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Histopathological examination of removed kidney allografts: Is it useful? A retrospective cohort study

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SUMMARY

The incidence and relevance of histological findings in removed allografts is unknown. In this study, we investigated the outcome of routine histopathological examination of removed allografts. We performed a retrospective cohort study in patients with kidney graft failure ≥3 months after transplantation. In this cohort, 244 allograft nephrectomies were performed. We routinely sent removed grafts for histopathological examination. In 197 cases, a pathology report was available for analysis. In 21 of the 197 grafts, gross necrosis precluded adequate interpretation. Signs of rejection were reported in 163 of the remaining 176 allografts. Recurrences of the original disease were found in 13 cases. These were all known from prior biopsies. Relevant secondary findings were present in eight cases: renal cell carcinoma ($n = 2$), urothelial cell carcinoma, candida pyelonephritis ($n = 2$), post-transplant lymphoproliferative disease, polyomavirus inclusions, and membranous nephropathy. All conditions were diagnosed before graft nephrectomy, except for one case of papillary renal cell carcinoma of 0.8 cm. As expected, signs of acute and/or chronic rejection are the main histopathological finding in grafts that are removed after late graft failure. Unexpected secondary findings are very rare. Therefore, it is justifiable to restrict histopathological examination of removed kidney allografts to specific cases.

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Introduction

When a kidney transplant fails, a decision must be made whether to remove the graft or to leave it *in situ*. In literature, the rate of allograft nephrectomy varies widely between 9% and 75% reflecting the lack of a standard policy [1–10]. In case of early graft failure (within 3–6 months after transplantation) or primary nonfunction, the graft is usually removed to avoid systemic and local effects of acute rejection and to allow complete withdrawal of immunosuppressive medication. A substantial proportion of these early failures present with decreased or absent blood flow within 48 h after kidney transplantation. When the kidney is deemed nonviable, it will be removed. In these cases, histopathological examination may reveal vascular abnormalities (renal vein or arterial thrombosis) or—rarely—hyperacute rejection.

In case of later graft failure, the graft is usually left *in situ* unless there is an indication for allograft nephrectomy. The most common indication is graft intolerance syndrome, which includes fever, pain and/or

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swelling of the graft, and hematuria. Other potential indications are infectious complications, severe hypertension, refractory nephrotic syndrome, proven or suspected graft malignancy, and the need to create space for retransplantation. From previous studies, it can be concluded that most allograft nephrectomies are performed within 12 months of graft failure [1–10].

In many centers, the removed grafts are routinely sent for histopathological examination. However, histopathological examination of removed grafts is time-consuming and costly, while the utility in terms of incidence and relevance of various histological findings in removed grafts is unknown. Goral *et al.* retrospectively studied 53 grafts from patients with graft failure at least three months after kidney transplantation. They reported the presence of extensive inflammation and ongoing immunologic activity in removed grafts, in both symptomatic patients and patients who underwent allograft nephrectomy to create space for retransplantation. Extensive inflammation with a considerable amount of interstitial fibrosis and tubular atrophy (IF/TA) was also present in grafts left *in* situ for many years after failure [11]. The presence of inflammation and IF/TA in failed grafts is not surprising however, and after graft removal, these findings do not have any clinical consequence for the patient.

In some cases, there will be a specific indication for histopathological examination, for instance, when a malignant lesion is expected and information about tumor margins is relevant. However, in most cases of graft nephrectomy a specific question for the pathologist will be absent. We therefore question whether routine histopathological examination of all transplant nephrectomy specimens of patients with late graft failure (>3 months after transplantation) is useful. To answer this question, we retrospectively studied the findings of histopathological examination of removed kidney grafts in a large cohort of unselected patients. We focused on the incidence and relevance of unexpected findings and tried to determine their clinical consequences. Based on our results, we aim to identify indications for histopathological examination of removed grafts and to define cases in which the likelihood of added value of histopathological examination is very small.

Materials and methods

Study population

The composition of our study population is shown in Fig. 1. Between 1968 and 2018, a total of 4570 kidney transplantations were performed in the Radboud university medical center. Both children and adults were included. We excluded patients when the graft failed within three months after transplantation or in case of primary nonfunction, because histopathological examination of these grafts is valuable for determining causes of graft failure. In 889 patients, the graft failed after a survival of more than 3 months after transplantation. In these cases, the policy of our center has been to leave the graft *in situ* unless there is an indication for graft nephrectomy (e.g., graft intolerance syndrome). In some patients with two nonfunctioning grafts *in situ,* one of these grafts had to be removed to create space for a subsequent transplant. We identified a total of 244 late allograft nephrectomies. The 197 cases with available pathology reports were included in our analysis.

Histopathological examination

Removed grafts were routinely sent for histopathological examination. Gross examination was done in a routine manner and included measuring weight and longitudinal slicing for identification and description of cortical or medullary abnormalities. Additionally, the vascular pole was transversely sliced in order to assess the presence of vascular abnormalities. Histological examination after hematoxylin and eosin and Periodic Acid Schiff staining was routinely performed for the following areas: normal-appearing parenchyma, focal abnormalities, and the large hilar vessels (transverse sections of the vascular pole). Immunohistochemistry and/or electron microscopy were only performed when indicated by clinical data or histological findings. Histopathological examinations were performed in a standardized manner by pathologists with extensive experience in renal pathology. All reports contained an extensive description of macroscopic and microscopic findings.

Data collection

Patient characteristics (age, gender), date of transplantation, graft failure and graft nephrectomy, and reasons for graft nephrectomy were retrieved from the local transplant registry and patient records. Graft failure was defined as start of dialysis therapy or pre-emptive retransplantation.

Graft intolerance was defined as the presence of one or more of the following clinical criteria in the absence of another plausible explanation: fever, malaise, hematuria, painful, swollen graft, or persistent leukopenia or thrombocytopenia.

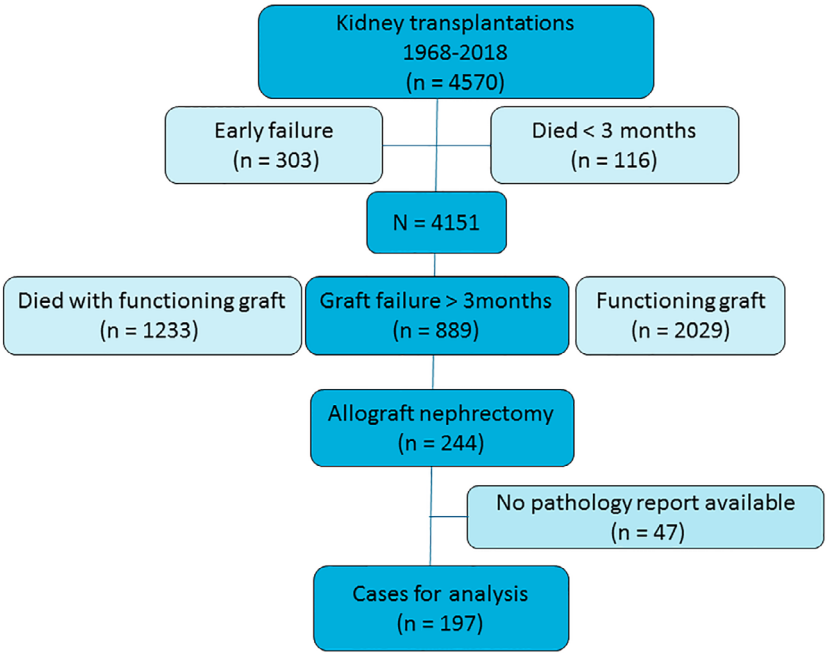

Figure 1 Study profile.

We studied all available pathology reports and scored the presence of acute and/or chronic rejection, thrombosis/necrosis, tubulo-interstitial nephritis, and signs of chronic calcineurin inhibitor (CNI) toxicity (arteriolar hyalinosis, striped interstitial fibrosis). When there was a suspicion of a malignant process, we evaluated whether the diagnosis was confirmed. In confirmed cases, we recorded the reported type and size of the malignant process. Furthermore, we carefully reviewed the reports for signs of recurrence of native kidney disease and for other secondary findings.

Histopathological slides are kept in storage in the pathology department for 10 years. In addition to reviewing available reports, we randomly selected 20 of the 42 patients who had a graft nephrectomy between 2010 and 2018 to confirm the original findings.

In case of a recurrence or other secondary finding, patients' records were retrieved to investigate whether these findings were new or already known, for instance, based on a prior kidney transplant biopsy. Finally, we studied the clinical consequences of unexpected secondary findings.

Statistical analysis

Characteristics of our study population are presented as means with standard deviation, medians with range or frequencies where appropriate. Analyses were performed using IBM SPSS Statistics 25.

Results

Study population

The characteristics of our patient cohort are described in Table 1. The median age at time of transplantation was 31 years. The median duration of graft survival was 32.6 months, and the median interval between graft failure and graft nephrectomy was 2.8 months. Most patients (75%) were on dialysis prior to graft nephrectomy, and in 50 cases (25%), the graft was removed before the first dialysis session. Graft intolerance syndrome was the most common indication for graft nephrectomy (55% of cases), followed by recurrent urinary tract infections (9.6%), to enable withdrawal of immunosuppression (6.1%), and to create space for retransplantation (5.1%). This percentage was stable throughout the studied decades with the exception of the last decade (2010-2018) in which a larger proportion was removed to create space for retransplantation.

Main histopathological findings

In 21 of 197 kidneys (10.7%), the presence of severe atrophy or gross necrosis due to infarction or thrombosis precluded an adequate histological interpretation. In 163 of the remaining 176 cases (93%), clear signs of acute and/or chronic rejection were present. Rejection was classified as chronic in 86 patients, acute in 15

Table 1. Characteristics of the study population.

Study population (n = 197)	
Male/female	115/82
Median age at the time of kidney transplantation (years)	31 (1–69)
Decade of kidney transplantation	
1968–1969	1 (0.4%)
1970–1979	24 (12.2%)
1980–1989	55 (27.9%)
1990–1999	66 (33.5%)
2000–2009	40 (20.3%)
2010–2018	11 (5.6%)
Median graft survival (months)	32.6 (3–461)
Decade of graft nephrectomy	
1970–1979	12 (6.1%)
1980–1989	43 (21.8%)
1990–1999	45 (22.8%)
2000–2009	55 (27.9%)
2010–2018	42 (21.3%)
Median time from kidney transplantation to allograft nephrectomy (months)	30.3 (3–336)
Median time from kidney graft failure to allograft nephrectomy (months)	2.8 (0–128)
Indication for graft nephrectomy	
Graft intolerance syndrome	110 (55.3%)
Recurrent urinary tract infections	19 (9.6%)
To enable withdrawal of immunosuppression	12 (6.1%)
To create space for retransplantation	10 (5.1%)
Hypertension	9 (4.6%)
Refractory nephrotic syndrome	8 (4.1%)
Malignancy	7 (3.6%)
Kidney stones	2 (1.0%)
Thrombosis	5 (2.5%)
Other indication	8 (4.1%)
Indication unknown	7 (3.6%)

patients, and both chronic and acute in 62 patients. In the remaining 13 cases, no signs of acute or chronic rejection were reported (Fig. 2).

In 14 cases, rejection was accompanied by a recurrence of the primary disease (n = 4), signs of calcineurin inhibitor (CNI) toxicity (n = 4), or other secondary findings (n = 6) that are described below.

Histopathological findings in removed grafts without signs of rejection were recurrence of primary disease (n = 9) and thrombotic microangiopathy (n = 2). In one case, the recurrence of focal segmental glomerulosclerosis (FSGS) was accompanied by CNI toxicity. Furthermore, there were single cases of severe candida pyelonephritis and extensive BK nephropathy.

Reassessment of histopathological specimen of 20 randomly selected patients gave no additional relevant information.

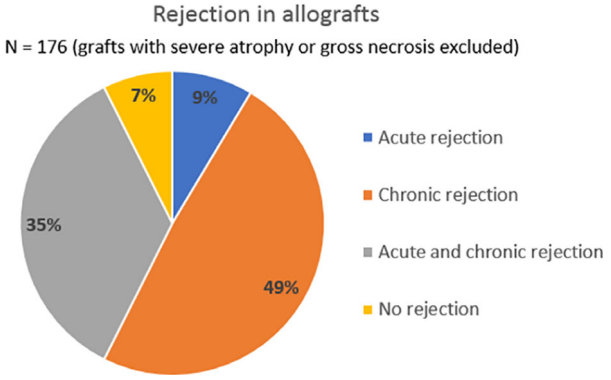

Figure 2 Distribution of type of rejection in 176 allograft nephrectomy specimens.

Recurrences of native kidney disease

Histological signs of recurrence of the native kidney disease were found in 13 patients. In six patients, this was membranoproliferative glomerulonephritis (MPGN), in six cases FSGS, and in one case, there was a recurrence of oxalate nephropathy in a patient with a primary hyperoxaluria. In all cases, the recurrence was already diagnosed by graft biopsies prior to the nephrectomy. Consequently, histological examination of the removed graft did not lead to new insights with respect to the pretransplant cause of end-stage renal disease in any patient.

Secondary findings and clinical consequences

Relevant secondary findings are listed in Table 2. There were four grafts in which a malignant disease was found. In three of these cases, the presence of the malignancy was already known based on previous CT scans and biopsy results. In only one case, concerning a small papillary renal cell carcinoma of 0.8 cm, this was an unexpected finding. In the latter patient, the indication for graft nephrectomy was to create space for a third transplantation. After the finding of the renal cell carcinoma in the nephrectomy specimen, a CT scan of chest and abdomen was performed for cancer staging, which showed no signs of metastases and no treatment was given. However, retransplantation was withheld for 9 months.

In two patients with known systemic candida infection, the presence of candida was observed in the removed graft. There was one nephrectomy specimen showing severe BK nephropathy which was also known from prior graft biopsy. In a single patient, membranous nephropathy was diagnosed in the removed graft,

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Table 2. Relevant secondary findings in histopathological examination of removed kidney allografts.

Relevant secondary findings	Expected	Consequence
Malignancy		
Urothelial cell carcinoma of the pyelum	Yes	Retransplantation withheld for 2 years
Renal cell carcinoma (8 cm), infiltrative with lymph node metastasis	Yes	Malignancy was reason for graft failure. Retransplantation withheld
Papillary renal cell carcinoma (0.8 cm)	No	CT scan. Retransplantation withheld for 9 months
Post-transplant lymphoproliferative disease (PTLD); no other localizations of lymphoma	Yes	No additional treatment necessary. Retransplantation withheld for 1 year
Infections		
Candida abscesses	Yes	Intravenous antifungal therapy, retransplantation withheld
Candida pyelonephritis	Yes	No change of existing treatment
Polyomavirus inclusions	Yes	No consequence
Other		
Membranous nephropathy	Yes	No consequence

which was the third transplant for this patient with an unknown primary disease (probably chronic pyelonephritis, no signs of nephrotic syndrome). Prior kidney biopsy showed acute rejection and membranous nephropathy. Despite antirejection therapy with corticosteroids, there was a persistent nephrotic syndrome resulting in graft failure and subsequent graft nephrectomy.

Graft removal because of a malignant disease

In seven patients, allograft nephrectomy was performed because of a malignancy (Table 1), and in five of them, a malignant lesion in the graft itself was presumed to be present. In these cases, histopathological examination of the graft was performed to confirm the diagnosis, and to report tumor size and resection margins. In three patients, the presence of a malignancy was confirmed by histological examination (Table 2), while in two patients the nephrectomy specimen showed no signs of malignancy. In one of these patients, non-Hodgkin lymphoma was expected because of lymphoid infiltrates in a prior biopsy specimen. Examination of the graft showed severe rejection, but no localization of a malignant lymphoma. In another patient, a malignant process was expected because of hematuria with a CT scan showing an abnormal aspect of the graft and enlarged lymph nodes surrounding the graft. However, no malignancy was found in the removed graft or dissected para-iliac lymph nodes.

Two patients underwent graft nephrectomy because of a urological malignancy outside the graft.

The first patient had a clear cell renal carcinoma in one of the native kidneys but not in the allograft, and the second patient had extensive surgery because of a carcinoma of the bladder, and as part of this procedure, the graft was removed and showed no signs of malignancy.

Discussion

We performed a retrospective analysis of the utility of histopathological examination in 197 allograft nephrectomies performed between 1968 and 2018. Our study confirms the presence of signs of acute and/or chronic rejection in nearly all nephrectomy specimens. Our most important observation is that unexpected secondary findings were very rare, and generally did not have any consequences for patient management. In our cohort of nearly 200 patients, the only unexpected finding with clinical relevance was a papillary renal cell carcinoma of 0.8 cm. Without histopathological examination of the removed graft, this lesion would have been missed. Probably, this would not have caused any harm to the patient, because the tumor was small and there were no signs of lymph node involvement or metastases.

To our knowledge, this study represents the largest series of pathology reports of allograft nephrectomies. The high rate of histological signs of rejection is in accordance with the results of two other smaller studies on this subject [11,12]. However, the proportions of acute and chronic rejection varied in these two studies and our cohort. In the study of Goral *et al.*, 53 graft nephrectomy specimens from patients who had an allograft nephrectomy later than three months post-transplant were examined according to the Banff classification [11]. Acute rejection was present in 89%

of the grafts and was classified as grade 2B or even grade 3 in most patients. Notably, 24% of the patients with late nephrectomy in this study were not on any immunosuppressive agent at the time of allograft nephrectomy. Furthermore, almost all patients in this cohort (51/53) were symptomatic (hematuria, graft pain and/or fever) and finding acute rejection in this particular group is not surprising. In contrast, Zargar *et al*. reported histological signs of acute rejection in only 1 case of 39 allograft nephrectomies performed more than six months post-transplant in symptomatic patients. In our cohort, 55.3% were symptomatic with a graft intolerance syndrome. The prevalence of histological signs of acute rejection in removed grafts of symptomatic patients was 46.3%, which is in between the prevalence in the two prior studies. It is important to notice that alloimmune injury could have been involved in the process of graft failure but could also be the result of the reduction in immunosuppression after graft failure. The median time between graft failure and allograft nephrectomy was 2.8 months, and was more than 1 year in 19% of the cases. Anyhow, the presence of acute and/or chronic rejection is an expected finding in grafts that are removed after late graft failure, especially in cases where the treatment with immunosuppressive drugs has already been discontinued and in the presence of a graft intolerance syndrome. In these circumstances, a more detailed phenotyping of the rejection process would not provide information leading to changes in the clinical management of the patient. For similar reasons, we did not reassess the pathological changes in the removed grafts according to the current Banff classification.

The greatest strength of our study, next to the size of the cohort, is the precise assessment of the clinical relevance of the histopathological findings. We had access to the medical records of all patients who were included in this study. Taking the information from the patient's history and prior graft biopsies into account, we concluded that the routine histopathological examination of the removed graft did not provide new information on the cause of graft failure or on the type of native kidney disease. Although 13 of the 176 nephrectomy specimens showed signs of recurrence of a native kidney disease, in all cases the recurrence was already diagnosed by graft biopsies prior to the nephrectomy. We acknowledge that this can also reflect an active policy of our center regarding performing indication biopsies in transplant patients with worsening kidney function or proteinuria.

Findings that were unexpected based on medical history or prior graft biopsies were very rare. In two patients with systemic Candida infection, the removed graft also showed signs of Candida infection. In one of these patients, this was reason to extend antifungal therapy and withhold retransplantation until antifungal therapy was completed. In the other patient with Candida infection, the treatment was not affected. There was a single case where a small renal cell carcinoma was detected which prompted some delay of a subsequent transplantation.

Current imaging techniques have considerably higher sensitivity to detect relevant kidney lesions [13]. In our opinion, routine histopathological examination of the removed allograft has little added value when no abnormalities are observed on preoperative ultrasound or CT scan. Histopathological examination can therefore be limited to patients in whom the cause of graft failure is unclear, or where there is suspicion of a recurrence of the original kidney disease which has not been diagnosed before in a graft biopsy. This proposal is applicable in case of meticulous post-transplant surveillance. It is possible that in centers with a less active follow-up of patients histopathological examination of removed allografts can reveal new information, such as an unknown primary disease, or a recurrence of the original disease that gives prognostic information concerning the chance of a recurrence in a subsequent graft.

A separate category of patients are those who are known to have a malignant disease or in whom a malignancy is suspected. In these cases, we strongly advocate histopathological examination of the removed graft to confirm the diagnosis and to assess tumor size and lymph node involvement. In our series, a malignancy was present or suspected in seven cases and histopathological examination of the removed graft proved to be relevant for the treatment of the patient in all cases.

Sending of an explanted organ to the pathology department is a widely used standard procedure. However, this should not impede the questioning of its usefulness. Withholding routine histopathological examination of the removed grafts has the obvious advantage of reducing resource utilization. Absolute cost savings are limited because the number of graft nephrectomies is small, but processing of the removed grafts for adequate histopathological examination requires specific expertise which may be difficult to provide especially during out-of-office hours. Moreover, the histopathological examination of the graft requires a pathologist with extensive experience in kidney graft

pathology since the presence of severe chronic injury can complicate the assessment of more subtle lesions.

Conclusion

As expected, signs of acute and/or chronic rejection are the main histopathological findings in grafts that are removed after late graft failure. Unexpected and clinically relevant secondary findings are very rare. In our opinion, routine histopathological examination is not indicated, and probably not cost-effective. On the other hand, if the cause of graft failure is unclear, when there is suspicion of a recurrence of the original kidney disease, or when the patient has a malignancy or systemic infection, histopathological examination of the removed graft can provide useful information.

Authorship

KLWB: participated in research design, data analysis and writing of the paper. EJS: participated in data analysis and supervised the writing of the paper. LBH: participated in research design, contributed to and supervised the writing of the paper.

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Conflicts of interest

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