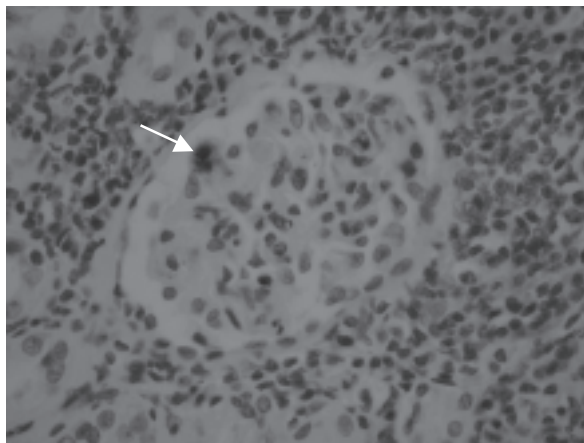
Of the 10 cases with no discernible evidence of rejection in the allografts, 7 were nephrectomies performed within 7 days post-transplant due to arterial



(a)

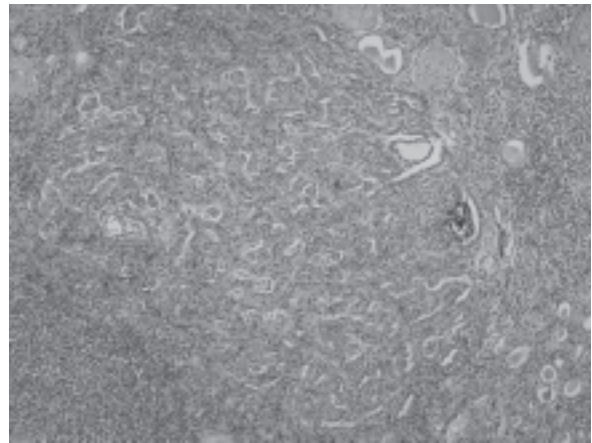


(b)

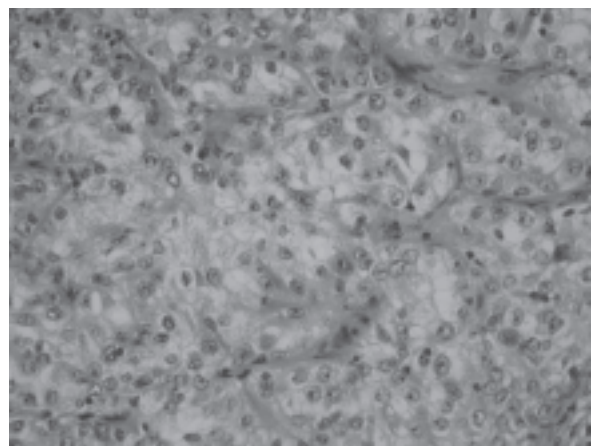


(c)

Fig. 2. Owl-eye nuclear inclusions and granular cytoplasmic inclusions in tubular epithelial cells, arrowed (H&E, original magnification $\times 400$) (Fig. 2a). Glomerulus shows CMV nuclear and cytoplasmic inclusions in cytomegalic visceral epithelial cells, arrowed (H&E, original magnification $\times 400$) (Fig. 2b). Immunohistochemistry for CMV showing positive reactivity within infected cells, arrowed (immunoperoxidase, original magnification $\times 400$) (Fig. 2c).



(a)



(b)

Fig. 3. Small tubulopapillary lesion lined by cells with bland nuclei and with occasional psammoma bodies (H&E, original magnification $\times 200$) (Fig. 3a). Features of RCC with solid alveolated architecture, higher nuclear grade and pale to clear cytoplasm (H&E original magnification $\times 400$) (Fig. 3b).

or venous thromboses with infarction. Of the remaining 3 nephrectomies which were performed at 5 years post-transplant, rejection could not be assessed in 2 cases due to extensive infarction in the sampled sections of renal tissue in one case, while only sections with widespread abscess formation were available for review in the second case, with no viable renal parenchyma for further evaluation. The third case showed arterial nephrosclerosis with diffuse global and segmental glomerulosclerosis; mesangial proliferation within non-sclerotic glomeruli and the immunohistochemical presence of mesangial IgA deposits suggested de novo/recurrent IgA disease.

There were 2 cases of IgA nephropathy (9 years and 10 years post-transplant); 2 cases with focal and segmental glomerulosclerosis (5 years and 7 years post-transplant) and 1 case of membranous nephropathy

(10 years post-transplant) observed in grafts that also revealed acute on chronic rejection. CMV infection was diagnosed histologically in 2 cases (2 months and 10 months post-transplant), with acute rejection in addition to abscess formation also being found in the former; while acute on chronic rejection accompanied the CMV alterations in the latter. Cytopathic changes of cytomegalic cells with intranuclear owl-eye inclusions were discovered in the tubular epithelium in one case, while the other case demonstrated the characteristic virally affected cells in glomerular visceral epithelium. Nuclear inclusions appeared somewhat amphophilic while cytoplasmic inclusions were more acidophilic. The diagnostic cells were very focal in both cases (Fig. 2) with confirmation on CMV immunohistochemistry.

Interestingly, multicentric renal cell carcinoma was found in a graft nephrectomy carried out at 15 months post-transplantation from a 34-year-old female recipient. Indication for graft nephrectomy was for clinically suspected graft infection 2 months after her graft had failed. At the time of renal transplantation 15 months earlier, the cadaveric donor kidney was noted to be scarred. A biopsy had been taken then, disclosing arterial nephrosclerosis with 21% globally sclerotic glomeruli, and no renal neoplasm was observed. The graft nephrectomy specimen appeared macroscopically congested and revealed multiple yellowish spots within the cortex, ranging from 0.2 to 0.5cm in diameter. Microscopically, the smaller nodules were fairly circumscribed but unencapsulated, comprising tubulopapillary proliferations having delicate fibrovascular cores covered by closely packed columnar and polygonal cells with enlarged irregular nuclei, some showing small but prominent nucleoli. Occasional mitotic figures were noted. The cells contained clear to amphophilic cytoplasm, and psammomatous calcifications were occasionally seen. The larger nodules showed more solid architecture with higher grade nuclei (Fuhrman nuclear grade 3). There was a tiny focus (<1mm diameter) of tubulopapillary proliferation in the vicinity, lined by uniform nuclei, suggesting a possible precursor lesion to the more established carcinoma (Fig. 3). The rest of the renal parenchyma showed arterial nephrosclerosis with features of acute on chronic rejection.

DISCUSSION

Pathological changes encountered in graft nephrectomies evaluated in our study were related to the duration from transplantation. Most nephrectomies performed under 1 week showed haemorrhagic infarction due to arterial or venous thromboses. After

that period, acute rejection was the most common finding. Chronic rejection was usually encountered in nephrectomies beyond 1 year post-transplantation. This is comparable to another study on 60 allograft nephrectomies in 1228 consecutive kidney transplantations which found that the time of transplant nephrectomy varied according to the causes of transplant kidney dysfunction.⁶

At the Singapore General Hospital, a total of 648 cadaveric and 278 live donor renal transplants have been performed as of 1 July 2003. Graft survival at 5 and 10 years was 80.1% and 66.9% for cadaveric donors; while that for live donors was 92.4% and 81% respectively (unpublished).

It is not unexpected to find no significant difference in severity of acute rejection between early and late nephrectomies in this study because the graft nephrectomy population is already preselected for cases that are symptomatic. Also, cessation of immunosuppression is often instituted once a clinical decision for nephrectomy is made, allowing the acute rejection process to develop to an approximately similar degree in many of the grafts. Likewise, the absence of histological alterations of cyclosporine nephrotoxicity in this graft nephrectomy series may also be attributable to several factors: cessation of immunosuppression prior to nephrectomy; relative non-specificity of light microscopic changes; or that cyclosporine is often not a prime reason for irreversible allograft failure.

In this study, chronic rejection was observed in 3 nephrectomy cases seen before 1 year post-transplantation: at 4 months, 7 months and 10 months respectively, accounting for 3 of 30 (10%) of all cases that disclosed changes of chronic rejection. All were categorised as Banff grade IIb. These findings are in keeping with results from another study that reported Banff chronic nephropathy lesions in up to 24% of grafts as early as 3 months after transplantation.⁷ The majority of chronic rejection alterations in our current study, however, occurred in grafts removed after 1 year post-transplantation.

It is readily noticeable in this study that the presence and severity of acute and chronic vascular changes were not uniform in the renal parenchyma, hence there is a real potential for sampling error in a renal biopsy specimen. Even though the presence of interstitial haemorrhage and/or infarction was correlated to a severe 'v' score, these alterations could also be seen in cases with mild or no acute vascular rejection. This is in accord with the updated Banff 97 classification

which no longer considers interstitial haemorrhage and/or infarction alone (v0*) adequate to presumptively score v3.⁵

It was not in the scope of this study to investigate the degree of concordance in applying the Banff 97 scheme. Nevertheless, the relatively clear definitions set out⁵ enabled the classification to be used with a fair amount of ease. In light of recent documentation of interobserver variation in categorising renal allograft changes, it would be prudent to establish local experience in the interpretation of renal allograft biopsies for prognostic and clinical management purposes.⁸

There were only 2 cases of CMV infection diagnosed histologically in this series. CMV infection can lead to interstitial nephritis that may be difficult to distinguish from acute cell-mediated rejection. It is also associated with an increased risk of allograft dysfunction and loss, with some studies documenting an increased risk of acute rejection.⁹

The solitary case of renal cell carcinoma (RCC) found in this study showed multiple tubulopapillary lesions ranging from tiny lesions, in keeping with the presumed precursor adenoma, to established carcinoma. The relatively rapid development of carcinoma and the observation that the allograft was scarred at the time of transplantation raised the possibility of donor transmitted malignancy. Papillary adenomas are believed to be the precursors of carcinoma in patients on long-term haemodialysis. Thoenes *et al* defined adenoma as any tumour up to 1.0cm in size with grade 1 cytology (small uniform nuclei with delicate or condensed chromatin, inconspicuous nuclei, absent mitotic figures) and included clear cell tumours in this definition. However, Grignon *et al* considered all lesions composed of clear cells to be malignant regardless of size.¹⁰ While the risk of RCC is increased in endstage native kidneys of renal transplant recipients, de novo RCC in a renal allograft is a rare event and has special implications in renal transplant recipients. A study involving 27 German transplant centres revealed 30 out of 10,997 renal donor grafts (0.273%) to harbour RCC detected at the time of preparation before transplantation. RCC developed in 16 recipients (0.145%) 3 to 12 years after renal transplantation, with the tumour size ranging from 2 to 2.8cm.¹¹ There has been a case report of metastatic renal cell carcinoma resulting in death of a heart transplant recipient 7 months post-transplantation when the donor had been previously documented to have a 17mm tubulopapillary adenoma in one kidney.¹²

Lastly, since many graft nephrectomies showed large areas of haemorrhage and infarction, proper sampling of viable areas is crucial in order to yield more informative pathological conclusions.

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