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University of Alberta

Clinical application of Banff Schema in baseline and follow up biopsies

By

Huijian Wang 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

In

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Clinical application of Banff Schema in baseline and follow up biopsies** by **Huijian Wang** in partial fulfillment of the requirements for the degree of **Master of Science in Experimental Pathology.**

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Abstract

Donor age is an very important factor in influencing the long-term kidney graft survival. Using the Banff Schema to measure the chronic histologic changes in older donor kidneys, we have demonstrated that it is the higher prevalence of vascular lesions in older donor kidneys that are responsible for their inferior outcome while age by itself is not. Caution should be exercised in accepting kidneys with lesions showing CV or moderate to severe degrees of AH due to the high incidence of delayed graft function and reduced graft survival attributed to these lesions. The percentage of glomerulosclerosis is not useful in predicting the incidence of delayed graft function or survival. Also given the sampling error for glomerulosclerosis, we believe that caution should be exercised when using the selection criteria for older donors which rely mainly on the fraction of sclerotic glomeruli in order to avoid unnecessary discarding of valuable grafts.

This thesis is dedicated to the two greatest teachers—Dr. Kim Solez and Dr. Carl M. Kjellstrand

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LIST OF ABBREVIATIONS

G	Glomerulitis
CG	Chronic transplant glomerulopathy
T	Tubulitis
CT	Tubular atrophy
I	Interstitial mononuclear infiltrate
CI	Interstitial fibrosis
V	Vasculitis
CV	Chronic intimal thickening
AH	Arteriolar Hyalinosis
DGF	Delayed graft function
UNOS	United network for organ sharing

CHAPTER 1

Introduction

Advances in technical procedures and the development of new immunosuppressive drugs have made organ transplantation possible for many patients throughout the world. However, the shortage of cadaveric organ donors remains the major concern, imposing a severe limit on the number of patients who could benefit from the therapy. In the US, from 1988 to 1994, the waiting list grew by 76% (1) and the median waiting time for a kidney increased from 394 days to 728 days (2). Currently, over 33,000 potential kidney recipients are on the waiting list in the US (3). Many people will die or remain on dialysis because the organ supply falls drastically short of the actual demand. Between 1988 and 1994, the number of reported deaths has doubled among those waiting for organs (for 1994, kidneys:3.6%, kidney-pancreas:3.5%)(1).

In the early years of organ transplantation, strict criteria were applied when selecting potential donors. The accepted age for donation of a kidney to an adult recipient initially ranged from 16 to 50 years (4). Because of the progressive disparity between the number

of patients in need of a transplant and the number of cadaveric kidneys available for transplantation, increasing numbers of kidneys have been recovered for transplantation in the US from donors that are not considered ideal. (for example, donors over the age of 55 (5,6)). As a reflection of this, the number of organ donors in the US has increased approximately 20% over the past 5 years (1,7). Between 1988 and 1992, the percentage of donors >55 years increased from 5.4% to 10.7%. Kidneys that were recovered from these donors, but not transplanted, increased from 15% to 23%, a 53% growth in the discard rate (5). Transplant centers have repeatedly evaluated and updated their donor selection criteria in an effort to expand the donor pool without compromising safety.

In the initial report of the United Network for Organ Sharing (UNOS) Registry, graft survival with kidneys from donors >55 years was approximately 10% lower at 1 year and 14% lower at 2 years than that with kidneys from the ideal donor group (16 to 45 years)(5). In a recent update of the UNOS data on the effect of donor age on the outcome of cadaveric kidney transplants, the best results were achieved with donor age 18 to 34 years. Graft survival rates for cadaveric kidneys from donors aged 50 to 64 years were 8.3%,

10.2%, and 12.2% lower at 1,2 and 3 years respectively posttransplantation when compared with the survival rates of kidneys from donor age 18 to 34. For donors older than 65 years, graft survival rates were 14.1%, 18.4%, and 20.9% lower after 1,2,3 years compared with young donors(age 18 to 34 years) (8). The results for living donor kidneys showed less striking effects of age on graft survival. Among kidneys transplanted from living donors, the best graft survival rate was achieved with donor age 11 to 17 years. By comparison with this group, graft survival rates for living donors aged 50 to 64 years and >65 years were 5.0% and 7.7% lower at 3 years posttransplantation (8).

It is generally accepted that increasing disease in older donors and the changes that normally occur in the kidney with age are responsible for the reduced allograft survival after transplantation (9). The kidneys of older donors exhibit a series of changes characterized by senescence of the glomeruli and tubules, as well as vascular changes. Glomerular changes start at the end of the third decades, and a steadily progressive glomerulosclerosis occurs with age. By the sixth decade, the number and the size of nephrons is reduced by 50%. The tubules also suffer gradual deterioration, characterized by

loss in length and volume. An increased amount of interstitial fibrosis is also present. The vascular changes produce decreased renal blood flow. A global decrease in renal mass, primarily cortical, may also occur. It has been calculated that the average weight of a single kidney in Caucasians at age of 40 is 432±37g and at the age of 60, 368±20g(10). These anatomical changes may be aggravated further by atherosclerosis, hypertension and diabetes, all of which are more common in older individuals.

Reflecting these anatomical changes are reduced kidney functions. The glomerular filtration rate begins to decrease (approximately 1ml/min per year) by the end of the third decade, at a predictable linear pattern, with each advancing year. By the sixth decade, the glomerular filtration rate is reduced by 50%. This decline in renal function is usually hidden by normal serum creatinine levels, which may increase in only very few individuals.

Take into account these characteristic changes in renal morphology in the elderly, it is necessary to establish specific selection criteria for donors from this group in order to exclude those with pathologic changes beyond the normal aging effects on kidneys. It is crucial to differentiate the normal "innocent" aging process from

the pathological changes that may aggravate the anatomical and functional changes of normal aging kidneys. However this task is limited by two major factors:

First is the lack of the consistent relationship between morphologic features and clinical course. This problem may lie within the uniform extraction of appropriate morphologic features. As a codified set of features that are highly standardized and reproducible (11,12), the Banff schema offers an approach to classifying native renal biopsies as well as allograft renal biopsies (13). Since wedge baseline biopsies are routinely performed 30-60 minutes following revasculization of renal transplants, we examined the potential utility of the Banff Schema for determining prognosis of patients.

The second is sampling error. Rapport, Converse and Billingham stated in 1971 that "Renal biopsy of renal transplants has been generally abandoned because rejection injury is not distributed uniformly throughout the transplanted kidney"(14). The sampling error considerations noted by Rapaport are still important today. The size of the renal biopsy is important as some lesions can be quite localized, particularly those involving glomeruli (15). Thus we studied the influence of sample size on the prognostic accuracy and

reproducibility of renal transplant biopsy to determine the minimal size required for prognostic use.

The Banff schema has become the international standardization of nomenclature and criteria for the histologic diagnosis of renal allograft rejection. We studied the clinical application of the Banff Schema in follow up biopsies to predict which lesions may lead to poor long-term term graft outcome. We hope that this study may shed light on further modifications of the Banff Classification, which will reduce unnecessary over or under immunosuppression, and so in improved cost control, graft survival and patient care.

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CHAPTER 2

THE INFLUENCE OF SAMPLE SIZE ON THE PROGNOSTIC ACCURACY AND REPRODUCIBILITY OF RENAL TRANSPLANT BIOPSY

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INTRODUCTION

The introduction of renal biopsy into clinical medicine by Alwall in 1944 (1) has greatly advanced the understanding of renal diseases. While an accurate diagnosis can sometimes be rendered in a biopsy containing only a single glomerulus (2), the overall sample size needed to generate accurate prognostic information is more controversial. While several authors find fairly good correlation between the biopsy and natural outcome in a variety of renal diseases (3-7), others find it much less useful (8-10), and in some instances the renal biopsy did not provide more information than could be obtained by simple routine clinical investigation (11).

The size of the renal biopsy is obviously of importance as some lesions can be quite localized, particularly those involving the glomeruli (12). Although there are statements in the literature as to how many glomeruli are needed for a satisfactory biopsy (13-16), this information is mainly personal impression. We found two articles (17,18) that carefully analyzed the size needed, either for prognostic information, or for reproducibility of findings in successive biopsies: Kellow and co-workers (17) in 1959 found reproducibility of 76%,

comparing results from core biopsies to those obtained at autopsy, but did not specifically look at biopsy size. Corwin et al (18) deduced the minimal size of biopsy from the mathematical model of binomial distribution.

Biopsies of transplanted kidneys offer a unique opportunity to study this problem. If baseline biopsies are obtained, it has been shown that chronic lesions, e.g. glomerulosclerosis, in such kidneys can prognosticate the outcome. Secondly, if subsequent biopsies are obtained in the transplanted kidney within a reasonably short time, one can compare the results from these biopsies to the baseline, and if paired kidneys are used and baseline biopsies are obtained from both kidneys in the pair, one has an opportunity of studying reproducibility between the two biopsies.

At The University of Alberta Hospital Renal Transplant Center, surgical baseline wedge biopsies are regularly performed in transplanted kidneys, and follow up needle biopsies are frequent. Furthermore, clinical follow up of these patients is almost complete. We felt this offered a unique possibility to study the influence of renal biopsy sample size both on ability to predict outcome of transplant, and on reproducibility.

MATERIAL AND METHODS

From July 1991 through April 1995, 199 renal transplants complete with baseline wedge biopsy were performed at University of Alberta Hospital. The observation period ended November 1, 1995. All transplants had been observed for 6 months or until loss of transplant function, defined as a return to dialysis or death of patient.

We investigated the influence of sample size on outcome in a study of 199 patients, all of whom are included in the analysis. We had follow up biopsies on 114 of the 199 transplanted kidneys, and these cases were used in a second study of the influence of size on reproducibility, comparing baseline to subsequent core biopsies. In 59 pairs of kidneys we had baseline biopsies on both kidneys, and these 118 kidney biopsies form the basis for a third comparison of reproducibility between the biopsies from the paired kidneys.

PATIENTS

There were 70 female patients and 129 males. The mean age was 42 ± 13 years (range 11 - 69). Of 199 transplants, 165 were first transplants, 29 second, and 5 third. The mean age of the donors was

37 ± 15 years (range 4 - 71 years). There were 145 cadavers and 54 living donors.

PREPARATION OF BIOPSY

Intra-operative baseline biopsies were performed by obtaining a small wedge from the renal cortex during the time between vascular anastomosis and ureteral anastomosis. Follow-up needle biopsies were obtained under ultrasound guidance using a Truecut® needle in a biopsy gun. The needle size was #16 and usually three cores were obtained.

The renal biopsy specimens for light microscopy were fixed in 10% buffered formalin and then embedded in paraffin. Sections of 3 μm thickness were cut and then stained with hematoxylin and eosin, PAS, silver-stain, and Masson trichrome stain.

SCORING OF BIOPSIES

The number of globally sclerosed glomeruli was counted and then compared with the total number of glomeruli and reported as a percentage. We also scored interstitial fibrosis/tubular atrophy and arteriolar hyaline thickening as binary data (absence/presence). According to Banff schema (19), the presence of interstitial fibrous

and tubular atrophy was defined as more than 5% of area involvement by the process. Multiple sections of each biopsy were always read.

TREATMENT

All patients received triple therapy with prednisone, azathioprine, and cyclosporine. After transplantation, daily cyclosporine levels were performed and the dose regulated according to blood levels, aiming at the concentration of 300 μg/L for the first two weeks and then slowly decreasing the dose, aiming at a concentration of 100 μg/L at the end of one year. In 49 cases, cyclosporine was not used early because of delayed graft function. Rejection episodes were treated with intravenous methyl-prednisolone, and if resistant, with intravenous OKT-3.

OUTCOME OF RENAL TRANSPLANT

In total, 33 kidneys were lost, 11 due to patient death, 14 from rejection, 5 from technical complications, and 3 from other causes. Twenty kidneys were lost within 6 months. There was no significant difference in long-term outcome between related and cadaver kidneys. The four year cumulative survival for living donors was 90%

versus 80% for cadaver donors ($p = 0.120$). Data were therefore pooled for all donors.

STATISTICAL METHODS

The data were entered on Statview IV Statistical Program, version 4.5 and SPSS 6.1 for the Macintosh computer. Analyses used were Fisher's exact probability test, linear regression analysis, Cohen's kappa coefficient and Cox Proportional Hazards Analysis. A probability of < 0.05 was regarded as significant. All data are mean ± standard deviation. The number of glomeruli per biopsy was used to decide the biopsy sample size.

The Influence of Lesion and Sample Size on Outcome of Renal Transplants

This was studied using Cox Proportional Hazards Analysis by successively excluding cases depending on the number of glomeruli in the biopsy. The first analysis included all biopsies, the second only those with more than 6 glomeruli, and then, more than 9, 14, 24 and 29 glomeruli successively. In this study, the three lesions (percent glomerulosclerosis, interstitial fibrosis/tubular atrophy, and arteriolar hyaline changes) were co-variates. The dependent outcome variable

in this study was transplant function defined as patient being alive and not on dialysis.

The Influence of Sample Size on Reproducibility of Findings

In the study of reproducibility of the morphological changes, we studied the influence of the size of biopsy by successively excluding cases as described above, and doing repeated chi-square or Fisher's exact analyses, Cohen's kappa determinations for association and linear regression analyses. Percent sclerotic glomeruli was always studied as a continuous variable, but we studied interstitial fibrosis and arteriolar hyaline changes both as continuous variables graded from 0 to 3 and as categorical variables, using either four groupings (0,1,2 and 3), or as binary variables - absent or present. Best accuracy always occurred in the latter two lesions with the binary absence/presence analyses, and only those results will be reported.

Because new lesions potentially could appear or old ones disappear in subsequent biopsies, we also did all analyses comparing baseline to subsequent core biopsy in two ways: first we included all biopsies independent of the time between the baseline and

subsequent biopsy, then we included biopsies only if performed within 60 days after the baseline biopsy.

RESULTS

GENERAL

In the baseline biopsy we identified four lesions, glomerulosclerosis, arteriolar hyalinosis and interstitial fibrosis/tubular atrophy. Because the last two lesions were always overlapped, they were analyzed together. The number of glomeruli in the baseline wedge biopsy was 16.4 ± 12 (range from 1 - 72). In the follow up biopsy the number of glomeruli was 10.4 ± 5.6 (range 1- 34) (see Figure 1). In 60/199 (30%) of the baseline and 29/114 (25%) of the follow-up needle biopsies, there was some glomerulosclerosis. Mean percent sclerotic glomeruli were 4 ± 7 (range 0 - 43) and 4 ± 9 (range 0 - 58) in baseline and follow up biopsy respectively. Arteriolar hyalinosis was present in 31% of baseline biopsies and 37% of follow up biopsies, There was no relation between the findings of arteriolar hyalinosis and the number of glomeruli (R=0.075, P= 0.303). Interstitial fibrosis was present in 40% and 35% respectively.

INFLUENCE OF SAMPLE SIZE ON PROGNOSTIC RELIABILITY

Percent glomerulosclerosis

When all baseline biopsies were related to outcome in Cox Proportional Hazards Analysis there was no predictive value of percent glomerulosclerosis (RR=0.998, CI 0.959-1.038, p=0.915). However, as biopsy size was increased by excluding cases with few glomeruli, accuracy increased and reached a maximum when samples including >25 glomeruli were used (RR=1.056, CI 1.010-1.105, p=0.017). These relations are demonstrated in Table 1 and Figure 2. Thus, in order to predict outcome from glomerular changes, our data indicate that samples with fewer than 25 glomeruli are unreliable.

Arteriolar Hyalinosis

Arteriolar hyalinosis was the best predictor of transplant failure when all biopsies were included. In kidneys without hyalinosis the RR=0.435, CI=0.219-0.863, p=0.017. No improvement in prognostic accuracy was obtained when including only larger samples. This was done by excluding samples with retrospectively fewer than 7, 10, 15, and 20 glomeruli, as for the analyses of the number of glomeruli.

Interstitial fibrosis/tubular atrophy

There was no correlation between interstitial fibrosis/tubular atrophy and long-term outcome, regardless of sample size. Statistical significance was not reached in any of the Cox Proportional Hazards analyses.

Comparison of glomerulosclerosis and arteriolar hyalinosis in predicting outcome

In stepwise Cox Proportional Hazards Analysis, arteriolar hyalinosis was more important in predicting outcome, until only samples with >25 glomeruli are included, at which point percent glomerulosclerosis became the better predictor of outcome.

SAMPLE SIZE AND REPRODUCIBILITY OF BASELINE TO SUBSEQUENT TRANSPLANT CORE BIOPSIES

Glomerulosclerosis

The reproducibility of glomerulosclerosis analyzed by linear regression between baseline and subsequent biopsies is

demonstrated in Table 2 and Figure 3. We successively included larger and larger samples by excluding cases with too few glomeruli, and also divided our material to include all biopsies, independent of the length of time between the two biopsies, or to include only those biopsies that were done within 60 days in order to exclude new lesions on the glomeruli. Better correlation is found in the analysis including only biopsies done within 60 days, and shows that precision, demonstrated both by increasing R values and decreasing p values, increased in all samples until only samples with 10 or more glomeruli were included. Beyond this, statistical precision is lost because there are only few cases that include more than 10 glomeruli in both biopsies. Correlation coefficient was only moderate, always less than 0.5, in statistically significant analyses.

Arteriolar Hyalinosis

We analyzed arteriolar hyalinosis by both sample size and time between biopsies with Cohen's kappa and Fisher's exact probability test (Table 3). Best statistical significance was obtained when all samples were included, while agreement increased only moderately when sample size was increased. There was no increase in

agreement, and always less statistical significance when a time limit of <60 days between biopsies was used: there was reproducibility in only two-thirds of the cases; in one-third, a lesion was found in only one of the two biopsies.

Interstitial fibrosis/tubular atrophy

There was no significant relation in interstitial fibrosis/tubular atrophy between baseline and subsequent biopsy in any combination of time limit or sample size.

COMPARISON OF BASELINE BIOPSIES IN PAIRED KIDNEYS

Percent glomerulosclerosis

The results of agreement of glomerulosclerosis in paired baseline biopsies studied by linear regression analysis, are demonstrated in Table 4. There was no correlation when all biopsies were included ($r = 0.217$, $p = 0.1081$), however, precision increased when sample size was increased, and when samples with only >14 glomeruli were used statistical significance occurred, ($r = 0.83$, $p < 0.001$). However in only 16 cases did both biopsies include >14

glomeruli, and the results were greatly influenced by a single outlier: if excluded r = 0.025, p= 0.858 (Fig. 4).

Arteriolar hyalinosis

Including all 54 paired biopsies with arteriole there was agreement in 80%, Cohen's kappa = 0.55 of the biopsies (p=0.0001). Both precision and probability decreased as the sample number decreased by excluding samples based on number of glomeruli (Table 5).

Interstitial fibrosis/tubular atrophy

There was no reproducibility in interstitial fibrosis/tubular atrophy between paired baseline biopsies, independent of sample or time.

COMPARISON OF FINDINGS IN THE BASELINE/BASELINE AND BASELINE/CORE ANALYSES

For glomerulosclerosis, there was much better agreement between the two baseline biopsies (Table 4) than between the baseline and subsequent core biopsy (Table 2). Thus, the R - value for >14 glomeruli between the two baseline biopsies is 0.83, compared to 0.56 for baseline to core biopsy. On the contrary, there

is no great difference in the reproducibility of arteriolar hyalinosis. The Cohen's kappa is 0.45 (Table 5) versus 0.47 (Table 3) for baseline/baseline and baseline/core biopsy respectively, when samples including > 14 glomeruli are considered.

DISCUSSION

In this study, we have examined the importance of sample size in renal transplant biopsy in three different ways. The succinct findings are, that to make any prognostic conclusions from glomerulosclerosis, >25 glomeruli are necessary, but for arteriolar hyalinosis sample size is not important. Interstitial fibrosis/tubular atrophy does not seem to carry prognostic information.

All three separate analyses of glomerulosclerosis, its predictability on outcome, its reproducibility between baseline and subsequent biopsies, as well as the comparisons of the two baseline biopsies, are in agreement. More than 25 glomeruli are necessary to predict outcome from glomerulosclerosis, and more than 14 to result in a coefficient of determination (R^2) of > 0.25 in the comparison of baseline to baseline or to subsequent biopsies. To us, these data

indicate that glomerulosclerosis is a localized lesion in the kidney, and that many more glomeruli are necessary for evaluation of lesions in the glomeruli than generally assumed.

Arteriolar hyalinosis is different. In all three analyses, the highest precision was reached statistically when all samples were included. The statistical significance rapidly declined as the number of samples was reduced, subsequent to the increase in sample size. We interpret these findings as showing that arteriolar hyalinosis is a diffuse lesion, and that almost any renal biopsy in which a blood vessel is found is diagnostic for this lesion. There were discordant findings in 10% of the comparison of baseline to baseline and in 20% - 30% of baseline to subsequent core biopsy. These findings at first appear to be in disagreement with the observation by Bell (20) who found renal arteriolar hyalinosis to be unevenly distributed in autopsies. However, he studied only hypertensive patients, and such patients are often excluded by transplant teams. Thus, the age-related lesions seen by us may be more diffuse, and the agreement between baseline to baseline and baseline to core biopsy, were better. The 50% discrepancy in the findings between paired baseline

and core biopsy is compatible with sampling error. It can also be due to the qualitative difference between the more superficial baseline wedge biopsy and the deeper core biopsy It can also be due to factors which cause new arteriolar hyaline change (cyclosporine/hypertension) or resolution of lesion of donor organs (14,15).

Interstitial fibrosis/tubular atrophy was of no value in predicting the outcome of the kidneys, regardless of sample size. This observation is at variance, particularly with the observation by Wehrmann and associates (3). These different findings probably reflect the fact that scarring, on a vascular atherosclerotic basis, as present in our patients, is quite patchy and therefore highly prone to sampling error, while it may be more diffuse in glomeulonephritis, the disease present in Wehrmann's patients. There were no correlations between interstitial fibrosis/tubular atrophy either between baseline and subsequent biopsies, nor in paired baseline biopsies.

We believe that this evaluation of the importance of size of the renal biopsy clarifies the many differences that have been found between morphological changes in the kidney, and outcome (3-15).

Our findings agree well with the conclusions of Corwin et al (18) and Madaio (21). Based on a mathematical model of binomial distribution, they thought at least 20 glomeruli were necessary for reasonable clinical prediction of focal glomerulosclerosis and of the severity of lupus nephritis. They did not discuss other findings such as hyalinosis or fibrosis. Our findings are quantitatively different from those of Gaber and co-workers, who found samples containing > 10 glomeruli to be prognostically important (22). Their analysis is different from ours, in that time to graft loss was not considered and the sample size of renal biopsy much smaller.

Each of the three analyses performed by us has certain strengths and weaknesses. In our trial to relate outcome to morphological changes there are many other factors that influence the fate of a transplanted kidney, and these clinical factors such as tissue-type, drug toxicity, and acute renal failure, make this analysis weak. In the comparison of baseline to core transplant biopsy, obviously new lesions may appear secondary to insults against the kidney after transplantation or old lesions perhaps disappear. However, as we focused only on chronic changes and also used a 60-day time limited analysis, we believe that the data here are

reliable. It appears to us unlikely that any of these chronic changes should either form or disappear within the 60-day time limit of our analysis (14,15). The comparison of a superficial wedge biopsy to the deeper core biopsy may be affected by quantitative differences. These may include a higher percentage of sclerosed glomeruli in the superficial wedge biopsy than in the deeper core biopsy obtained with a needle (23). In fact, we found that glomerulosclerosis was less reproducible in the comparison of baseline wedge biopsy to core needle biopsy than hyalinosis.

The easiest study to interpret is the comparison of two paired baseline biopsies. However, although the latter is morphologically the most sound, it suffers from the disadvantage of containing the fewest number of biopsies. Therefore statistical instability markedly increased as increasing sample sizes were included with subsequent decrease in the number of biopsies.

Perhaps the greatest strength in our three analyses is, that although there may be some quantitative differences, all of them essentially showed the same findings: for judgment of glomerular changes a large number of glomeruli, between 15 and 25 are necessary; for prognostication and reliability of arteriolar hyalinosis,

almost any size renal biopsy will suffice; and finally, that interstitial fibrosis/tubular atrophy has little value either for prognosis or reproducibility.

The second limitation of our study is that only chronic changes in the kidney were studied. Since there was no acute disease found in the baseline biopsies of the transplanted kidneys, one cannot extrapolate our findings to biopsies of acute ongoing disease in the kidney. It has been shown that a single glomerulus may suffice to make diagnosis (2). However similar quantitative influences found by us may potentially be present in acute diseases of the kidney. Thus, the correlation between histology and renal function and acute tubular necrosis has always been poor (24-26). This poor correlation and some of the confusion of the prognostic reliability and the controversy if different lesions of lupus nephritis can change into each other (4,5,9,11), may be partially explained by too small sample size in analyses. Since the earliest counting of the number of glomeruli obtained in a needle biopsy, the total number found, as a mean, is ten (2). This has remained unchanged and is the same as we found our in our core biopsies. These 10 glomeruli represent only 0.001% of the one million glomeruli in the kidney.

A practical conclusion from our findings is that the average size of baseline biopsies should be increased so that better representation is obtained. The size of the biopsy needle and the number of cores that should be obtained in a needle biopsy of the kidney, also needs reconsideration. Based on our observations in transplants and Corwin's general mathematical analyses, we conclude that there must be a minimum of 20-25 glomeruli for reliable prognosis and probably at least 15 for true representation of chronic changes.

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TABLE 2-1

Included Biopsies	N	P	R.R.	C.I.
All cases	197	0.915	0.998	0.959 - 1.038
> 6 glomeruli	172	0.822	1.005	0.965 - 1.046
> 9 glomeruli	142	0.699	1.008	0.967 - 1.051
>14 glomeruli	97	0.173	1.031	0.987 - 1.078
>19 glomeruli	69	0.062	1.043	0.998 - 1.091
>24 glomeruli	49	0.017	1.056	1.010 - 1.105

Relative risk, confidence intervals and significance of percent glomerulosclerosis predicting long-term graft function related to sample size, expressed as number of glomeruli in baseline biopsy. Cox Proportional Hazard Analyses. The larger the sample-size, the higher is the relative risk of graft-loss, as a function of percent glomerulosclerosis. Statistical significance, as defined by a $p < 0.05$, is reached first when only samples including > 24 glomeruli are included

TABLE 2-2

Cases Included	No Time Limit			< 60 Days Between Biopsies		
	N	R	P	N	R	P
ALL	108	0.17	0.073	80	0.24	0.034
>6 glomeruli	66	0.33	0.006	51	0.40	0.003
>9 glomeruli	41	0.46	0.003	31	0.49	0.005
>14 glomeruli	12	0.57	0.053	10	0.56	0.095
>19 glomeruli	2	-	-	2	-	-

Reproducibility of glomerulosclerosis in baseline and subsequent biopsies and its relation to sample size and time between biopsies
Linear regression analysis.
Although the R value increases with an increasing number of glomeruli in the biopsies, this becomes statistically insignificant in samples with over 14 glomeruli as statistical precision is lost due to the small number of samples with large number of glomeruli

TABLE 2-3

	No Time Limit				<60 Days Between Biopsies			
Cases Included	N	Percent Agreement	Kappa	P	N	Percent Agreement	Kappa	P
All	104	67	0.34	0.002	76	68	0.38	0.003
>6 glomeruli	69	68	0.33	0.012	53	66	0.29	0.043
>9 glomeruli	46	67	0.31	0.041	31	67	0.31	0.132
>14 glomeruli	15	73	0.46	0.080	12	75	0.45	0.076

Reproducibility of arteriolar hyalinosis in baseline and subsequent biopsies, and its relation to sample size and time between biopsies, analyses by Cohen's kappa. Probability by Fisher's exact test

TABLE 2-4

Cases Included	N	R	P
All	56	0.22	0.108
>6 glomeruli	42	0.26	0.091
>9 glomeruli	31	0.34	0.061
>14 glomeruli	16	0.83	<0.001

Reproducibility of glomerulosclerosis between paired baseline biopsies and its relation to sample size.
Linear regression analysis.

TABLE 2-5

Cases Included	N	Percent Agreement	Kappa	P
All	54	80	0.55	0.0001
> 6	41	78	0.53	0.004
> 9	31	71	0.32	0.185
>14	17	71	0.47	0.162

Reproducibility of arteriolar hyalinosis between paired baseline biopsies and its relation to sample size. Analysis by Cohen's kappa. Probability by Fisher's exact test.

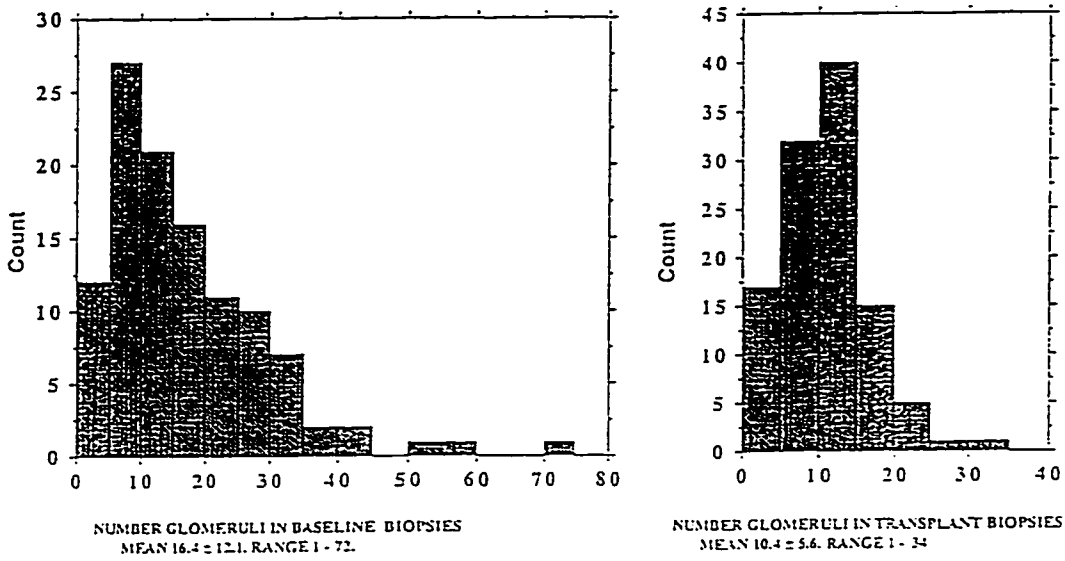

FIGURE 2-1

Histogram of number of glomeruli per biopsy in baseline and subsequent transplant biopsy.

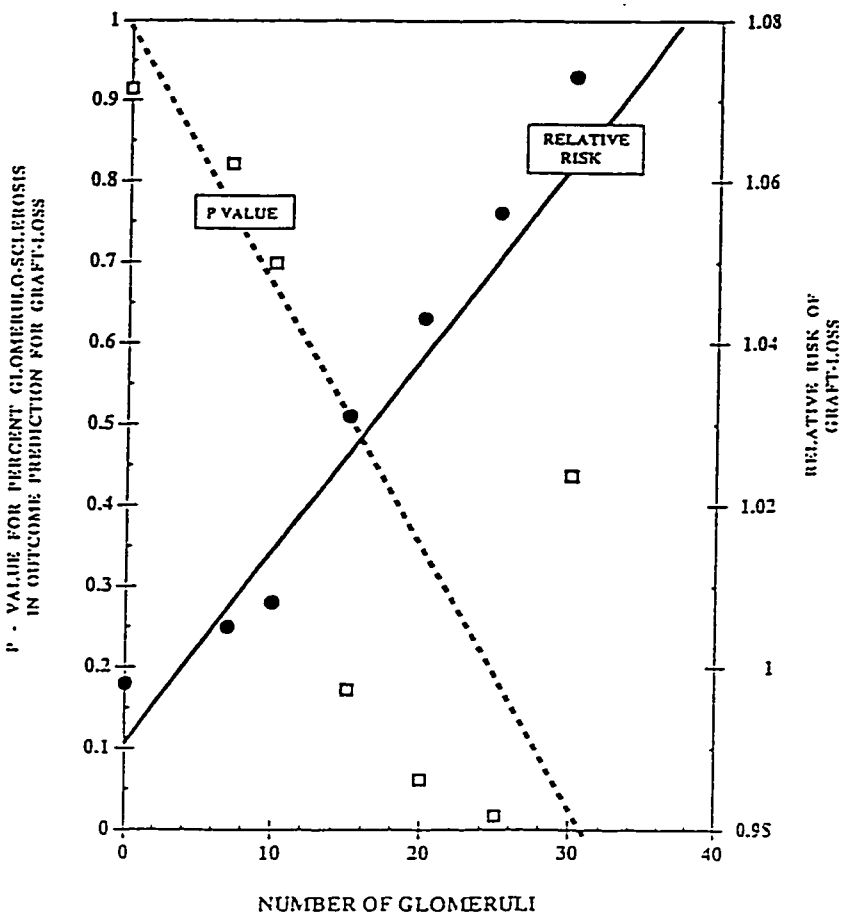

Figure 2-2

The probability value for glomerulosclerosis (open squares, hatched line) and relative risk (closed circles, solid line) of subsequent transplant failure plotted versus the number of glomeruli per biopsy included in the Cox Proportional Hazard analyses. As only biopsies with more glomeruli are included, the p value decreases and the relative risk increases. The data indicate that at least 25 glomeruli are necessary for reliable prediction ($p<0.05$). A p value < 0,05 is reached first when samples containing > 24 glomeruli are included. See text for details.

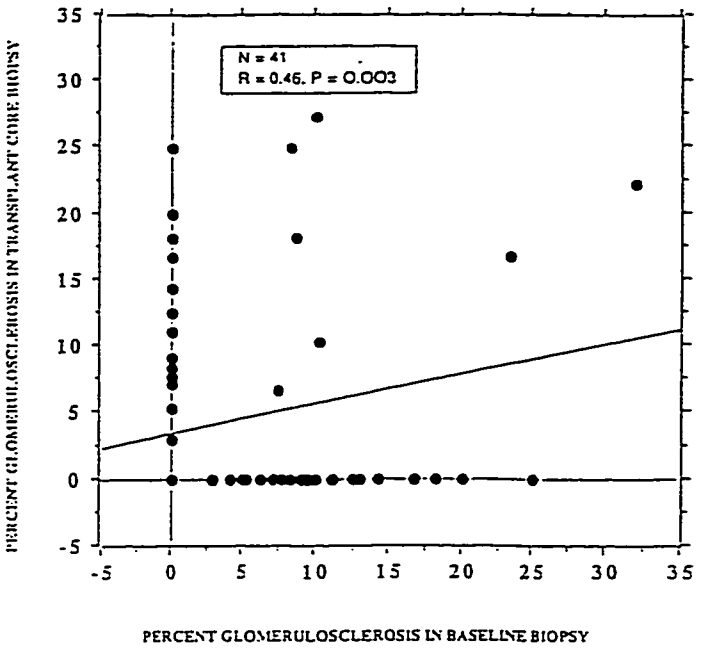

Figure 2-3
Percent glomerulosclerosis in transplant core biopsies, analyzed by linear regression as the dependent variable of the percent glomerulosclerosis in the baseline biopsy. Only 41 samples without time exclusion and all containing >9 glomeruli per biopsy are included. Although there is statistical significance, in 16 (39%) of the samples > 10% glomerulosclerosis was found in one of the biopsies, while there was none in the other paired biopsy.

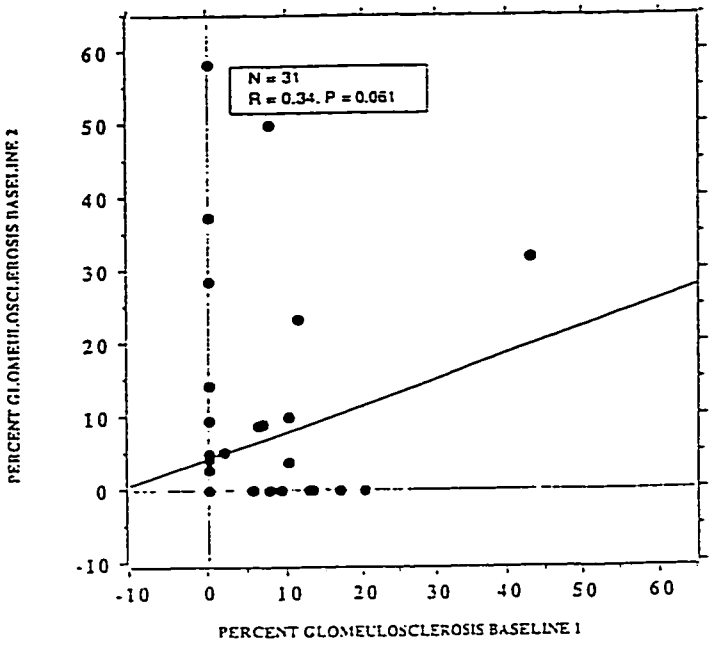

Figure 2-4

Percent glomerulosclerosis in paired baseline biopsies, studied by linear regression, and including only biopsies with more than 9 glomeruli. There is poor agreement, and the R decreases and the p value increases greatly, by excluding the single outlier with 44% and 32% sclerosis.

CHAPTER 3

PROGNOSTIC VALUES OF BASELINE BIOPSIES

INTRODUCTION

The increasing disparity between the number of patients in need of a renal transplant and the number of cadaveric kidneys available has resulted in the use of organs from donors previously considered to be suboptimal. These donors included those over the age of 50, and those with hypertension, vascular disease or diabetes. The use of these marginal donors has been the main reason for an approximate 20% increase in the number of organ donors over the past five years (1,2). In a retrospective review of UNOS, between the year 1988 to 1993, the most dramatic increase among donors occurred in the age group 60-65 years, from 10.7% (1988) to 17.7% (1993) (3). A 1993 UNOS survey of 241 US kidney centres detected trends toward liberalization with regards not only to age, but also in term of diseases such as diabetes or hypertension in the donor's medical history (4).

With the reduction in the incidence of early graft loss, a result of current immunosuppressive protocols, attention has shifted to

focusing on factors that affect long-term graft survival. Early immunologic events such as acute rejection are clearly important. However there is evidence that non-immunologic parameters may also influence graft outcome. Donor characteristics such as age, race, gender and donor-recipient size mismatch have been postulated to affect graft survival, perhaps through "nephron under-dosing". Of these variables, older donor age appears to be a particularly powerful predictor of both long-term graft function and survival. Most studies have demonstrated that the use of older donor kidneys is associated with higher serum creatinine at all time points post-transplant (5). The progressive difference in survival comparing the older donor group with the ideal donor group has become a concern (6). Although early reports suggested that graft survival was not adversely affected by age (7-9), subsequent data has convincingly shown that graft survival is lower with kidneys from donors over the age of 50 years (6,10). Older donor kidneys appears to be particularly susceptible to ischemic injury, resulting in a higher incidence of delayed graft function and loss from primary non-function (11). Unfortunately the transplant community must continue to utilise organs from marginal donors in order to maintain, if not increase,

current levels of cadaveric renal transplant activity.

In the face of this dilemma, some have suggested that a biopsy at the time of organ retrieval may be a useful method of assessing the quality of the donor organ from the older cadavers. But it is further complicated by our limited knowledge on the distinction between abiotrophic involutional process and disease-related process. The percentage of glomerulosclerosis is often cited as an important determinant of organ quality and graft outcome. However the data supporting this recommendation has been relatively sparse. In an effort to establish histologic criteria which would assist in evaluating kidneys procured from marginal donors, we examined baseline biopsy material to quantify the extent of glomerulosclerosis and score the degree of tubulointerstitial fibrosis and vascular lesions (arteriolar hyaline thickening and chronic fibrous intimal thickening). These histologic parameters were analysed taking into account their relation to age. Their effects on initial function and graft outcome were also assessed to distinguish between "innocent" changes and pathologic lesions, which are responsible for the reduced allograft survival.

MATERIALS AND METHODS

Preparation of biopsies

Histologic material was obtained from superficial wedge biopsies of the renal cortex performed 30-60 minutes following revascularization of renal transplants. Between July 1991 and December 1997, 297 such biopsies were performed and constitute the study series. Serial 3-μm sections from paraffin-embedded tissue samples were stained with hematoxylin-eosin, periodic acid-Schiff, silver, and Masson trichrome stain. The number of globally sclerosed glomeruli were counted and then compared with the total number of glomeruli and reported as a percentage. The other three lesions were graded according to Banff Schema (12). The degree of tubular atrophy and interstitial fibrosis (ATR) was graded as mild, moderate and severe. Arteriolar hyalinosis (ART-HY) was graded with "0" indicating no PAS-positive hyaline thickening, "1" identifying the presence of mild-moderate PAS-positive hyaline thickening in at least one arteriole, "2" identifying moderate to severe PAS-positive hyaline thickening in more than one arteriole, and "3" signifying severe PAS-positive hyaline thickening in many arterioles. Fibrous intimal

thickening of donor arteries (VASC) was also graded as mild, moderate, and severe, with severe indicating complete occlusion of the lumen. A pathologist (Kim Solez) blinded to clinical events reviewed all biopsies.

Donor and recipient data

Demographic and outcome data was collected from the renal transplant database maintained at the University of Alberta and the Canadian Organ Replacement Register. Recipient outcome parameters reviewed were the immediate function of the allograft and graft survival. All recipients were followed for a minimum of 12 months or until graft loss. Delayed graft function was defined as the need for dialysis during the first 7 days posttransplantation, excluding those grafts lost immediately due to technical failures or hyperacute rejection. Graft failure was defined as the reinstitution of chronic dialysis support, re-transplantation, or death.

Immunosuppressive protocols

Triple immunosuppression utilising steroids (0.5 mg/kg/d methylprednisolone), azathioprine (1-1.5 mg/kg/d), and cyclosporine (10 mg/kg/d) was initiated immediately post-transplantation aiming for cyclosporine trough levels of 300-400mcg/L using whole blood TDX

assay. Induction with OKT3 (2.5-5 mg/d) or polyclonal anti-lymphocyte antibody (Minnesota anti-lymphoblast globulin or Merrieux anti-thymocyte globulin) was reserved for those recipients with delayed graft function or felt to be at high immunologic risk. The immunosuppressives were tapered after the first month to maintenance doses of prednisone 5-10 mg/d and cyclosporine doses resulting in trough levels of 125-200 μg/L. Suspected rejection episodes were treated with pulse methylprednisolone (250-500 mg/d) for 3 to 4 days. Steroid-resistant rejection was confirmed by renal biopsy prior to the institution of OKT3 or ALG/ATG.

Statistical analyses

The data were entered on the Statview IV statistical program, version 4.5 for the Macintosh computer. Analyses used were the unpaired Student's t-test for univariate analyses of continuous variables, and the chi-square test for categorical data. Kaplan-Meier cumulative survival curves, with Mantel-Cox analyses were used for analysis of graft survival. Multivariate analyses of factors influencing graft survival were done using Cox Proportional Hazard analyses. Results were considered to be statistically significant for p values of < 0.05. All data are expressed as mean ± standard deviation. For factors

influencing delaying graft function, the data was transferred to SPSS version 6.1 and analysed by binary regression analysis.

RESULTS

Recipient and donor demographics

Of the 297 recipients, there were 99 females and 198 male patients. The mean age was 43 ± 14 years (range 4-72). There were 239 first transplants, 46 second transplants, 11 third transplants and 1 fourth transplants. The mean age of the donors was 37 ± 15 years (range 4-71 years). All were cadaver donors.

In total, 67 kidneys were lost, 15 due to patient death, 27 from rejection, 6 from NF, 7 from other causes, 2 from PTLD, 3 from RecD and 7 from technical complications.

The effect of donor age on graft outcome

Increasing donor age was associated with increased risk of graft loss. The mean donor age of allografts lost through the follow-up period was 40 ± 17 years compared a donor age of 35 ± 15 years for those grafts surviving. The influence of age can also be seen in a Kaplan-Meier plot of graft survival by lumping the donor ages as 10-45 and >45 groups (figure1). Graft survival is significantly poorer

either in age>45 group with a P value of 0.002.

Donor age also influenced initial allograft function. Those recipients experiencing delayed graft function (ATN) received donor kidneys from donors with a mean age of 42 ± 16 years compared to mean donor age of 34 ± 15 years for those allografts with immediate function ($p<0.0001$). In donor age group 10-50, 24% had ATN while 43% had ATN in donor age group >50 ($P= 0.0023$). Of note, delayed graft function is itself a powerful predictor of graft loss (Figure 2,$P<0.0001$)

Histologic Features

General features

In the implantation biopsies the number of glomeruli was 23±17 (range 1-100). The mean percentage of sclerotic glomeruli (only including biopsies containing more than 9 glomeruli was 4±7 (range 0-58) and interstitial fibrosis/tubular atrophy was present in 144 out of 297 biopsies. Arteriolar hyalinosis and chronic fibrous intimal thickening was present in 123 out of 295 and 36 out of 223 biopsies respectively.

Relation to age

Donor age was significantly correlated with percentage of glomerulosclerosis ($r=0.37$, $p<0.0001$) as shown in Figure 3 (only including biopsies containing more than 10 glomeruli). As demonstrated in table 1, one is more likely to get all four chronic lesions as age increases. Table 1 shows the mean age of various chronic lesions. The percentage of glomerulosclerosis is divided into four groups: group 1: 0%; group 2: 1%-9%; group 3: 10%-19% and group 4: >19%. The other three lesions are divided into four groups: group 1: none; group 2: mild; group 3: moderate and group 4: severe. For all four lesions, the mean age of group 1 (absence of the lesion) is significantly different from that of all other three groups (presence of the lesion). But the mean age differences among groups 2,3,4 are not statistically significant at all except for that between group 2 (mild) and 4 (severe) in AH lesion with p value of 0.04. Using correlation matrix, we further examined the correlations among four chronic lesions. Better results are achieved when the sample size was increased. When only biopsies containing more than 24 glomeruli are included, percentage of glomerulosclerosis (continuous variable) is

moderately related to CI/CT (R=0.496) and arteriolar hyalinosis is moderately related to chronic fibrous intimal thickening (R=0.446).

The relationship between histologic parameters and long-term graft survival

Given that percentage glomerulosclerosis and the presence or absence of three chronic lesions (ATR, VASC and AH) in baseline biopsies correlated closely with increasing donor age, we included all four lesions as well as donor age, transplant number and ATN in graft survival analysis. First we include all biopsies for analysis. As table 2 shows, of all the factors, ATN is the most important and the only factor influencing graft survival using multivariate analysis (p=0.02). Surprisingly age itself and transplant number did not influence the graft survival with a p value of 0.52 and 0.99 respectively. None of the histologic parameters influences outcome significantly. Then the analysis was performed again excluding those biopsies containing less than 25 glomeruli. ATN is still the most important factor influencing graft survival (P=0.004). But the chronic fibrous intimal thickening becomes important in influencing outcome with p value of 0.026 in addition to ATN. None of the other factors including donor

age and transplant number predicts outcome. The percentage glomerulosclerosis did not correlate with long-term graft survival when treated as a continuous variable in two analyses above. Further we divide those biopsies containing more than 9 glomeruli into either 0,1-19, >19 groups or <20, >19 groups. Comparing survival curves of various groups, none is significantly different from the others (P value for 0,1-19, >19: 0.7136; P value for <20, >19 group is 0.6616).

The relationship between histologic parameters and delayed graft function (DGF)

Since ATN is the most important factor in influencing graft survival, we further included all the four histologic parameters besides age and transplant number in the binary logistic regression analysis to search for parameters which cause aged donor kidneys to be more susceptible to DGF. First we included all the biopsies in the analysis. The overall agreement in this model is 74%. Of all the factors we included, the chronic fibrous intimal thickening is the most important predictor of DGF (P=0.0036) followed by arteriolar hyalinosis (P=0.02). Donor age itself, transplant number, and the other two factors (glomerulosclerosis and CI/CT) are not important statistically.

We did the same analysis on samples containing more than 24 glomeruli. The overall agreement is 74%. Chronic fibrous intimal thickening is still the most important predictor of DGF (P=0.005) while arteriolar hyalinosis is knocked out. Still all the other factors including donor age itself are not important. In order to avoid the confounding effect of pairing, we further randomly excluded one of the paired donor kidneys and did the same analysis. Chronic fibrous intimal thickening and arteriolar hyalinosis are still the two important risk factors for DGF when all biopsies are included. When you only included biopsies containing more than 24 glomeruli, chronic fibrous intimal thickening are only risk factor for DGF (P=0.047).

Actually 21 out of 36 kidneys (58%) with presence of chronic fibrous intimal thickening developed DGF while only 41 out of 187 kidneys without that lesion (22%) developed DGF ($p<0.0001$). 6 out of 8 kidneys with severe degrees of AH lesion developed DGF. 24 out of 58 kidneys (41%) with moderate or severe degrees of presence of AH lesion developed DGF comparing to 36 out of 172 kidneys without AH lesion (21%) with a p value of 0.001.

DISCUSSION

It has been postulated that the pre-existing structural and functional alteration in the aged kidney makes it less likely to stand ischemia or other insults and that the aged kidney is less likely to maintain long-term adequate function after transplantation. In agreement with other studies, our study also shows that delayed graft function is the most important factor in determining long-term graft survival. The kidneys from donors older than 50 have a higher incidence of delayed graft function compared to kidneys from ideal 15-45-year old donors. Using Banff Schema to measure the chronic histologic changes in old kidneys, we have demonstrated that chronic fibrous intimal thickening and arteriolar hyalinosis lesions are responsible for higher incidence of delayed graft function in older donor kidneys while age by itself is not. Yet the model is not very predictive (74% overall) of delayed graft function indicating that we are also missing other important factors in our analysis such as donor blood pressure, presence or absence of hypotension, urine output at the time of transplantation and tissue typing etc. The percentage of glomerulosclerosis, either treated as a continuous variable or

subgroups is not useful in predicting the incidence of delayed graft function or graft survival even when an adequate biopsy containing at least 24 glomeruli has been obtained.

UNOS registry studies (6,13) demonstrated a 7% and 15% difference in the one or two-year survivals between kidneys from young versus older donors. Blumke et al (14) even reported a 20% difference in the one-year survival between grafts between grafts from donors younger and older than 50 years, a difference triple as much as that from the UNOS registry report. Our studies also confirm the inferior outcome of grafts from older donors both in short-term (delayed graft function) and graft survival. It is generally accepted that increasing disease in older donors and the senescent changes that normally occur in the kidney are responsible for the inferior performance of old donor kidneys compared to kidneys from ideal 15-45-year old donors. The criteria to identify subgroups of older donors that should be excluded from the donor pool become extremely important.

Yet the help from the kidney function test is limited. Specific tests of function such as glomerular filtration rate measured by creatinine clearance are not practical. Furthermore, serum creatinine

may inaccurately indicate normal renal function, as it ignores the effect of a decreased or subnormal muscle mass (15). As coexistent with the progressive functional decline since the fourth decade (15-18), there is a decrease in renal cortical mass that begins by the fifth decade, causing loss of 30% of renal weight by the ninth decade.

Aged kidneys certainly get more chronic lesions besides glomerulosclerosis. Thus we used the Banff schema to partially quantify the involvement of three chronic lesions: Interstitial fibrosis/tubular atrophy, arteriolar hyalinosis and chronic fibrous intimal thickening. There is a significant difference in mean age between the absence and presence of all three chronic lesions we have scored (Interstitial fibrosis/ tubular atrophy, arteriolar hyalinosis and chronic fibrous intimal thickening). However, there is no significant difference in mean age among groups with presence of various degrees of chronic lesions except for the difference between groups with mild and severe degree of arteriolar hyalinosis. Also the large age deviation within each group suggests that age alone could not be used to assess the probability of degrees of chronic lesions.

In aged kidneys, the vascular state is extremely important in predicting the incidence of delayed graft function. The indicator of

large vessels, chronic intimal thickening, is the most important parameter among all parameters we studied in determining the incidence of DGF. Kidneys with presence of chronic fibrous intimal thickening have very high incidence of DGF (58%). Arteriolar hyaline thickening is also a component of age-related histologic change in the kidney. Evaluating this parameter in baseline biopsies has been particularly emphasized in order to assist with the interpretation of etiology of this lesion on subsequent biopsies especially assessment of cyclosporine toxicity (19,20). Although both Marcussen, Solez et al (21) and Mihatsch et al (22) have documented poor reproducibility in assessment of the degree of AH lesion, most pathologists agree on whether the AH lesion is present or not. In previous study (23), we have shown that AH lesion predicted transplant failure well, when all biopsies were included. In this study, arteriolar hyalinosis is also an important predictor of DGF secondary to the chronic fibrous intimal thickening. Actually 6 out of 8 kidneys with severe degrees of AH lesion developed DGF and 24 out of 58 kidneys (41%) with moderate or severe degrees of presence of AH lesion developed DGF compared to 21% DGF rate in 172 kidneys without AH lesion. We suppose that the already fragile vessels with chronic fibrous intimal

thickening and arterioles has difficulty enduring the dramatic hemodynamic changes happening after harvest and more readily deteriorate after transplantation. Of note, age itself doesn't cause delayed graft function. It is the higher prevalence of vascular lesion in older donor kidneys that is responsible for their inferior outcome. Great caution should be exercised in accepting kidneys with the lesion of chronic fibrous intimal thickening or moderate or severe degrees of arteriolar hyalinosis due to the high incidence of delayed graft function and later inferior graft survival. Interstitial fibrosis/tubular atrophy was not useful in predicting graft outcome in our study.

Glomerulosclerosis is a mark of irreversible nephron damage and it is one important index of chronicity. In our study, there is a clear relationship between the extent of glomerulosclerosis and age. Data derived from autopsies and nephrectomies concluded that glomerulosclerosis was not present in significant degree (<10%) until the age of 40 (24). Kaplan also reported a clear linear relationship between the percentage of glomerulosclerosis and age ($r=0.90$ and $P<0.01$). Many reports have highlighted the tremendous inter-individual variability in the percentage of glomerulosclerosis within a given age range, suggesting that age alone could not be used to

assess the probability of significant glomerulosclerosis. Thus the recommendation using percentage of sclerotic glomeruli besides age appears reasonable.

It was not until the report of Gaber et al (5), that there was objective data to support the recommendation that donor allografts containing more than 20% glomerulosclerosis should not be transplanted. This group was able to demonstrate that allografts with >20% sclerotic glomeruli on post-revascularization biopsies were associated with a 4-fold increased risk of delayed graft function and a 5.5-fold increased risk of graft loss when compared to allografts without glomerulosclerosis. Of note however, there were only 8 cadaveric donor organs with >20% glomerulosclerosis in this analysis, which included both live and cadaveric donors. However we were not able to demonstrate a statistically significant relationship between this histologic parameter and graft failure. In our studies, only 30% of kidneys with >20% glomerulosclerosis develop DGF and later failed, which is not significantly different from the kidneys with less than 20% glomerulosclerosis. There are certainly some methodological differences between Gaber's study and ours. Gaber used short-term serum creatinine while we use long-term graft

survival as indicator of graft outcome. It is also possible that the glomeruli sampled in a small superficial wedge biopsy are not representative of the pattern of glomerulosclerosis throughout the donor organ. Subcapsular glomeruli appear particularly susceptible to the forces resulting in sclerosis. The number of glomeruli obtained was only 23±17 in our study. Thus the pathologist and transplant physician should assess the adequacy of the biopsy sample prior to deciding the fate of kidneys from donors thought to be marginal. Also glomerular scars are not permanent monuments to damaged nephrons, when in fact resorption and disappearance have been shown to occur during the course of chronic renal disease (25).

Our study stresses the importance of vascular status in donor kidneys. We recommend the routine practice of baseline biopsies and the examination of vascular lesions especially when selecting marginal donors. Caution should be exercised when accepting the kidneys with lesions showing chronic fibrous initial thickening or moderate to severe degree of arteriolar hyalinosis due to the high incidence of delayed graft function and reduced graft survival attributed to these lesions. Glomerulosclerosis or age by itself are not important predictors of graft outcome. Many important clinical data

were not available for our studies, which may explain the only moderate accuracy of prediction of delayed graft function in our study. These include: donor blood pressure, presence or absence of hypotension, serum creatinine level and urine output at time of transplant etc.

The data compiled in our center are still too scanty to draw certain conclusions. Given the difficulties in gathering large numbers of patients in a single center, multicenter longitudinal studies, either prospective or retrospective, appear necessary. In this regard, Banff Schema will facilitate uniform quantification.

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Pathological lesions	AGE:Mean±SD(Cases)			
	Group 1	Group 2	Group 3	Group 4
Percentage Glomerulosclerosis*	30.7±13.6 (143)	42.6±12.8 (68)	45.0±14.4 (21)	47.4±11.3 (10)
Tubular Atrophy and Interstitial Fibrosis	30.7±13.7 (162)	42.8±12.1 (102)	46.8±12.1 (33)	0
Arteriolar Hyalinosis	30.8±14.3 (172)	39.2±14.5 (65)	45.5±12.4 (50)	54.3±4.6 (8)
Fibrous Intimal Thickening**	33.7±15.1 (187)	44.1±14.5 (23)	49.3±7.8 (12)	61.1 (1)

*include only the biopsies containing >9 glomeruli
** can only be assessed in 223 cases

Table 3-1

The mean age of various groups in four lesions. All biopsies are divided into four groups for four lesions. For percentage of glomerulosclerosis: group 1: 0% ; group 2:1%-9%; group 3:10%-19% and group 4:>19%. For the other three lesions: group 1:none; group 2:mild; group 3:moderate and group 4: severe. For all four lesions, the mean age of group 1 (absence of lesion) is significantly different from that of all other three groups(presence of lesion). But the mean age difference in group 2,3,4 are not statistically significant at all except for that between group 2 and 4 in arteriolar hyalinosis lesion with p value of 0.04(ANOVA analysis).

Model Coefficients for FU_T(m)
Censor Variable: F_censor
Model: Proportional Hazards

	DF	Coef	Std. Error	Coef/SE	Chi-Square	P-Value	Exp(Coef)
Tx#	1	.175	.271	.647	.418	.5177	1.192
D_AGE	1	-2.345E-4	.013	-.018	3.249E-4	.9856	1.000
%GL_SC	1	.008	.023	.333	.111	.7393	1.008
ATROPHY	2	•	•	•	.121	.9414	•
0	1	-.051	.570	-.089	.008	.9291	.951
1	1	-.152	.534	-.285	.081	.7755	.859
ART_HY	3	•	•	•	1.082	.7813	•
0	1	-.175	.845	-.207	.043	.8358	.839
1	1	.226	.844	.268	.072	.7885	1.254
2	1	-.094	.844	-.112	.013	.9108	.910
VASC	3	•	•	•	3.309	.3464	•
0	1	-2.179	1.338	-1.628	2.649	.1036	.113
1	1	-1.806	1.373	-1.315	1.729	.1886	.164
2	1	-1.661	1.372	-1.211	1.466	.2260	.190
ATN: N	1	-.782	.342	-2.286	5.224	.0223	.457

Confidence Intervals for FU_T(m)
Censor Variable: F_censor
Model: Proportional Hazards

	Exp(Coef)	95% Lower	95% Upper
Tx#	1.192	.700	2.028
D_AGE	1.000	.975	1.026
%GL_SC	1.008	.963	1.055
ATROPHY: 0	.951	.311	2.905
ATROPHY: 1	.859	.302	2.445
ART_HY: 0	.839	.160	4.395
ART_HY: 1	1.254	.240	6.561
ART_HY: 2	.910	.174	4.757
VASC: 0	.113	.008	1.561
VASC: 1	.164	.011	2.426
VASC: 2	.190	.013	2.796
ATN: N	.457	.234	.895

Table 3-2
Influence of various parameters on graft survival. Of all the factors, ATN (delayed graft function) is the most important and the only factor influencing graft survival(CI:0.23-090). Age itself and transplant number(Tx#) don't influence the graft survival significantly. Also none of histologic parameters influences outcome significantly.

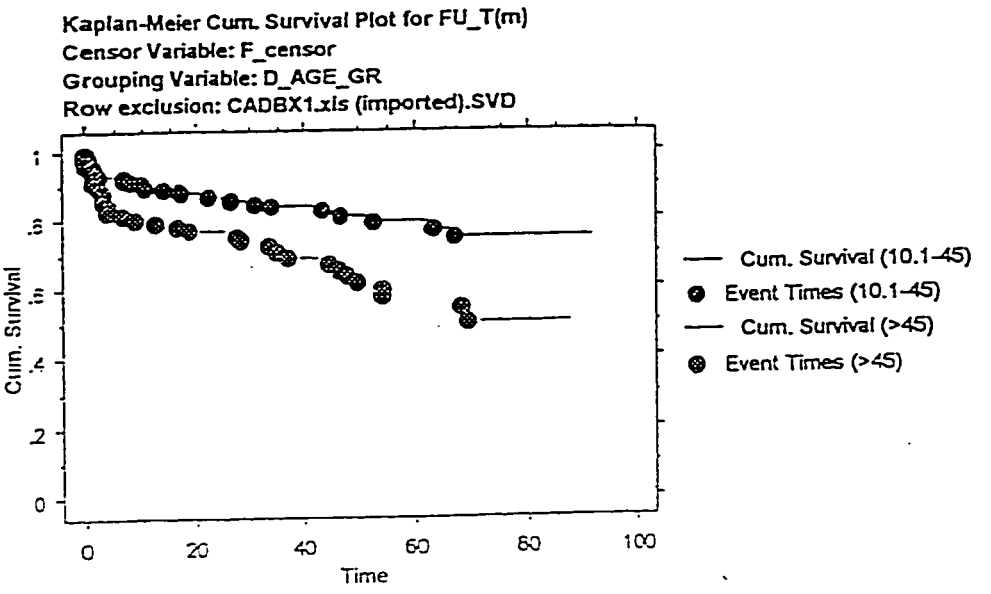

Figure 3-1

The effect of donor age on graft survival (10 cases of age less than 10 are excluded). Graft survival is significant poorer in age >45 group with a P value of 0.0020 (using Mantel-Cox test).

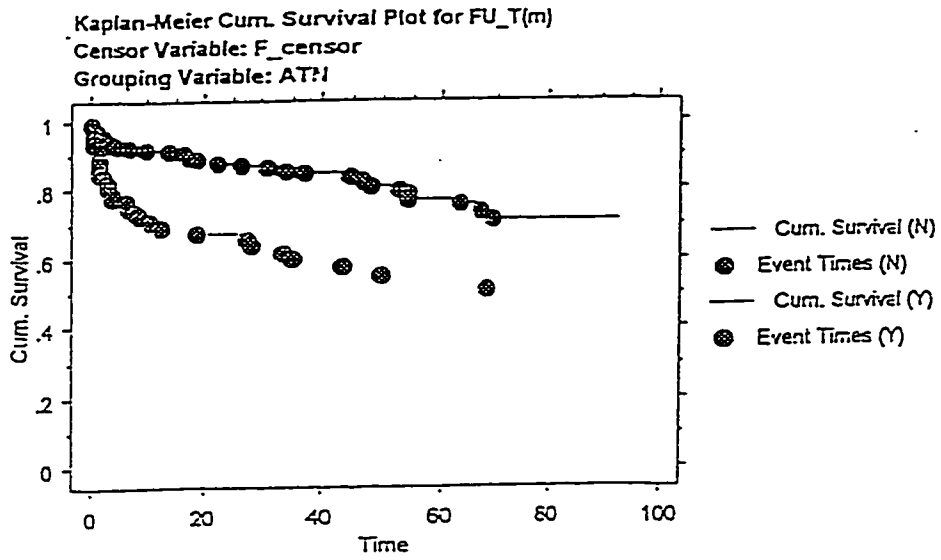

Figure 3-2

The effect of DGF on graft survival. The Graft survival is significant poorer in DGF(Delayed graft function) group with P value of 0.0042(using Mantel-Cox test).

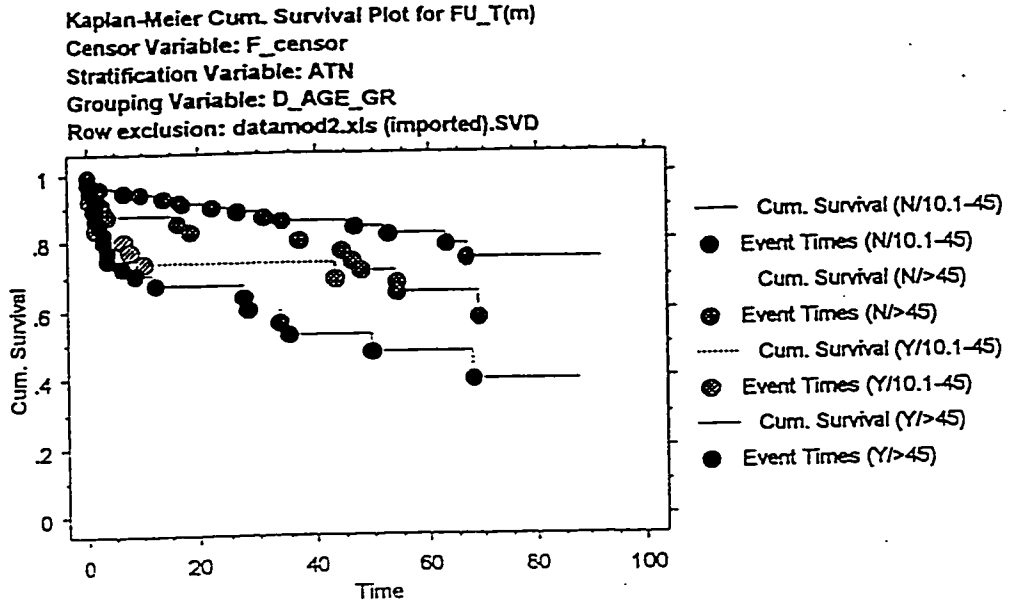

Figure 3-3

The Kaplan-Meier curves of various groups(Group 1: No ATN+ Age<45; group 2: ATN+ Age<45; group 3: No ATN+ Age>45 4. ATN+age>45) The group ATN+Age>45 has the worst outcome. However, the difference between the group(ATN+Age<45) and the group (NO ATN+Age>45) is not statistically significant.

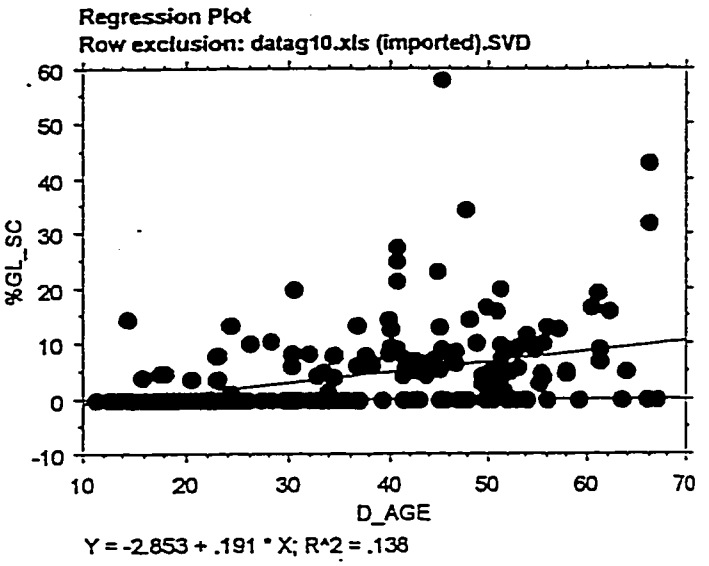

Figure 3-4

The relation of donar age to percentage of glomerulosclerosis in the graft. In linear regresion analysis, the donor age is closely related to percentage of sclerotic glomeruli with r value of 0.37 and P value <0.0001. Note that the samples containing less than 10 glomeruli are excluded in this analysis to minimize sampling error.

CHAPTER 4

The clinical application of Banff Schema in follow up needle biopsies

INTRODUCTION

Renal biopsies play a critical role in the management of transplant recipients. The value of renal allograft biopsies to recipient care has been emphasized by the widespread use of nephrotoxic immunosuppressants and other agents that compound the difficulty in establishing the clinical diagnosis of renal dysfunction episodes (1-3). Yet the pivotal diagnostic role, the utility of biopsy was largely limited by the subjective grading of histologic lesions of rejection and evaluation of the relative extent of injury of renal compartment targeted by rejection. This limitation is broken by the Banff schema that has become the international standardization of nomenclature and criteria for the histologic diagnosis of renal allograft rejection (4). It is the first critical step in promoting international uniformity in the reporting of renal allograft pathology. There have been many studies showing its clinical reproducibility and validation (5-12). But most

studies have involved grafts less than 3 years posttransplantation. The goal of our study is to examine the correlation between the lesions in Banff classification and long-term clinical outcome. We hope to determine which types of lesions show resistance to therapy and therefore lead to poor graft outcome. In that way, it may shed light on further modifications of Banff classification.

MATERIAL AND METHODS

From July 1991 through April 1995 295 core biopsies have been done out of 156 renal transplants. The observation period ended November 1,1995 and all transplants were thus observed for 6 months or until loss of transplant function, defined as a return to dialysis or death of patient.

PREPARATION OF BIOPSY

Needle biopsies were obtained under ultrasound guidance using a Truecut® needle in a biopsy gun. The needle size was #16 and usually three cores were obtained.

The renal biopsy specimens for light microscopy were fixed in 10% buffered formalin and then embedded in paraffin. Sections of 3μ

m thickness were cut and then stained with hematoxylin and eosin, PAS, silver-stain, and Masson trichrome stain.

SCORING OF BIOPSIES

Glomerulitis (G), Chronic transplant glomerulopathy (CG), interstitial infiltration (I), interstitial fibrosis (CI), tubulitis (T), tubular atrophy (CT), intimal vasculitis (V), chronic fibrous intimal thickening (CV) and arteriolar hyaline change (AH) were scored according to the semiquantitative guideline of the Banff classification (4). Multiple sections of each biopsy were always read.

Mild acute rejection indicated the presence of foci of moderate tubulitis (5-10 mononuclear cells per tubular cross section/10 tubular epithelial cells) in cases with significant interstitial infiltration (>25% of parenchyma affected). Moderate acute rejection was determined as the presence of significant interstitial infiltration with foci of severe tubulitis (>10 mononuclear cells per tubular cross section/10 tubular epithelial cells) and/or mild or moderate intimal arteritis. Severe acute rejection included the cases with severe intimal arteritis in many arterial cross-sections and/or "transmural" arteritis with fibrinoid change and necrosis in medial smooth muscles. Recent infarctions and interstitial hemorrhage without other obvious cause was also

regarded as evidence for severe acute rejection. Glomerulitis was defined as presence of mononuclear cells in peripheral loops of glomerular capillaries with focal or diffuse endothelial swelling. CADI score is the sum of CG+CI+CT+CV.

TREATMENT

All patients received triple therapy with prednisone, azathioprine, and cyclosporine. After transplantation, daily cyclosporine levels were performed and the dose regulated according to blood levels, aiming at the concentration of 300 mcg/L the first two weeks and then slowly decreasing the dose, aiming at a concentration of 100 mcg/L at the end of one year. In 49 cases, cyclosporine was not used early because of delayed graft function. Rejection episodes were treated with intravenous solu-medrol, and if resistant, with intravenous OKT-3.

STATISTICAL METHODS

The data were entered on Statview IV Statistical Program, version 4.5. Analyses used were Cox Proportional Hazards Analysis with further forward and backward stepwise analysis. A probability of < 0.05 was regarded as significant. All data are mean ± standard deviation.

RESULTS

General features

210 biopsies contained more than 6 glomeruli and blood vessel for assessment by Banff Schema and thus were used for further analysis.

Relation between the pathologic lesions and graft survival

Nine lesions in the Banff Schema were entered in the Cox proportional hazards analysis to assess their predictive value of graft survival. As table 1 shows, of nine lesions, both Glomerulitis (p=0.009,RR=1.8) and Vasculitis (p=0.05,RR=1.7) decrease the graft survival significantly. Tubulitis, interstitial mononuclear infiltrate and all chronic lesions do not influence graft survival significantly. Certainly the acute rejection score in Banff Schema does not influence the graft survival independently for it is based on the individual scores of nine lesions. Further using the forward and backward stepwise analysis, the vasculitis is the most important lesion (P=0.0009) and the glomerulitis also predict the graft survival independent of vasculitis with a p value of 0.01 when all other lesions are knocked out. As figure 1 shows, the effect of glomerulitis adds to

the effect of vasculitis in decreasing graft survival i.e. group with both lesions has the worst outcome.

For glomerulitis, 6 out of 9 cases (67%) with moderate or severe degrees of glomerulitis lost the graft compared to 32% graft loss rate of 185 cases without the lesion. Figure 2 shows the survival curves of groups with various degrees of glomerulitis with a p value of 0.002. However the graft survival curve between the group with mild degree of glomerulitis and the group without the lesion is not statistically different (P=0.3502). 12 out of 22 biopsies (55%) with the lesion of glomerulitis also have vasculitis with p value of 0.0001.

For vasculitis, 12 out of 20 cases (60%) with moderate or severe degrees of vasculitis lost the graft compared to 32% graft loss rate for 151 cases without the lesion. Figure 3 shows the survival curves of groups with various degrees of vasculitis with a $p<0.0001$. The graft survival curve between the group with a mild degree of vasculitis and the group without the lesion is not statistically different. Only 20% of the biopsies with vasculitis also contained glomerulitis.

DISCUSSION

Of nine lesions intrinsic in Banff Schema, the vasculitis lesion is the most important factor for decreasing graft survival. Glomerulitis also decreases graft survival independent of vasculitis. Even though the modern immunosuppressive therapies are satisfactory in treating tubulitis and interstitial mononuclear infiltrate, new therapy is needed to prevent the undertreatment of moderate or severe degrees of either vasculitis or glomerulitis. The fact that the chronic lesions failed to have independent prognostic value indicates that future work will need to define the lesions of chronic rejection rigorously.

Intimal arteritis is the pathognomonic lesion of acute rejection, as first noted by Dammin (13). The biologic and diagnostic significance of this lesion in acute cell mediated rejection is well established (14-19). In agreement with most studies (20-22), our study shows that vasculitis is a consistent predictor of graft failure even in the context of newer, more potent anti-rejection therapies. It supports the Banff schema for stressing vasculitis as a main criteria

for severity of rejection and need for more aggressive and innovative treatment.

Although scoring for glomerular inflammatory lesions has been incorporated into Banff Schema, it was stated that the significance of the glomerulitis was not clear. Rejection glomerulitis is defined by intraglomerular margination of mononuclear cells, endothelial swelling and glomerular thrombosis or necrosis. In agreement with many studies (23-30), the glomerulitis, independently of other factors carries a dismal prognosis using the multivariate analysis. Although Olsen et al (31) and Hansen et al (32) showed that moderate or severe glomerulitis doesn't change the allograft's one year survival, the lesion may get under-treated with conventional therapy thus decreasing allograft long-term survival. Marcussen et al (33) indicates that glomerulitis is the least reproducible lesion in the Banff Schema especially for the differentiation between G0 (normal) and G1 (mild). This may explain partly the fact that there is significant difference between the group with mild degree of glomerulitis and the group without that lesion.

Because of the close correlation between the glomerulitis and vasculitis, we first believed that glomerulitis would be dependent on

vasculitis and not independently predict survival. It turned out to be not the case. In our study the glomerulitis does influence the graft survival quite independent of vasculitis. The injury caused by glomerulitis may increase the immunogenicity of glomerulus and initiate a vicious cycle of the injury response that destroys the whole glomerulus.

It is widely accepted that CMV is probably neither a necessary nor a sufficient stimulus for developing glomerulitis (25,30,34-38). The finding that the glomerulitis is closely related to the vasculitis suggests that they have the similar underlying mechanisms. Trpkov et al. suggested that the endothelium of the arteries, glomeruli, and the microcirculation is the main target of anti MHC class I antibody mediated rejection. But the fact that 45% of biopsies with glomerulitis did not have the vasculitis indicate that there must be other mechanisms such as cyclosporine nephrotoxity (39). Given that the glomerulitis is an important factor influencing the long-term allograft outcome, the underlying mechanism and further modification of the scoring system to improve its reproducibility need further attention.

In Banff Schema tubulitis and interstitial mononuclear infiltrates are also the important criteria for diagnosing acute rejection in Banff

Schema. But the finding that these two lesions didn't decrease graft survival indicates that the modern immunosuppressive therapies are satisfactory in treating either lesion. This may also explain the fact that the cases with mild degrees of either glomerulitis or vasculitis do not have inferior outcome. To our surprise, none of the chronic lesions influenced the graft survival. In our center, we do not perform the protocol core biopsies in long term follow up of stable renal transplant recipients. Also the follow up time may still not be long enough to reach statistical significance. But our findings at least suggest that the criteria for scoring the chronic allograft lesions are not as well defined as that for acute lesions and need further modification.

Of note, the prognostic values of histologic lesions are also influenced by inaccuracy due to sampling errors (40) which are partly influenced by distribution patterns of various lesions. Here we talk only about the use of the histologic lesion in predicting graft outcome. Actually changes in renal function are a relatively sensitive indicator of kidney rejection once kidney allograft function commences, so it is better to combine the functional and structural data to make a secure

diagnosis rather than relying solely on histologic grounds in the functioning kidney transplant.

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Model Coefficients for TIME TO FAIL
Censor Variable: FAILURE
Model: Proportional Hazards
Row exclusion: follow up biopsies

	DF	Coef	Std. Error	Coef/SE	Chi-Square	P-Value	Exp(Coef)
G= GLOMULAR.1	1	.581	.223	2.606	6.789	.0092	1.787
CG= CHRON.1	1	.100	.224	.447	.200	.6548	1.105
I=INT INFL.1	1	-.050	.212	-.237	.056	.8126	.951
CI=INT FIBR.1	1	-.038	.502	-.076	.006	.9392	.962
T=TUBULITIS.1	1	.115	.184	.626	.392	.5314	1.122
CT=TUB ATR.1	1	.239	.472	.505	.255	.6134	1.270
V=VASCULITIS.1	1	.514	.264	1.948	3.794	.0515	1.671
CV= FIBR INT TH..1	1	-.039	.162	-.240	.057	.8106	.962
AH=ART.HYAL..1	1	-.069	.164	-.422	.178	.6729	.933
AC REJECTION 1	1	-.064	.164	-.393	.155	.6942	.938

Confidence Intervals for TIME TO FAIL
Censor Variable: FAILURE
Model: Proportional Hazards
Row exclusion: follow up biopsies

	Exp(Coef)	95% Lower	95% Upper
G= GLOMULAR.1	1.787	1.155	2.766
CG= CHRON.1	1.105	.713	1.713
I=INT INFL.1	.951	.627	1.441
CI=INT FIBR.1	.962	.360	2.576
T=TUBULITIS.1	1.122	.782	1.610
CT=TUB ATR.1	1.270	.503	3.204
V=VASCULITIS.1	1.671	.997	2.803
CV= FIBR INT TH..1	.962	.700	1.321
AH=ART.HYAL..1	.933	.677	1.286
AC REJECTION 1	.938	.680	1.292

Table 4-1
Influence of various lesions in follow up biopsies on graft survival. Both the presence of glomerulitis(P=0.009, RR=1.8,CI=1.2-2.8) and vasculitis(P=0.05, RR=1.7,CI=1-2.8) decrease the graft survival significantly.

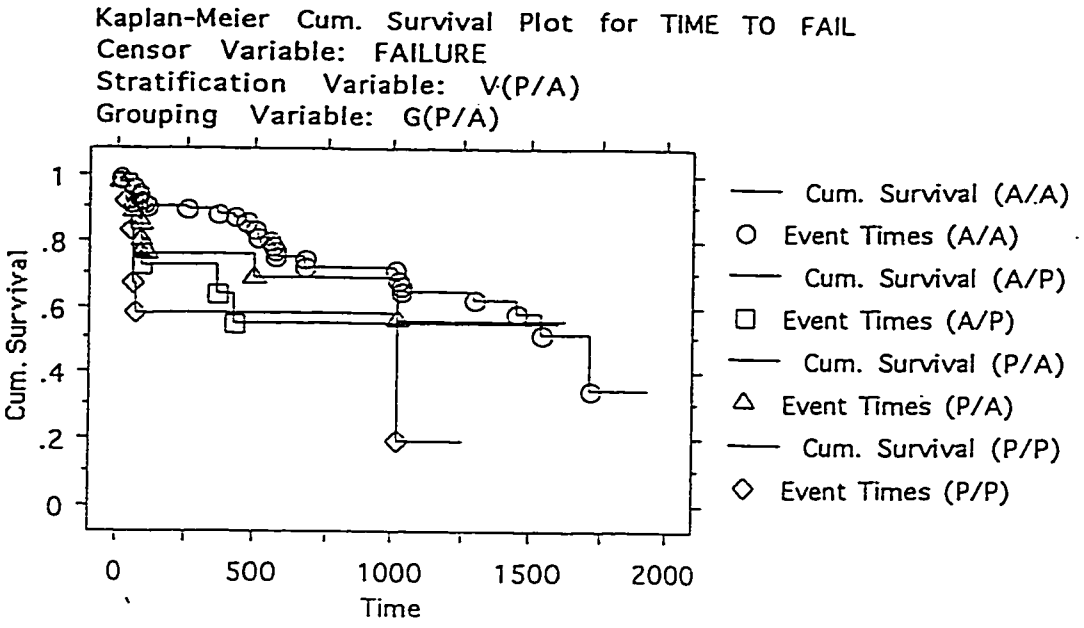


Figure 4-1

The Kaplan-Meier curves of various groups (O: No vasculitis+ NO glomerulitis; □: No vasculitis + glomerulitis; Δ: Vasculitis + No glomerulitis; ◊: Vasculitis + glomerulitis) The effect of glomerulitis is additive to the effect of vasculitis in decreasing graft survival and the group with both lesions has the worst outcome.

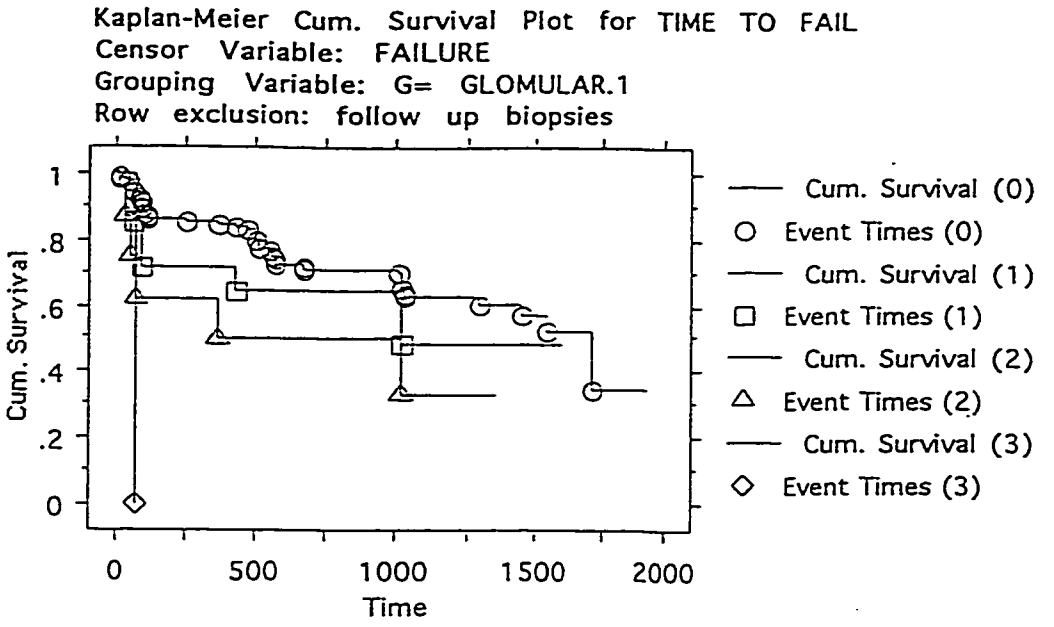


Figure 4-2

The effect of glomerulitis on graft survival. The survival curves of groups with various degrees of glomerulitis are significantly different with P value of 0.0020 (using Mantel-Cox test). 6 out of 9 grafts with moderate or severe degrees of glomerulitis lost the graft compared to 32% loss rate of 185 grafts without the lesion.

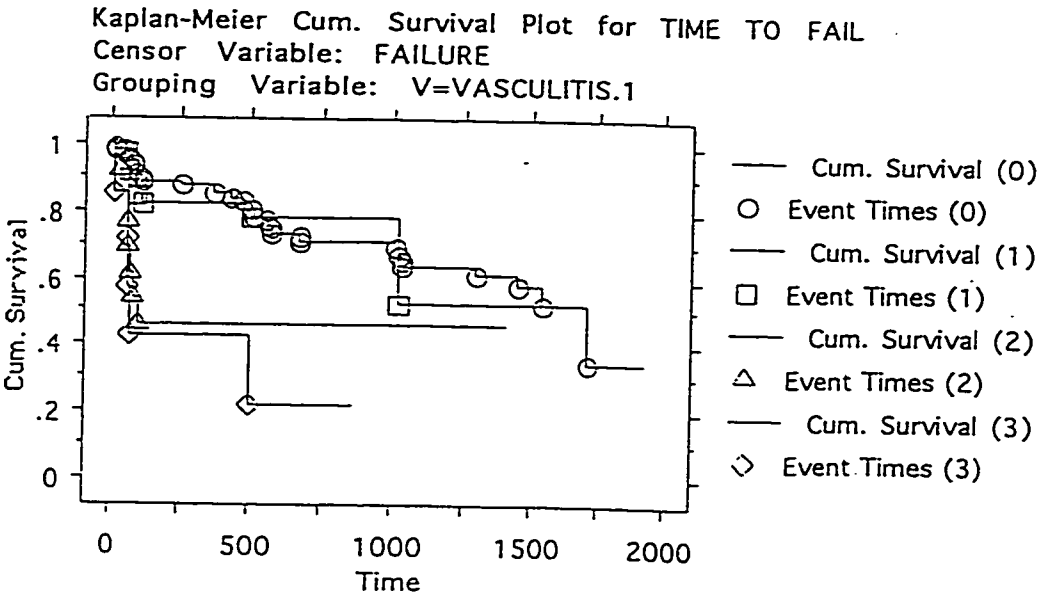


Figure 4-3

The effect of vasculitis on graft survival. The survival curves of groups with various degrees of vasculitis are significantly different with a $p<0.0001$(using Mantel-Cox test). The worst outcome is seen in the groups with moderate or severe degrees of vasculitis.

CHAPTER 5

Conclusions

The pathologist and transplant physician should assess the adequacy of all biopsies of potential donor organs prior to deciding on their use should they belong to donors thought to be marginal. Some practical findings from our investigations are that the average size of baseline biopsies should be increased so that better representation is obtained and that there must be at least 20-25 glomeruli for assessment of glomerulosclerosis. Also all biopsies should contain blood vessels in order that vascular lesions (such as chronic fibrous intimal thickening and arteriolar hyalinosis) could be used for reliable determination of prognosis.

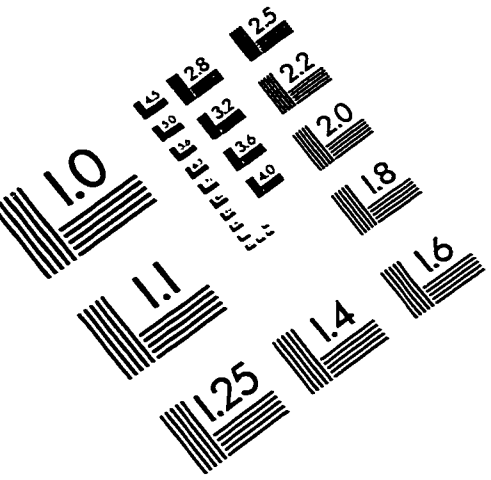
Many important clinical data were not available for our studies. These include: donor blood pressure, presence or absence of hypotension, serum creatinine level and urine output at the time of transplant. These are conceivably important in terms of outcome. Delayed graft function is the most important factor in determining long-term graft survival and the kidneys from donors greater than age 50 has a higher incidence of delayed graft function compared to

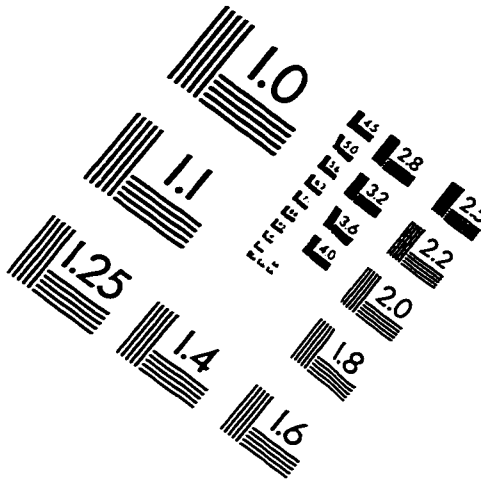
kidneys from ideal 15-45-year old donors. Using the Banff Schema to measure the chronic histologic changes in older donor kidneys, we have demonstrated that it is the higher prevalence of vascular lesions in older donor kidneys that are responsible for their inferior outcome while age by itself is not. We presume that the already fragile vascular system in older donor kidneys has difficulty enduring the dramatic hemodynamic changes occurring after harvest and more readily deteriorate after transplantation. Caution should be exercised in accepting kidneys with lesions showing chronic fibrous intimal thickening or moderate to severe degrees of arteriolar hyalinosis due to the high incidence of delayed graft function and reduced graft survival attributed to these lesions. The percentage of glomerulosclerosis, either treated as continuous variable or subgroups, is not useful in predicting the incidence of delayed graft function or survival even when an adequate biopsy containing at least 24 glomeruli has been obtained. Also given the sampling error for glomerulosclerosis, we believe that caution should be exercised when using the selection criteria for older donors which rely mainly on the fraction of sclerotic glomeruli in order to avoid unnecessary discarding of valuable grafts.

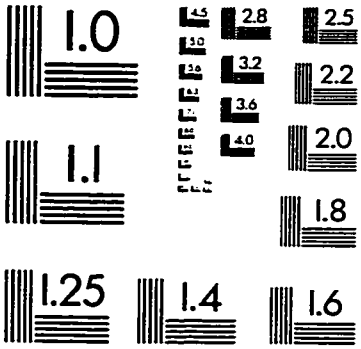
The data compiled in our center are still too scanty to definitively mandate the histologic selection criteria for older donor kidneys. Given the difficulties in gathering large numbers of patients in a single center, multicenter longitudinal studies, either prospective or retrospective, appear necessary. In this regard, the defined criteria of the Banff schema will certainly facilitate inter-institutional studies.

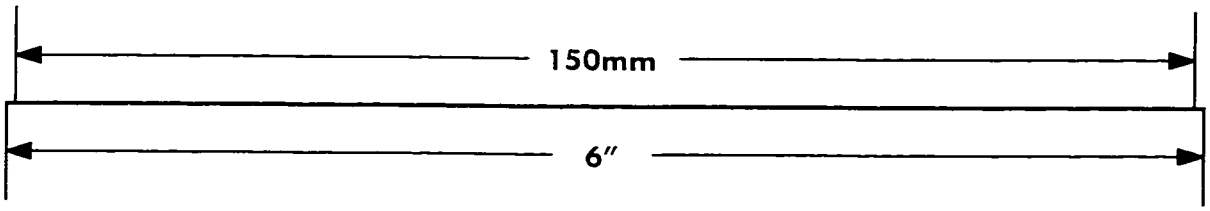
In the analysis of follow up biopsies, glomerulitis caused poor graft outcome, independent of vasculitis. This finding further stresses that more attention should be paid to glomerulitis and the elucidation of its mechanisms. It also indicates the need for the more aggressive treatment of moderate to severe degrees of glomerulitis or vasculitis. The fact that none of the chronic lesions in follow up biopsies has prognostic value indicates the need for a more defined approach to the diagnosis of chronic rejection.

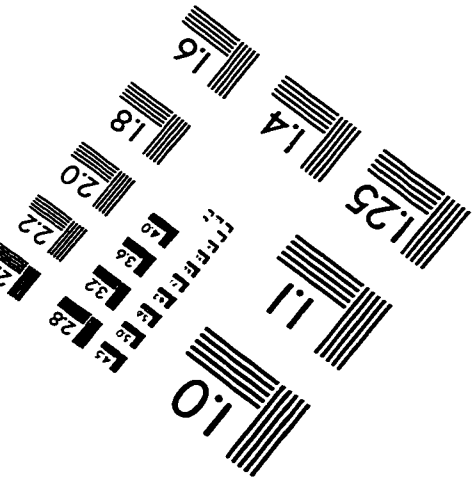
IMAGE EVALUATION TEST TARGET (QA-3)

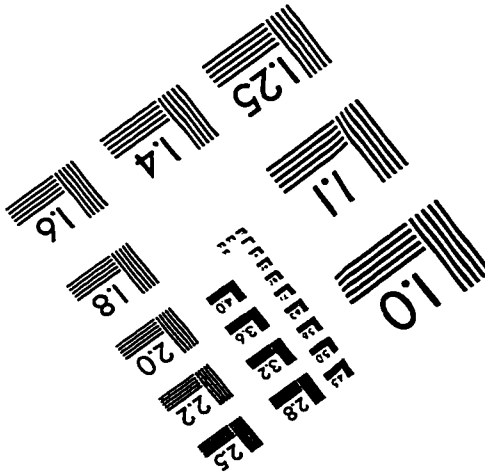












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