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Percutaneous renal graft biopsy: a clinical, laboratory and pathological analysis

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ABSTRACT

Context: Renal allograft biopsies have been used as a good method for monitoring the evolution of kidney transplants for at least 20 years.¹ Histological analysis permits differential diagnosis of the causes of allograft dysfunction to be made.

Objectives: To correlate the data of urinalysis and serum creatinine with histological diagnosis of renal graft in a group of renal transplant patients.

Design: Accuracy study, retrospective analysis.

Setting: A university tertiary referral center.

Sample: 339 percutaneous allograft biopsies obtained from 153 patients. Blood and urine samples were obtained before the graft biopsy.

Main Measurements: Laboratory evaluation and histological analysis (light microscopy, immunofluorescent electronic microscopy).

Results: Most of the biopsies (58.9%) were performed during the first month post-transplant. An increase in serum creatinine was associated with acute tubular and/or cortical necrosis. Proteinuria and normal serum creatinine were associated with glomerular lesions. Non-nephrotic range proteinuria and an increase in serum creatinine were associated with chronic rejection.

Conclusion: Evaluation of serum creatinine and urinalysis can be useful in suggesting the histological graft diagnosis.

Key words: Kidney transplantation. Renal biopsy. Acute rejection. Acute tubular necrosis. Glomerulonephritis.

INTRODUCTION

Renal allograft biopsies have been used as a good method for monitoring the evolution of kidney transplants for at least 20 years.¹ Histological analysis permits differential diagnosis of the causes of allograft dysfunction to be made. Such an evaluation can lead to the avoidance of the use of additional immunosuppressive drugs,^{1,2} thereby reducing the incidence of complications. The usual position of an allograft in the iliac fossa renders it easily palpable and accessible to safe needle biopsy.¹ However, the risk of accidents leading to loss of the allografts is still around 1%.³ Sufficient material for histological evaluation is generally obtained in about 90% of the cases.³

In the present study, we analyzed 339 percutaneous needle allograft biopsies from 135 patients and compared these results with the clinical presentation and laboratory data obtained before the biopsy as well as with the incidence of complications.

METHODS

From November 1986 to December 1991, 339 percutaneous biopsies were obtained from 135 renal transplant patients.

The usual immunosuppressive protocol included azathioprine (2 mg/kg/day) and prednisone (2 mg/kg/day) for recipients of a transplant from a living related HLA-identical sibling donor, and cyclosporine (7 mg/kg/day) for recipients of a transplant from a living related non-identical or cadaver donor. Prednisone was progressively reduced to 10 mg/day at the third month post-transplant, and the cyclosporine dose was adjusted to maintain blood levels around 100 to 200 ng/dl as determined by a monoclonal antibody radioimmunoassay.

Acute rejection episodes were treated with a pulse of methylprednisolone (500 mg/day, IV), for three days.

Laboratory evaluation. Blood and urine samples were collected before renal biopsy. The urinary analysis involved sediment and biochemical tests. When proteinuria was positive, 24 hour urine samples were collected for the quantification of protein. The blood samples were analyzed for their creatinine (Jaffé method) and cyclosporine levels as well as their ability to coagulate.

Indications for percutaneous needle allograft biopsy. Percutaneous needle allograft biopsy was indicated when at least one of the following criteria was present:

1. An increase in the serum creatinine level to more than 25% above basal.
2. Signs and symptoms of acute rejection, including fever, edema, arterial hypertension, renal pain, body weight gain, eosinophilia, oliguria and elevation of serum creatinine levels.
3. Cadaver kidney recipients with oliguria or

anuria and/or stable serum creatinine levels above normal values (> 2 mg%).

4. Unsuccessful treatment of the acute rejection with methylprednisolone.
5. Abnormal urinalysis with glomerular hematuria, proteinuria in isolated samples and/or hematic or leukocytic casts.
6. Twenty-four hour proteinuria above 1 g/day.

Biopsy Procedure. Prior to renal biopsy, the patients had their blood pressure controlled and their blood coagulation parameters determined. In addition, the allografts were evaluated by renal ultrasound. If other causes of renal dysfunction such as vascular or ureteral obstruction were conclusively discarded, the renal biopsy was performed. Vin-Silverman-Franklin or discardable Tru-Cut Travenol[®] needles were used. Usually the needle was positioned in the convex lateral border in the superior pole and, after local anesthesia, was introduced in a perpendicular position, followed by the removal of one or two tissue fragments. Post-biopsy hematuria was monitored by the visual inspection of urine samples on three separate occasions.

Histological Analysis. Renal biopsy fragments were considered adequate if they contained cortical or cortical/medullar junction tissue. Samples containing only medullar tissue were inadequate for diagnosis, and another biopsy was performed. The biopsy material was processed in the University Department of Pathological Anatomy. Fragments were examined by light microscopy following HE, PAS and Masson staining. Immunofluorescent staining was done with antiserum to IgM, IgG, IgA, C3, C1q, kappa and lambda. Some samples were examined by electron microscopy.

Statistical Methods. Statistical analysis was carried out using the chi-square test and unpaired Student's t test.

RESULTS

From November 1986 to December 1991, 339 needle allograft biopsies were obtained from 135 renal transplant patients (95M, 40F), out of a total of 247 such transplants performed during

Table 1 - Number of percutaneous graft biopsies performed per patient during study period

donor	patients	number of graft biopsies	relationship biopsy/patient
identical HLA	14	26	1.85
non-identical HLA	57	119	2.08
cadaver	64	194	3.03 *
Total	135	339	2.51

* p < 0.05 (chi-square test)

this period. The number of biopsies per patient (Table 1) was significantly greater in individuals receiving a kidney from cadaver donors ($p < 0.05$). In 64 such recipients, 194 biopsies were done, compared to 145 biopsies in 71 patients receiving a kidney from related living donors (14 HLA-identical and 57 non-identical).

The indications for renal biopsy were graft dysfunction in 272 instances (80.2%), anuria in 34 (10.0%) and abnormal urinalysis in 33 (9.8%). Graft dysfunction ($n = 272$) was associated with oliguria in 74 instances (27.2%) and no response to rejection treatment in 15 (5.5%). In the patients for whom allograft biopsy was indicated by abnormal urinalysis, the most frequent abnormality observed was isolated proteinuria (in 20 biopsies) followed by proteinuria in association with glomerular hematuria in six biopsies. Isolated glomerular hematuria was the cause of renal biopsy on seven occasions (Table 2). One hundred and eighty-three biopsies (53.9%) were done during the first month post-transplant, with a gradual reduction in the number thereafter. In the first year after the renal transplant, 302 biopsies (89%) were performed (Table 3). Adequate material for analysis was obtained in 306 fragments (91.1%). Severe hemorrhagic complications occurred in four cases, and led to graft loss in two.

Acute rejection was observed in 136 fragments (40.1%), 106 of them during the first two months post-transplant and four after the first year. In this group, renal dysfunction was the main indicator of renal biopsy in 127 instances, followed by anuria in seven and abnormal urinalysis in two. Acute cellular rejection (ACR) without a vascular component or acute tubular necrosis was observed in 57 biopsies. ACR associated with acute tubular necrosis was seen in 47 instances and was associated with vascular rejection in 32 fragments. No difference in the incidence of ACR was observed between recipients of cadaver or living-related donor organs.

Acute tubular necrosis (ATN) was the second most frequent diagnosis (66 biopsies, 19.4%). As with ACR, acute tubular necrosis was

observed mainly during the first two months post-transplant (62 cases). The histological diagnosis of ATN was more frequent in cadaver donor recipients. In individuals with ATN, the indication for biopsy was renal dysfunction in 52 instances, anuria in 12 and abnormal urinalysis in two.

Chronic rejection was observed in 40 fragments (11.7%). This diagnosis became more frequent after the sixth month post-transplant (Table 3). Renal dysfunction was again the major indicator of the need for a biopsy. In 26 fragments, a glomerular lesion was detected.

Glomerulonephritis (GN) was diagnosed in 27 biopsies (7.9%). Focal and segmental glomerulosclerosis was the most frequent diagnosis, occurring in ten biopsies from eight patients. Mesangiocapillary type I GN was observed in seven biopsies from four patients, and type III in two biopsies from two patients. Membranous GN occurred in two fragments from two patients. In one patient, the diagnosis was mesangial proliferative GN. In this group, proteinuria (7.71g, SD 8.82) was greater than in others, while serum creatinine (2.36mg/dl, SD 1.99) was the lowest among the various groups. In living donor recipients, the diagnosis of GN was more frequent than in cadaver ones.

Acute vascular rejection was observed in 14 fragments (4.1%) from 11 patients. Graft loss occurred in all patients within two months after diagnosis. All biopsy indications were based on renal dysfunction. The urinalysis (proteinuria below 1 g/l, hematuria < 50 RBC per high power field) and serum creatinine levels (6.45 mg/dl) were similar to ACR group.

Table 2 - Indications for percutaneous graft biopsies

Indications	number (%)
Graft dysfunction	272 (80.2%)
- with oliguria	74
- no response to anti-rejection treatment	15
Anuria	34 (10.0%)
Abnormal urinalysis	33 (9.8%)
- isolated proteinuria	20
- proteinuria + glomerular hematuria	6
- isolated glomerular hematuria	7

Cortical necrosis was the diagnosis in 13 fragments (3.8%), and in 12 of them the indication for renal biopsy was anuria. All biopsies were done in the first month post-transplant. A total of seven patients were included in this group and all the grafts were removed.

DISCUSSION

Percutaneous needle allograft biopsy may be performed in order to evaluate the renal function of kidney transplant patients. The position of the allograft in the iliac fossa permits easy access for the biopsy procedure. The kidney can be localized by bimanual palpation and hemostasis is facilitated by the use of a compressive technique.³ Adequate material for histological analysis, containing cortical and cortical/medullar junction material is obtained in 80% to 100% of renal biopsies. Renal biopsy accidents that result in kidney loss are rare, occurring in about 1% of the cases. In the present study, the incidence of allograft loss after percutaneous needle biopsy was 1.5% (2 graft losses for 135 patients).

The major indication for kidney transplant biopsy was the differential diagnosis of acute rejection and renal dysfunction. This indication was based on the increase in serum creatinine levels. Probable differential diagnoses included acute vascular rejection, chronic rejection, acute tubular necrosis and cyclosporine nephrotoxicity. Matas¹ suggested that 40% of renal transplant patients presented no changes in their immunosuppressive

therapy after renal allograft biopsy. In the present study, unchanged immunosuppression was observed in 38% of the cases.

The usefulness of urinalysis as an indicator for allograft biopsy is controversial. Hematuria has no value either as an indicator for renal biopsy or in the prognosis of renal function. Proteinuria is an important marker and has been extensively studied.^{4,5,6,7} Massive proteinuria after transplant is frequent during the first three months,⁴ with spontaneous remission during evolution. Persistent proteinuria occurs in about 30% of transplants, and is positively correlated with the presence of glomerular lesions, which is indicative of chronic rejection or glomerulonephritis. Persistent proteinuria is one of the most frequent indicators for renal biopsy. In the present study, proteinuria was used as an indicator for allograft biopsy in 7.6% of the cases, and was present in 21.6% of the renal biopsies.

Abnormal urinalysis was indicative of allograft biopsy in 10% of the cases, especially after the third month post-transplant. The histological diagnoses were chronic rejection and glomerular disease.

Acute tubular necrosis (ATN) occurs in about 30 to 60% of cadaver kidney recipients and in about 10% of recipients from living donors.⁸ The former incidence may be associated with extended periods of cold organ storage that can promote tissue ischemia and acute tubular necrosis. The hemodynamic status of the donor can also influence the onset of acute tubular necrosis.^{8,9} In our study, ATN occurred in 29.3%

Table 3 - Time post-transplant when graft biopsies were performed

Days	ACR	AVR	ATN	CR	GN	other	total
1 — 30	92	7	51	0	0	33	183
31 — 60	14	7	11	0	3	4	39
61 — 180	22	0	3	6	8	6	45
181 — 365	4	0	1	14	7	9	35
after 1st year	4	0	0	20	9	4	37
Total	136	14	66	40	27	56	339

ACR = acute cellular rejection, AVR = acute vascular rejection, ATN = acute tubular necrosis, CR = chronic rejection, GN = glomerulonephritis. *other: medullar (n=33), cortical necrosis (n=13), hemolytic uremic syndrome (n=3), Cyclosporine nephrotoxicity (n=2), chronic pyelonephritis (n=2), normal (n=1), granulomatosis (n=1), nephrosclerosis (n=1).

of the patients (74.3% received a kidney from a cadaver donor and 25.6% from a living donor). Urinalysis was not important in this group, since the presence of non-glomerular hematuria and leukocyturia could have been secondary to the post-operative period or to the presence of a bladder catheter.

Acute rejection is considered to be the most frequent cause of allograft dysfunction and usually occurs during the first three months post-transplant.^{1,2} In the present study, we observed acute rejection in 40.1% of the biopsies. The incidence of acute rejection was similar between recipients from cadaver or living related donors. Acute rejection associated with ATN was a frequent histological observation in our patients. This association may reflect antigen presentation by necrotic tubule cells which results in a rejection process.⁸

Acute vascular rejection can be secondary to the presence of humoral factors.¹⁰ Vascular rejection is considered a poor prognostic indicator, because the intimal infiltrate can lead to occlusive arteriopathy and tissue ischemia.¹¹ Individuals in this group had short survival since the aggressive immunosuppressive therapy can promote infectious complications and death of the patient. In our series, acute vascular rejection was associated with long-term graft loss in 100% of the cases. The histological analysis showed renal cortical ischemia and obliterative arteriopathy, secondary to intimal growth, suggestive of endothelial disease.

Chronic rejection occurs frequently after the sixth month post-transplant and progresses to chronic renal failure.¹² The condition occurs in about 17% of renal transplants, although in our series the incidence of chronic rejection was 11.8% (40 histological diagnoses in 21 patients). The clinical diagnosis was based on an increase in serum creatinine levels, usually in the presence of normal urinalysis. The average level of isolated proteinuria was about 1.31 g. Histological evaluation showed tubular atrophy, interstitial fibrosis and thickening of vascular walls, all of which suggest ischemic disease. Varying degrees of glomerular sclerosis were also present. The

differential diagnosis between transplant glomerulopathy, chronic rejection and segmental glomerulosclerosis can be difficult. Immunofluorescence and electron microscopy can be helpful in such cases.

Post-transplant glomerulonephritis is frequent, but classification as de novo, recurrent or indeterminate is difficult since the primary renal disease is rarely diagnosed. Such glomerulonephritis can be due to persistent systemic factors,¹³ donor glomerular disease,¹⁴ ischemic lesions, hypertension or secondary to acute rejection episodes.⁸ Following its introduction, cyclosporine has been found to be a further factor causing glomerular lesions^{15,16}

Histologically, glomerular lesions can be superposed since, as with ischemia, hypertension and immunological or toxic factors, the pathogenic mechanisms can lead to endothelial injury¹⁷ which in turn produces focal and segmental glomerulosclerosis, usually in the vascular pole of the glomerulus. These mechanisms can promote thickening of the basal membrane. In short, the histological analysis of biopsy fragments by optical microscopy alone, along with imprecise diagnosis of the primary renal disease, does not permit correct diagnosis of post-transplant glomerular disease.

The differential diagnosis of chronic rejection, cyclosporine-mediated nephrotoxicity and focal and segmental glomerulosclerosis requires immunofluorescence and electron microscopy. The serum levels of cyclosporine may also be useful in such diagnoses.^{17,18}

In summary, percutaneous needle allograft biopsy is an useful procedure in the follow-up of kidney transplants. Since such biopsies permit a differentiation between rejection and any other causes of allograft dysfunction, they should prove to be useful in determining adequate immunosuppressive therapy and in reducing the risk of complications.

The only laboratory data that generally correlated with the histological diagnosis was the elevation in serum creatinine levels associated with ATN or cortical necrosis. Proteinuria with normal creatinine levels was associated with

glomerular lesions, usually after the sixth month post-transplant. Late proteinuria in the non-nephrotic range associated with increased creatinine levels may be indicative of chronic rejection.

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RESUMO

Contexto: Biópsia percutânea do rim transplantado tem representado um bom método para monitorização da evolução funcional do enxerto há pelo menos 20 anos. A análise histológica nos permite o diagnóstico diferencial entre as várias causas de disfunção do enxerto. **Objetivos:** Correlacionar, em pacientes transplantados renais, o exame simples de urina e a creatinina sérica com achados histológicos do enxerto renal. **Tipo de Estudo:** Estudo da acurácia, análise retrospectiva. **Local:** Hospital das Clínicas da UNICAMP. **Amostra:** 339 biópsias renais percutâneas (de 135 pacientes transplantados) no período de janeiro de 1984 a dezembro de 1991. Amostras de sangue e de urina foram coletadas antes da biópsia renal. **Variáveis estudadas:** Avaliação laboratorial e análise histológica. **Resultados:** Em 94,1% das indicações de biópsia foi obtido material para análise histológica. Em 58,9% das biópsias a indicação ocorreu no primeiro mês de transplante. Elevação da creatinina sérica associou-se a necrose tubular aguda e/ou necrose cortical. Proteinúria com níveis de creatinina sérica dentro da normalidade associou-se a doenças glomerulares. Proteinúria (níveis não nefróticos) e aumento da creatinina estiveram associados a rejeição crônica do transplante. **Conclusão:** A análise da creatinina sérica e do exame de urina em pacientes transplantados renais pode sugerir o padrão histológico diferenciado de acometimento.