Using Implantation Biopsies as a Surrogate to Evaluate Selection Criteria for Living Kidney Donors

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Background. The acceptance criteria used for living kidney donors are largely theoretical, as they are not clearly linked to outcomes. The goal of this study was to use implantation biopsies as a surrogate outcome marker to evaluate our living kidney donor selection criteria.

Methods. One thousand six hundred sequential living kidney donor biopsies were performed between 2001 and 2011. Implantation biopsies were assessed by dedicated renal pathologists according to the Banff criteria. Biopsies with any chronic score of 2 or higher were deemed to have moderate to severe changes (MSC).

Results. MSC was present in 4% (n=65) of implantation biopsies and occurred across a wide range of age and other demographics. By multivariate analysis, donor age (odds ratio [95% confidence interval], 1.060 [1.035–1.086]; $P<0.0001$) and donor systolic blood pressure (SBP) (odds ratio [95% confidence interval], 1.022 [1.006–1.037]; $P=0.0060$) were associated with MSC. Donor gender, body mass index, diastolic blood pressure, glomerular filtration rate, and urinary microalbuminuria were not. MSC was further increased in donors older than 60 years with SBP>140 (30% [7 of 23]) and donors older than 60 years with SBP>140 and glomerular filtration rate above the 25th percentile (42.8% [3 of 7]). In donors younger than 60 years, combining factors did not show an increased prevalence of MSC. At follow-up, renal function was similar in donors with and without MSC.

Conclusions. MSC occurred sporadically in donors with varied characteristics. Although we did not detect patterns to support specific changes in our acceptance criteria, certain subgroups of donors might benefit from close follow-up.

Keywords: Implantation allograft biopsies, Living kidney donor, Selection criteria.

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Kidney transplantation remains the best available option for treating end-stage renal disease. Living kidney donation results in superior long-term patient/graft survival with decreased time to transplantation compared with deceased donation (*1*).

Increased living kidney donation has been accompanied by an interest in quantifying the risks associated with donation particularly related to kidney function. Although several studies appear to support the concept that kidney donation is relatively safe both in the short term and long term, morbidity and mortality still occur (*2–4*). Prior reports indicate that approximately 126 kidney donors have subsequently been listed for kidney transplantation with a mean time from donation to listing of 17.6 years (*5*). There has been an increased acceptance of donors with advanced age, hypertension, and obesity (*6–10*). This has increased the call for evidence-based donor acceptance criteria.

The fact that renal failure or death from stroke and heart disease are low-incident events in the years after living kidney donation means that establishing associations with these rare events and donor characteristics is difficult.

One important goal of the living-donor evaluation criteria is to identify potential donors with significant renal abnormalities at the time of transplantation that might affect the long-term outcomes for either the donor or the recipient. Thus, donor criteria commonly involve acceptable levels of glomerular filtration rate (GFR) and albuminuria or proteinuria.

Donor kidney biopsies also might supply useful information regarding the appropriateness of selection criteria, yet relatively few studies have examined this issue.

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In the current study, we analyzed histologic characteristics of kidney biopsies obtained at the time of living-donor kidney transplantation (implantation biopsies) using the Banff criteria. Our goal was to use the presence of moderate to severe changes (MSC) in implantation biopsies from living kidney donors as a surrogate "outcome." This would allow us to determine if there were certain donor characteristics that led to a higher prevalence of MSC that might then suggest the need for changes in our donor selection criteria.

RESULTS

One thousand six hundred sequential implantation biopsies were reviewed in this study. One hundred thirty (8.1%) donors were hypertensive, 297 (18.56%) had a body mass index (BMI) above 30, and 136 (8.5%) were older than 60 years.

In addition, 95.9% of the donors had either normal biopsies or showed minimal chronic changes (i.e., Banff chronicity score of 0 or 1 in all categories). In this normal/mild chronicity group, 829 had all scores of 0, 414 had only one score above 0, and 291 had more than one score above 0.

MSC was present in 4.1% (65 of 1600) of donors. The most common lesions were vascular, including cv (vascular fibrous intimal thickening, 78.4% [n=51]) and ah (arteriolar hyaline thickening, 20% [n=13]). Other lesions were very rare: ci (interstitial fibrosis, 4.61% [n=3]), ct (tubular atrophy, 4.61% [n=3]), and cg (allograft glomerulopathy [n=0]).

Scatterplots show that donors with MSC features demonstrated a wide range of blood pressures measurements, age, BMI, and microalbumin excretion at baseline that overlapped significantly with donors without MSC (Fig. 1).

Factors associated with MSC by univariate analysis are shown in Table 1. The mean age and systolic blood pressure (SBP) of donors with MSC was higher than those

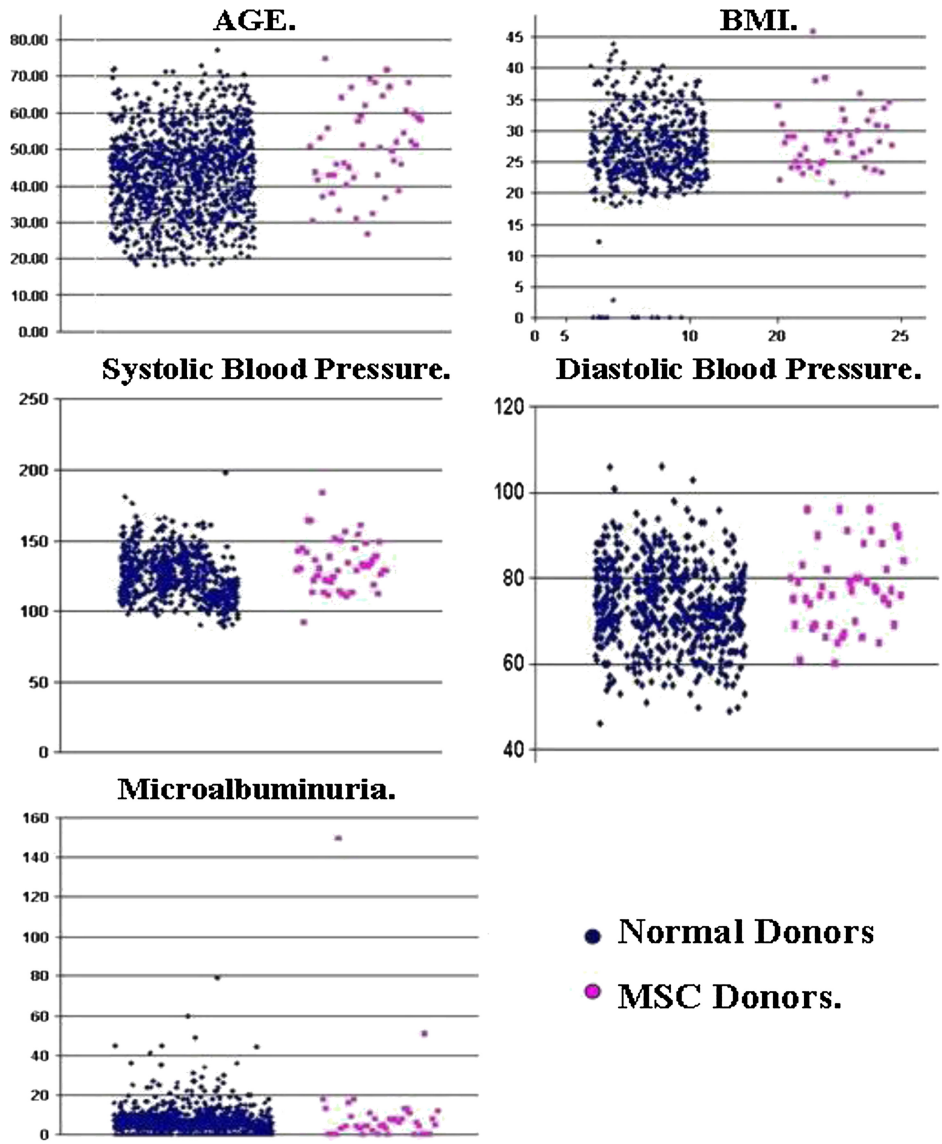

FIGURE 1. Distribution of age, BMI, SBP, DBP, and microalbumin excretion among normal and MSC donors.

TABLE 1. Univariate analysis in patient with and without MSC

Characteristics	No chronicity (n=1535) n	Mean±SD	Chronicity (n=65) n	Mean±SD	Odds ratio (95% confidence interval)	P
Donor age (years), mean±SD	1535	43.73±11.54	65	51.65±11.42	1.063 (1.039–1.087)	**<0.001**
Male, n (%)	1531	658 (43)	23	23 (35.4)	0.724 (0.431–1.216)	0.220
Donor height (cm), mean±SD	1107	171.2±9.53	45	168.44±8.75	0.969 (0.938–1.001)	0.057
Donor weight (kg), mean±SD	1129	81.33±16.86	46	79.75±17.29	0.994 (0.977–1.012)	0.534
Donor BMI (kg/m^2), mean±SD	882	27.53±4.74	64	28.26±4.91	1.032 (0.980–1.086)	0.238
Donor sitting SBP (mmHg), mean±SD	935	121.10±15.76	65	128.28±17.44	1.026 (1.011–1.041)	**0.001**
Donor sitting DBP (mmHg), mean±SD	936	73.75±9.43	65	76.09±9.93	1.026 (1.000–1.054)	0.054
Pretransplantation microalbumin (mg/24 hr), mean±SD	1129	8.55±6.56	49	10.14±7.97	1.027 (0.995–1.060)	0.104
Posttransplantation microalbumin (mg/24 hr), mean±SD	584	11.16±15.25	37	10.35±11.41	0.996 (0.971–1.022)	0.753
Donor pretransplantation iothalamate clearance (mL/min/1.73 m^2), mean±SD	1206	75.83±13.81	46	75.89±13.05	1.000 (0.979–1.022)	0.976
Donor pretransplantation MDRD GFR (mL/min/1.73 m^2), mean±SD	1519	78.88±15.46	65	78.14±13.38	0.997 (0.981–1.013)	0.702
4-Month donor posttransplantation MDRD GFR (mL/min/1.73 m^2), mean±SD	1515	55.28±11.31	64	53.47±9.66	0.984 (0.961–1.009)	0.205

without MSC. Other factors were not significantly different between MSC and non-MSC donors.

Factors associated with MSC by multivariate analysis were donor SBP and donor age (Table 2). This multivariable model was fit by considering all predictors ($P<0.1$) listed in Table 2, first entering the variable most strongly associated with chronicity, and then adding covariates until none could be added using a $P<0.05$ requirement for inclusion. Correlations between variables were also taken into account to prevent inclusion of highly correlated predictor variables in the model. The results demonstrate that older age ($P\leq0.0001$) and donor SBP ($P=0.006$) remain independently associated with chronicity.

In subgroup analyses, the prevalence of MSC was 11% (15 of 136) in donors older than 60 years versus 3.5% (50 of 1405) in donors 60 years or younger ($P=0.0003$). MSC was found in 4.4% (29 of 652) of donors with a GFR below the 25th percentile (iothalamate <66 mL/min) versus 3.81% (36 of 944) of those with a GFR of 66 mL/min or higher ($P=0.52$). The odds for MSC in the 25th, 75th, and 90th percentiles were not statistically significant ($P=0.63$, 0.73, and 0.24, respectively). MSC was seen in 12% of donors with SBP>140 mmHg versus 5.7% in those with SBP<140 mmHg ($P=0.01$).

Combining factors resulted in smaller groups but still was revealing—especially pointing to age as the main contributor to MSC (Table 3). Donors older than 60 years with SBP>140 had a MSC prevalence of 30% (7 of 23). Donors older than 60 years with SBP>140 and GFR above the 25th percentile had a prevalence of 42.8% (3 of 7). It is important to note that the number of donors older than 60 years with these combined factors was small.

Still, most donors with MSC were younger than 60 years (76.9% [50 of 65]) and had no "risk" factors.

There was no difference in graft survival in the two groups.

In addition, 98% of donors with and without MSC had follow-up at 4 months. Their renal function was similar.

In multivariate analyses after adjusting for blood pressure, donor age, gender, ethnicity, and BMI, only donor age was associated with 4-month postdonation GFR (estimate=−0.36; SE=0.05; $P<0.001$) in the non-MSC group.

In the MSC group, no significant variable associated with 4-month GFR.

Long-term follow-up data were obtained in 67% of donors with MSC. The follow-up period varied from 4 to 126 months (mean, 42.61±39.15). Using the most recent data, the mean GFR among the MSC donors was 55.98±11.64 mL/min (range, 34–85) and the mean serum creatinine was 1.2±0.27 mg/dL (range, 0.7–1.9).

One donor (65 years old) died because of natural causes 88 months after donation. None of the other donors developed renal failure or required transplantation.

TABLE 2. Multivariate regression model

Characteristics	Odds ratio (95% confidence interval)	P
Donor age	1.060 (1.035–1.086)	<0.0001
Donor SBP	1.022 (1.006–1.037)	0.0060

Factors associated with MSC.

DISCUSSION

This study used a surrogate marker—MSC on implantation biopsies—to assess our current donor acceptance criteria and evaluate donor characteristics that might lead to a higher rate of chronic histologic changes. MSC was uncommon in this large donor population (n=1600), with a prevalence of 4%. The most common changes were vascular lesions and arteriolar hyalinosis. Interstitial fibrosis and tubular atrophy were present only in three donors. MSC

TABLE 3. Presence of MSC with different combination of risk factors

	MSC, n (%)	*P*	
All donors	16/1600 (4.1)		
GFR<25th percentile (<66 mL/min)	29/652 (4.4)		0.52[a]
GFR>25th percentile (>66 mL/min)	36/944 (3.8)		
SBP>140	14/116 (12)		0.01[a]
SBP<140	51/885 (5.7)		
Age <60 years	50/1405 (3.5)		0.0003[a]
Age >60 years	15/136 (11)		
BMI>30	20/260 (7.7)		0.47[a]
BMI<30	44/688 (6.4)		
Age >60 years+SBP>140	7/23 (30)	0.0005[b]	0.21[a]
Age >60 years+SBP<140	8/51 (15.7)	0.02[b]	
Age <60 years+SBP>140	7/93 (7.53)	0.66[b]	0.47[a]
Age <60 years+SBP<140	43/781 (5.51)	0.003[b]	
Age >60 years+GFR<25th percentile+SBP>140	4/16 (25)	0.01[b]	0.62[a]
Age >60 years+GFR>25th percentile+SBP>140	3/7 (42.8)	0.008[b]	
Age <60 years+GFR<25th percentile+SBP>140	2/34 (5.9)	1[b]	1[a]
Age <60 years+GFR>25th percentile+SBP>140	5/58 (8.6)	0.58[b]	
Age >60 years+BMI>30	3/20 (15)	0.16[b]	0.52[a]
Age >60 years+BMI<30	12/49 (24.5)	0.0003[b]	
Age <60 years+BMI>30	17/228 (7.46)	0.83[b]	0.25[a]
Age <60 years+BMI<30	32/598 (5.35)	0.004[b]	

[a]*P* value obtained when comparing each pair.
[b]*P* value compared with the rest of the cohort.

occurred sporadically across a wide spectrum of age, baseline GFR, and blood pressure; however, multivariate analyses showed that increased donor age and increased donor blood pressure were independently associated with it.

The prevalence of MSC was higher in donors older than 60 years; however, most of the donors with MSC in this large cohort were younger than 60 years. Thus, relying on isolated individual variables would result in the denial of a large number of donors without MSC.

The odds of having MSC were higher in subsets of donors older than 60 years (especially the ones who were also hypertensive). These subgroups of donors were uncommon.

Although this group did well in the short term, we suggest that MSC rates in this range are worrisome and this group at the least deserves longer follow-up. This finding underscores the need to examine large numbers of donors to make valid assessments regarding the true rates of MSC. We plan to continue to accept these donors and to continue to assess their prevalence of MSC as the sample size increases.

We could not identify adverse effects of MSC during this period of follow-up. Although donors with MSC at baseline maintained good renal function during the follow-up, the long-term significance of MSC remains unknown. Graft survival in the recipients of MSC was similar to that of non-MSC recipients (data not shown). However, the outcome of renal transplants depends on so many factors that any connection between pretransplantation factors and posttransplantation events must be interpreted with caution.

The Banff criteria were not developed for the evaluation of native renal diseases. However, the Banff scoring system does include assessment of all "compartments" of the kidney and is likely to be abnormal in more advanced stages of chronic renal failure. The report includes a narrative in which the pathologist may give other data including the overall impression of the possibility of native disease. In this series, only one biopsy was suspicious for IgA nephropathy and no other biopsies were believed to show signs of native renal disease. More detailed studies such as immunohistochemistry and electron microscopy would be needed to rule out the occult presence of renal diseases such as IgA nephropathy and other glomerular diseases; however, these were not performed routinely. The renal biopsies in this study were read by a team of dedicated renal pathologists at the time of transplantation. Certainly, there can be some variability in the readings, but the assessment was unbiased.

Any study of living kidney donors has to deal with the fact that most donors do well—at least as far as most programs follow them. Negative outcomes are very low incident events. Thus, these data cannot be construed to suggest that donors with obesity or hypertension are not at higher risk for subsequent problems, including chronic kidney disease in the years after donation. Long-term follow-up and healthy lifestyle are needed to avoid complications.

Few studies have examined implantation biopsies from living donors (*11–15*). They demonstrated that mild histologic changes are common and that MSC and unrecognized renal disease are very uncommon. Mancilla et al.

found that 54.3% of donors showed features of chronicity with the vast majority of these findings categorized as mild (*11*). Goecke et al. observed histologic abnormalities in 16 of their living donors. Upon follow-up, they found an increased prevalence of hypertension compared with the general population, potentially a manifestation of these renal abnormalities. There was no proteinuria and no donors developed clinical nephropathy (*13*).

The current study supports these observations and also extends this experience in several important respects. First, the current study is from a more recent era and involved a substantially larger number of donors than prior studies. Second, this study added our standard posttransplantation recipient biopsy protocol to validate the presence of MSC. Finally, we specifically examined histology in donors with obesity, hypertension, and advanced age. Mild baseline fibrosis and tubular atrophy have been described in previous studies in these donors (*12, 13*).

Based on these data, we do not recommend a change in current selection criteria and would continue to accept donors with characteristics that might be perceived as risk factors (i.e., mild hypertension and obesity) for postdonation complications such as renal failure. We do recommend that donors with abnormal implantation biopsies be followed more closely and evaluated on a serial basis for the possible development of renal disease.

Going forward, it may be helpful to emphasize the importance of follow-up for donors determined to have MSC at donation.

MATERIALS AND METHODS

The study was performed with informed consent using a study protocol approved by the Institutional Review Board of the Mayo Clinic Foundation and Clinic (Rochester, MN). We performed a retrospective review of the medical records of living renal donors who underwent hand-assisted laparoscopic nephrectomy from January 2001 to March 2011 at the Mayo Clinic Rochester. Records of 1600 sequential living kidney donors who had an implantation biopsy were analyzed.

Living-Donor Screening Protocol

Potential donors were evaluated according to prespecified protocols using guidelines that remained relatively stable during the entire study period (Table 4). Protocol selection criteria included (a) GFR measurement with iothalamate renal clearance above the 5th percentile adjusted for age, (b) 24-hr urine microalbumin excretion less than 30 mg per day, (c) fasting plasma glucose (FPG) 110 mg/dL or lower adjusted for age, and (d) overnight ambulatory blood pressure measurements within the normal range or easily controlled with simple antihypertensive regimen.

Hypertension was defined as SBP≥140 mmHg and or diastolic blood pressure (DBP)≥90 mmHg in three different clinic visits and/or use of antihypertensive therapy and/or 18-hr ambulatory awake blood pressure monitoring showing a mean above 135/85 mmHg.

Donors with hypertension may be acceptable within the Mayo Expanded Donor Program if they fulfilled the following criteria: (a) age older than 40 years, (b) Caucasian ethnicity, (c) normal GFR and blood pressure levels with well-tolerated regimen: usually ACE/ARB with or without diuretic, and (d) patient agrees to follow-up through expanded donor program.

Exclusion criteria included age younger than 18 years, significant cardiac disease, poorly controlled hypertension with end-organ damage, viral hepatitis, malignancy, uncontrolled substance abuse, and psychiatric illness. Previous abdominal surgery was not a contraindication to laparoscopic nephrectomy.

TABLE 4. Donor selection criteria

Criteria	Evaluation	Acceptability
Urinary albumin excretion	24-hr urine microalbumin excretion	Excretion less than 30 mg per day
Measured GFR	Iothalamate clearance, or	- Iothalamate clearance more than or equal to lower 5th percentile for age and GFR >70 mL/min/SA/min
	24-hr creatinine clearance	Similar criteria for creatinine clearance
	Instructed to discontinue use of nonsteroidal anti-inflammatory drugs and/or cyclooxygenase-2 inhibitors before testing	
Blood pressure	Automated oscillometric blood pressure,	Normal blood pressure
	18 hr-ambulatory blood pressure monitor, and	Hypertension; acceptable
	Hypertensive therapy RN using standardized AHA criteria	(a) Age >40 years
		(b) Caucasian
		(c) Normal blood pressure with simple treatment regimen
		(d) Donor agrees to follow-up with Mayo expanded living-donor program
Blood glucose	FPG <126 mg/dL	Acceptable levels by age
	For borderline levels, 2-hr oral glucose tolerance testing and hemoglobin A1c	Age/FPG: <30 years/<102 mg/dL, 31–49 years/<106 mg/dL, and >50 years/<110 mg/dL
		Unacceptable candidates
		(a) FPG >126 mg/dL on two occasions
		(b) Female <30 years with history of gestational diabetes
BMI	BMI calculation based on height and weight	(a) Donor age <30 years: BMI<30 kg/m^2
		(b) Donor age 30–49 years: BMI<35 kg/m^2
		(c) Donor age 50 years or older: BMI<40 kg/m^2

Kidney Biopsy Assessment

Renal allograft implantation biopsies were obtained after vascular anastomosis and reperfusion.

Three 18-gauge core needle biopsies were obtained using an automated biopsy gun. Specimens were fixed in 4% formaldehyde and examined using hematoxylin, eosin, periodic–acid Schiff, and trichrome-stained sections. The biopsies were not routinely processed for electron microscopy or immunofluorescence.

Each biopsy was assessed by dedicated renal pathologists for specific signs of renal disease on light microscopy. The biopsies were scored routinely for acute and chronic changes using the Banff 2007 classification (*16*).

Moderate to Severe Changes

In addition to overall scoring, we focused on those biopsies that were deemed to have MSC, with scores of 2 or greater in at least one of the following categories: ah, cg, ci, ct, or cv.

All living kidney donors are encouraged to return for evaluation of GFR, blood pressure, and urinalysis between 3 and 12 months after donation.

Donors with MSC on implantation biopsies were further analyzed by reviewing all follow-up data available through their medical record.

Donor Follow-up

Living donors were routinely invited for a 4-month postdonation clinical assessment at our institution. Live donors with MSC on their implantation biopsy who did not participate with this early postdonation follow-up were contacted via telephone. Oral consent was obtained at the time of the telephone call to request a copy of their most recent laboratory reports. A Health Insurance Portability and Accountability Act (HIPAA) authorization form was mailed to the donor after the telephone interview. If the HIPAA form was not returned within another 2 to 3 weeks, a second telephone call was made to inquire as to the status of the form. Information obtained during (or subsequent to) the telephone conversation was used if a signed HIPAA authorization form was returned to the investigators.

Donors who did not have follow-up measurement of their renal function within the past 1 year were encouraged to obtain a serum creatinine along with a 24-hr urine microalbumin excretion measurement. A letter was sent to each patient requesting their local provider to obtain a serum creatinine and 24-hr urine microalbumin excretion and to record their height, weight, and blood pressure measurements. The patient or their local providers were reimbursed for the cost of the blood and urine testing.

Statistical Analysis

Frequency of donor characteristics including age, BMI, SBP, DBP, and estimated GFR (Modification of Diet in Renal Disease [MDRD]) were analyzed using descriptive statistics to include mean, range, and SD. Univariate nominal regressions were performed to identify association between donor characteristics and morphologic findings on the implantation biopsies. Variables on univariate analysis with $P \leq 0.1$ were included in a multivariate logistic regression analysis to determine preexisting donor characteristics that predicted morphologic changes on implantation biopsies.

JMP statistical software version 9.0.1 (SAS Campus Drive, Cary, NC).

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