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Henryk Karkoszka[1], Katarzyna Kwiecień[1], Marcin Adamczak[1], Magdalena Szotowska[1], Robert Król[2], Lech Cierpka[2], *Andrzej Więcek[1]

Histomorphometric analysis of glomeruli obtained by preimplantation needle biopsies of paired kidneys harvested from adult deceased donors. Important factor for kidney graft outcome**/***

Ocena histomorfometryczna kłębuszków nerkowych pobranych metodą przedimplantacyjnej biopsji igłowej z obu nerek od dorosłych dawców zmarłych. Istotny czynnik wpływający na czynność nerki przeszczepionej

[1]Department of Nephrology, Transplantation and Internal Medicine, Medical University of Silesia in Katowice
Head of Department: Professor Andrzej Więcek, MD, PhD
[2]Department of General, Vascular and Transplant Surgery, Medical University of Silesia in Katowice
Head of Department: Robert Król, MD, PhD

Keywords

preimplantation kidney biopsy, paired kidneys, histomorphometric analysis, graft outcome

Słowa kluczowe

biopsja przedimplantacyjna nerki, nerki pobrane od jednego dawcy, ocena histomorfometryczna, czynność nerki przeszczepionej

S u m m a r y

Introduction. Kidney transplantation is recognized as treatment of choice for most patients with end stage kidney disease. Long-term kidney allograft outcome may be determined among others by kidney morphology.

Aim. The aim of this study was: 1. to perform histomorphometric analysis of glomeruli obtained by needle biopsies of paired kidneys harvested from adult deceased donors, and 2. to assess the relationships between kidney weight, glomerular volume (GV) and glomerular density (GD) as well as 3. 1- and 3-years outcome of paired kidneys in relation to histomorphometric analysis, cold ischemia time (CIT) and donor-recipient HLA mismatch.

Material and methods. 56 kidneys obtained from 28 adult kidney donors were biopsied and weighted at time of transplantation. Total number of complete glomeruli, as well as percentage of normal (Norm), globally sclerosed (GS), segmentally sclerosed (SS), hyperperfused (Hyp) and ischemic (Isch) glomeruli were calculated. Mean glomerular volumes (MGV) were estimated from the maximal glomerular profile area according to the formula: $GV = 4/3\ \pi\ r^3$ (μm^3) and glomerular density (GD) was expressed as the number of non-globally sclerotic glomeruli per mm^2 of cortical area. CIT, incidence of immediate (IGF) and delayed graft function (DGF) and number of HLA-mismatches were assessed.

Results. Significant negative correlation was found between MGV and GD ($r = -0.31$; $p = 0.017$). A significant positive correlations between donor age and kidney weight ($r = 0.390$; $p = 0.0011$) and kidney weight and MGV ($r = 0.258$; $p = 0.044$) were observed. Significant negative correlations have been found between donor age and glomerular density as well as between kidney weight and glomerular density ($r = -0.306$; $p = 0.016$) and ($r = -0.394$; $p = 0.0016$), respectively. Additionally, a significant positive correlation was found between kidney weight and percentage of segmental glomerulosclerosis ($r = 0.261$; $p = 0.042$). DGF was observed more frequently in recipients with longer CIT (40.3 vs 25.2%; $p < 0.05$). Paired kidneys did not revealed significant differences in assessed histomorphometric indices. Significant positive correlation between donor age and 1- and 3-years creatinine concentrations ($r = 0.320$; $p = 0.016$ and $r = 0.311$; $p = 0.036$, respectively) was noted.

Conclusions. 1. Mean glomerular volume in kidney biopsy may serve as surrogate marker of glomerular number. 2. Negative relationships between kidney weight and both glomerular density, and percentage of glomerulosclerosis as well as between mean glomerular volume and glomerular density suggest that higher kidney weight in adults is mainly related to kidney hypertrophy. 3. Needle biopsy of only one of paired kidneys is sufficient to assess the initial quality of the organ and predict its outcome.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

Address/adres:

*Andrzej Więcek
Department of Nephrology,
Transplantation and Internal Medicine
Medical University of Silesia in Katowice
ul. Francuska 20/24, 40-027 Katowice
tel. +48 (32) 255-26-95,
fax +48 (32) 255-37-26
awiecek@sum.edu.pl

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S t r e s z c z e n i e

Wstęp. Przeszczepianie nerek jest metodą z wyboru leczenia dla większości chorych ze schyłkową ich niewydolnością. Odległa czynność wydalnicza nerki przeszczepionej jest między innymi uwarunkowana zmianami morfologicznymi w przeszczepianym narządzie.

Cel pracy. Celem pracy były: 1. analiza histomorfometryczna kłębuszków nerkowych uzyskanych metodą przedimplantacyjnej biopsji gruboigłowej z obu nerek od dorosłych dawców zmarłych, 2. ocena zależności między masą nerki a objętością (GV) i gęstością kłębuszków (GD) oraz 3. czynności pierwszorocznej i 3-letniej obu nerek pobranych od jednego dawcy i przeszczepionych różnym biorcom w zależności od ocenianych wskaźników histomorfometrycznych kłębuszków, wpływu czasu niedokrwienia zimnego (CIT) oraz niezgodności w układzie HLA między dawcą a biorcą.

Materiał i metody. Analizie poddano bioptaty 56 nerek pobranych od 28 dorosłych dawców zmarłych. Przeanalizowano liczbę kłębuszków kompletnych oraz odsetki kłębuszków prawidłowych (Norm), całkowicie zwłókniałych (GS), zwłókniałych segmentowo (SS), z cechami hiperfiltracji (Hyp) oraz niedokrwienia (Isch). Obliczono GD oraz średnią objętość kłębuszków (MGV). Obliczono CIT, odsetek bezpośredniego (IGF) i opóźnionego podjęcia czynności przez nerki przeszczepione (DGF) oraz niezgodność w zakresie układu HLA między dawcą a biorcą.

Wyniki. Wykazano znamienne, ujemne korelacje między MGV a DG ($p = -0,31$; $p = 0,017$), wiekiem dawcy a GD ($r = -0,306$; $p = 0,016$) oraz masą nerki a GD ($r = -0,394$; $p = 0,0016$). Stwierdzono znamienne, dodatnie korelacje, odpowiednio między: wiekiem dawcy a masą nerki ($r = 0,390$; $p = 0,0011$), masą nerki a MGV ($r = 0,258$; $p = 0,044$) oraz masą nerki a odsetkiem SS ($r = 0,261$; $p = 0,042$). DGF stwierdzono znamiennie częściej w grupie biorców z dłuższym czasem niedokrwienia zimnego (40,3 vs 25,2%; $p < 0,05$). Nie stwierdzono istotnych różnic w zakresie masy oraz ocenianych wskaźników histomorfometrycznych między obiema nerkami pobranymi od jednego dawcy. Stwierdzono dodatnią korelację między wiekiem dawcy a stężeniem kreatyniny w surowicy krwi po upływie pierwszego i trzeciego roku po przeszczepieniu, odpowiednio $r = 0,320$; $p = 0,016$ i $r = 0,311$; $p = 0,036$.

Wnioski. 1. Średnia objętość kłębuszków może być pośrednim wykładnikiem ich liczby. 2. Odwrotna zależność między masą nerki a gęstością kłębuszków i odsetkiem kłębuszków zwłókniałych segmentowo, jak również między średnią objętością kłębuszków a ich gęstością wskazuje, że większa masa nerki u dorosłych jest uwarunkowana głównie jej przerostem. 3. Biopsja igłowa jednej nerki jest wystarczająca do określenia charakteru i zaawansowania zmian histologicznych w drugiej nerce pobranej od tego samego dawcy i pozwala na ustalenie rokowania dotyczącego jej czynności w odległym okresie po transplantacji.

INTRODUCTION

Kidney transplantation is recognized as treatment of choice for most patients with end stage kidney disease. However, despite substantial progress during last few decades in field of transplantology, long term graft outcome is still unsatisfactory (1). Kidney allograft outcome is influenced by the quality of transplanted organ as well as multiple donor and recipient interactions which are non-immune and immune in their nature. Seron et al. (2) and Isoniemi et al. (3) have postulated that histological changes in posttransplantation protocol biopsies can be useful in predicting long term graft outcome. Other studies (4, 5) suggested that particular morphological changes detectable at the very early stages after transplantation or even in preimplantation biopsies, before chronic changes superimpose could even be more useful to assess organ quality and predict long term graft outcome. Donor biopsies usually do not reveal acute inflammatory lesions and the range of chronic lesions is even narrower than in protocol ones (6). Therefore some authors have proposed to evaluate donor biopsies with a very detailed semiquantitative scale in order to differentiate groups in this range of lesions (7, 8), while others have proposed to employ a morphometric analyses (9, 10). Although more laborious and time consuming than conventional histologic evaluation, morphometric methods reduces the subjectivity and variability in interpretation.

Results of some quantitative histopathological studies underscore the role of nephron number and glomerular size/volume as well as its variability in the pathogenesis of arterial hypertension and kidney failure (11, 12). It has been found that patients with essential hypertension are characterized by lower nephron number and higher glomerular volume (GV) in comparison to normotensive patients (13). Counting of total glomerular (nephron) number in the kidney is very laborious and time consuming method not useful in every day clinical practice. However, results of autopsy studies (13, 14) revealed an inverse relationships between kidney mass and glomerular volume as well as between glomerular volume and glomerular (nephron) number, so the GV could serve as surrogate of total glomerular number in clinical studies (14, 15). Calculation of glomerular number from biopsy material is not possible. However, it is thought that in these specimens estimation of glomerular density is a marker of glomerular number which determine the adaptive capacity of grafted kidney to its new conditions after transplantation. An inverse correlation between GV and

total glomerular number noted in autopsied kidneys of people without evidence of renal disease (13, 14) and between GV and glomerular density (GD) in IgA Nephropathy (IgAN) (16), Idiopathic Membranous Nephropathy (IMN) (17) and Minimal Change Disease Nephrotic Syndrome (MCDNS) (18) patients represent structural adaptation of glomeruli and their decreased functional reserve, susceptibility for subsequent renal injury and faster loss of function as well as worse response to therapy. Fogo et al. (19) documented in children with MCDNS, that the presence of glomerular hypertrophy in a biopsy is an indicator of an increased risk of progression to focal segmental glomerulosclerosis (FSGS). Koike et al. (18) showed that MCDNS adult patients with low GD (< 3.4/mm^2) had not only larger GV but also characterized by higher degrees of chronic histopathological changes, such as global glomerulosclerosis and interstitial fibrosis indices in comparison to high GD group (> 3.4/mm^2). Alperovich et al. (20) has been found that transplanted kidneys with higher GV characterized worse long term outcome in comparison to kidneys with lower GV. Therefore it was found, that variations in glomerular volume may influence the clinical course of various forms of primary glomerulonephritis as well as outcome of transplanted kidneys. Recent studies have underscore the importance of GD (the number of non-sclerotic glomeruli per renal cortical area of biopsy specimen) on kidney outcome in patients with different forms of primary glomerulonephritis. An individual value of GD in a diagnostic biopsy (≥ 10 glomeruli) showed approximately a 7-fold variation, even in patients with relatively well preserved kidney function (eGFR > 60 ml/min). Patients with IMN, IgAN and MCDNS and low GD characterized more rapid progression in comparison to patients with high (16-18). These results identified GD as an important histological predictor of kidney diseases progression. Summarizing the presented data, the value of pre-implantation kidney biopsy seems to be unquestionable. However, there is still no full agreement, whether histological changes available at transplantation provide only information concerning donor-derived organ injury or which lesions in pre-implantation biopsies allow the best prediction on outcome, and whether it is necessary to perform pre-implantation biopsies in both kidneys of the same donor (paired kidneys), or it will be representative to biopsy only one kidney.

Relationship between duration of cold ischemia time (CIT) and the incidence of DGF has been well established (21-24). However, there is still no clear consensus concerning the influence of CIT-induced DGF on long-term graft function and outcome (21, 22, 25-27). Recent studies of Kayler et al. (22) and summary of data by Terasaki (28) on paired kidneys have shown, that if no longer than 15h, CIT has no influence on long- term graft outcome between longer- and shorter-CIT groups. They concluded that the CIT--caused DGF was only the result of ischemic acute tubular injury (ATI), which as fully reversible state has no impact on long term graft outcome. In their study Louvar et al. (29) have been found a significant correlation within paired kidneys for the occurrence of DGF and kidney allograft failure. They noted, that within pairs of recipients from a common donor, when DGF occurred in one recipient, the adjusted odds for DGF in the recipient of the contralateral kidney was > 200% higher, suggesting that unmeasured donor genetic and biochemical factors, so called "nature" contribute more than CIT to a recipient risk for DGF and late graft outcome. On the contrary, results of the Collaborative Transplant Study (30) and analysis of US Renal Data System (31) have found greater risk of graft failure in recipients with CITs > 18h and continuous worsening of graft outcome associated with each 6h CIT increase, respectively. Notably, most of these studies did not included into the analysis kidneys weight and pre-transplant histology of the paired kidneys. Therefore, it is suspected, that CIT induced injury superimposed on already existing chronic changes are responsible for observed relationship between CIT and long term graft outcome and may be partially explain the differences in published results (22, 28). Taking into account the above mentioned facts, the integrated analysis which include not only pre-procurement kidney function but also its mass as well as histologic changes and morphometric characteristics in pre- (4, 5) and posttransplantat biopsies (2, 3) is useful to predict graft outcome.

AIM

The aim of this study was: 1. to perform a preimplantation histomorphometric analysis of glomeruli obtained by needle biopsies of paired kidneys harvested from adult deceased donors, and 2. to assess the relationships between kidney weight, glomerular volume (GV) and glomerular density (GD) as well as 3. 1- and 3-years outcome of paired kidneys transplanted into different recipients in context histomorphometric studies, influence of CIT and donor-recipient HLA mismatch.

MATERIAL AND METHODS

To minimize most transplant related differences influencing graft function and arising from local practices, only paired kidneys without any gross abnormalities (congenital or acquired), harvested from adult deceased donors and transplanted in a single center between January 1, 2005 and December 31, 2007 were included into the analysis. After procurement, all the kidneys were stored in "Viaspam" solution until implantation, without machine perfusion. In 28 deceased donors with adequate biopsy material (according to Banff criteria – at least 7 glomeruli and 1 artery section), weight of both kidneys were obtained. Kidney biopsies were performed on the back table after surgical preparation using semiautomatic needle "PRECISA" 16 G x 200 mm (Italy). For measurement of kidney weight an electronic scale was used. Kidney weight measurements were performed with precision of 1 g.

Kidney biopsies were fixed in 4% buffered formalin, processed and embedded in paraffin, then cut on 2-3 μm thick sections, and stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Masson's trichrome, periodic acid silver methenamine and Congo red. Immunohistochemistry for IgA, IgG and IgM was performed in each case. Only cases containing at least 7 glomeruli and 1 arterial section (measurement of six glomeruli provides a reliable estimate of glomerular size by the MPA method (32)) as well as corticomedullary junction and negative or unspecific immunostaining underwent subsequent histomorphometric analyses using "OLYMPUS BX51" microscope connected with "OLYMPUS CX50" camera (Olympus, Japan) and "cellSense Standard" softwere (Olympus, Japan). Total number of complete (being encircled by non-interrupted Bowman capsule except vascular and tubular poles) glomeruli, as well as numbers of normal (Norm), globally sclerosed (GS), segmentally sclerosed (SS), hyperperfused (Hyp) and ischemic (Ish) glomeruli were counted in the section containing the highest number of glomeruli. Glomeruli with a completely patent capillary network, relatively uniform capillary diameters, no excess of mesangium (no more than three nuclei per mesangial stalk), and no areas of sclerosis, adhesions to Bowman's capsule or podocyte alterations were defined as normal. Hypertrophic glomeruli were characterized by combinations of two or more of the following histologic features: a) evident increase in size in comparison to most surrounding glomeruli, b) a dilated hilar capillary region in comparison to most surrounding glomeruli, substantially wider than the lumen of it feeding afferent arteriole, usually with evident dilatation of the primary branches, c) widely patent, often distended capillary loops, in the absence of increase in mesangium or any focal adhesions, sclerosis or podocyte alterations, d) marked disparity in capillary loops. Mesangial increase and sclerosis with narrowing of glomerular capillaries and tuft adhesions to Bowman's capsule defined glomeruli of FSGS type. As ischemic were classified glomeruli which show progressive shrinkage of the tuft, compared with most surrounding glomeruli, with wrinkled capillary walls surrounding narrowed but still patent capillary loops in most areas. Globally sclerotic glomeruli characterized with no patent capillary loops (33). Results were expressed as the percentage of complete glomeruli.

Morphometric analysis of complete, nonsclerosed glomeruli was performed on PAS, Masson's trichrome and periodic acid silver methenamine stained sections at 200 x magnification. Glomerular diameters and maximal profile areas were measured. Glomerulus in the present study was defined as an area inside a Bowman's capsule containing tuft. Maximal glomerular volumes (maxGV) were estimated from the maximal glomerular profile area according to the formula: GV (μm^3) = 4/3 π r^3 where:

GV – glomerular volume (assuming that its 3-daimentional shape is a sphere),

r = GPA $\mu m^2/\pi$ where:

r – glomerular radius (calculated from the glomerular area, assuming that its shape is a circle),

GPA – glomerular profile area.

Glomerular density (GD) was calculated in the section containing the highest number of complete glomeruli and defined as the number of non-globally sclerotic glomeruli per mm^2 of renal cortical area of needle biopsy.

Statistical analysis

Results were expressed as the median and 95% confidence interval (CI). The significance of correlation between variables was assessed using the method of Spearman. Comparisons between paired kidneys and recipients of paired kidneys were performed using Mann-Whitney U test. A p value < 0.05 was considered as statistically significant.

RESULTS

Median of age and 95% CI of the whole donors group (N = 28) was 44.1 and 40.9-47.2 years, respectively (range 18-64). In 16 cases intracranial hemorrhage was a cause of death. Remaining twelve donors died due to brain trauma. Last serum creatinine concentration (median and 95% CI) before kidney procurement in the donors was 1.47 mg% (1.14-1.81), respectively. Weight of kidneys between donors ranged two fold, from 248 to 504 g, without significant differences between paired kidneys (tab. 1). Significant positive correlation between donor age and kidney weight ($r = 0.390$; $p = 0.0011$) was noted. Significant negative correlations have been found between donor age and glomerular density as well as between kidney weight and glomerular density (fig. 1) in the whole group of donors ($r = -0.306$; $p = 0.016$) and ($r = -0.394$; $p = 0.0016$), respectively. On the contrary, kidney weight correlated positively with mean MGV in the whole group of donors ($r = 0.258$; $p = 0.044$) (fig. 2). No significant correlation was observed between last serum creatinine concentration and donor age or kidney weight, respectively.

Tab. 1. Weight, glomerular density and mean glomerular volume of paired kidneys. Median and (95% CI)

Parameter	Kidney 1	Kidney 2	p
Kidney weight (g)	175 (167.8-189.1)	177 (171.7-192.4)	NS
GD (1/mm^2)	2.94 (2.66-3.6)	2.90 (2.65-3.43)	NS
GV (x $10^6 \mu m^3$)	4.56 (4.16-5.22)	4.28 (3.02-8.19)	NS

GD – glomerular density; GV – glomerular volume

Median and 95% CI of complete glomeruli in preimplantation kidney biopsies for donors was 17.35 (14.95-19.74) and did not differ significantly between paired kidneys (tab. 2). The percentage distribution of normal, global and segmental sclerosed as well as hyperperfused and ischemic glomeruli in preimplantation biopsies was as follows (Median and (95% CI)): 60.5 (54.89-66.15), 3.84 (1.97-5.71), 4.14 (2.52-5.76), 29.23 (24.29-34.18), 2.23 (0.69-3.77). Histological

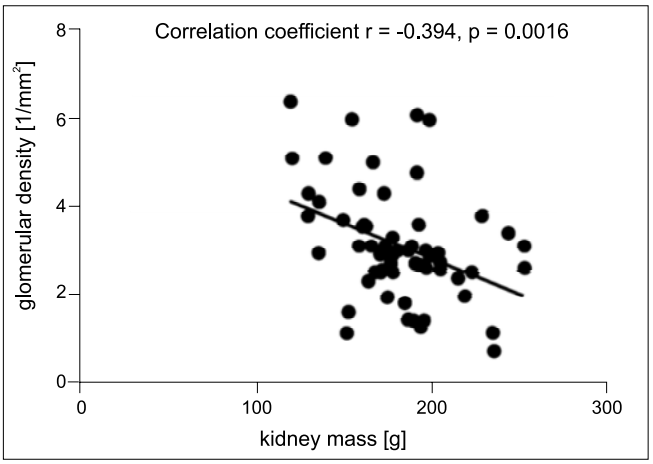

Fig. 1. Correlation between kidney mass and glomerular density in whole group of donors

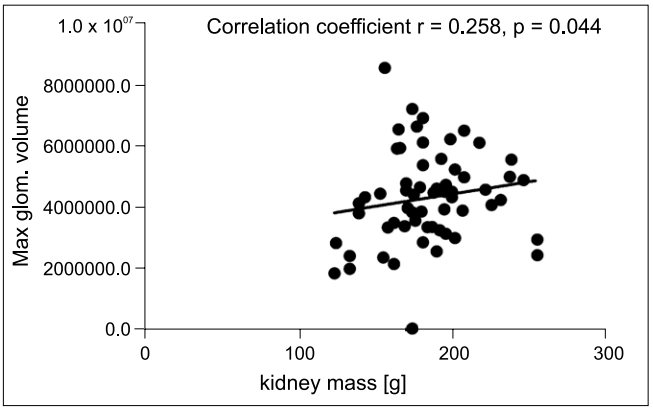

Fig. 2. Correlation between maximal glomerular volume and kidney mass in whole group of donors

assessment of paired kidneys are summarized on table 2. We did not reveal significant differences in percentage distribution of normal, globally and segmentally sclerotic as well as hyperperfused and ischemic glomeruli.

Tab. 2. The percentage distribution of normal, globally and segmentally sclerotic as well as hyperperfused and ischemic glomeruli of paired kidneys. Median and (95% CI)

Parameter	Kidney 1	Kidney 2	p
N. glom.	17.9 (15.1-23.9)	16.9 (13.2-20.7)	NS
Normal %	65.5 (55.3-70.2)	60.0 (49.5-67.0)	NS
GGS %	0.0 (1.1-5.8)	0.0 (1.2-7.2)	NS
SGS%	0.0 (1.6-5.9)	0.0 (1.9-7.0)	NS
Hyp %	30.0 (20.8-33.5)	30.8 (23.5-39.2)	NS
Isch %	0.0 (0.3-5.38)	0.0 (2.65-3.43)	NS

N. glom. – number of complete glomeruli per biopsy; GGS – global glomerulosclerosis; SGS – segmental glomerulosclerosis; Hyp – hyperperfused glomeruli; Isch – ischemic glomeruli

A significant positive correlation was found between kidney weight and percentage of segmental glomerulosclerosis (r = 0.261; p = 0.042) in the donors (fig. 3). No significant correlation was observed between donor age and particular histological glomerular changes.

The mean glomerular volume measured for the whole group of donors in preimplantation biopsies was 5.15 x 10^6 μm^3 (3.86-6.44) and did not differ significantly between paired kidneys (tab. 1). The variability in MGV in whole group of donors ranged 3.74 fold, from 1.86 x 10^6 to 6.97 x 10^6 μm^3. GD in the preimplantation biopsies for the whole group of donors was 3.07/mm^3 (2.82-3.38). No significant differences were noted between paired kidneys concerning to GD (tab. 1). A significant negative correlation was found between GD and MGV (r = -0.31; p = 0.017).

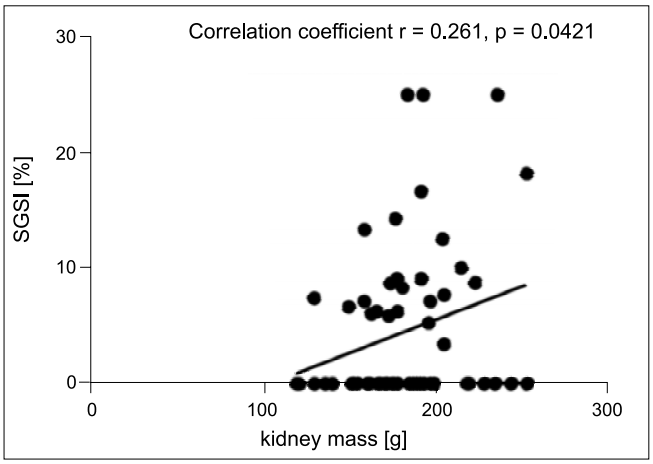

Fig. 3. Correlation between percentage of segment al glomerulosclerosis and kidney mass in whole group of donors

56 recipients (26F/30M) aged 45 (40.1-48.3) years received for the first time single kidney allografts. In each case, donor and recipients were ABO blood group compatible, Cross-match negative. None of the recipients was highly immunized (PRA < 25%). Initial immunosuppressive protocol consisted of Prednizone, Calcineurin inhibitor (CyA/Tac) and MMF/MPA. All recipients were randomly allocated into two groups of which the first comprised 28 patients (15F/13M) who received single of the paired kidneys with shorter CIT, and the second one consisted also of 28 recipients (12F/16M) of the remaining paired kidneys from the same donor with longer CIT. DGF was observed with significantly higher frequency in recipients with longer CIT in comparison to shorter CIT recipients (40.3 vs 25.2%; p < 0.05). Recipients of paired kidneys did not differ significantly neither for KW/RBW ratio nor for HLA-incompatibility. Clinical characteristic of both groups summarizes table 3.

Tab. 3. Clinical characteristic of recipients of paired kidneys. Median and (95% CI)

Parameter	Group I	Group II	p
Recipient age (years)	44 (41.5-48.3)	46 (40.1-47.4)	NS
CIT (min)	729 (674-935)	1080 (1015-1313)	p < 0.05 (0.00065)
IGF/DGF	21/7 (74.8%/25.2%)	17/11 (59.7%/40.3%)	p < 0.05
KW/RBW (g/kg)	2.51 (2.4-2.76)	2.75 (2.64-3.07)	NS
Number of HLA--A+B+Dr mismatches	4.0 (2.9-3.9)	4.0 (3.1-4.1)	NS

CIT – cold ischemia time; IGF – immediate graft function; DGF – delayed graft function; KW – kidney weight; RBW – recipient body weight; HLA – human leukocyte antigen

At the end of the first year after transplantation kidney graft function was assessed in 24 of the 28 pairs of recipients which received kidney allografts from the same donors (paired kidneys). During the first twelve months after the procedure three recipients died (2 – cardio-vascular events, 1 – septic complications), two recipients lost their grafts due to biopsy proven acute rejection episodes as a consequence of nonadherence to immunosuppressive drugs, one due to surgical complications, two recipients lost to follow up due to transfer to other centers. At the end of third year after transplantation additional three recipients (1 died due to cardio-vascular event, 1 died due to sepsis and one lost the graft due to biopsy proven acute rejection episode) were excluded from the analysis of the graft function. Finally, 3-years kidney graft function was evaluated in 21 pairs of recipients. As presented in table 4, 1- and 3-years eGFR did not differ significantly between recipients of paired kidneys.

Tab. 4. 1- and 3-years graft function of paired kidneys. Median and (95% CI)

Parameter	Kidney 1	Kidney 2	p
1-year eGFR ml/min (N = 24)	50.2 (44.5-57.6)	37.0 (36.2-48.3)	NS
3-years eGFR ml/min (N = 21)	51.74 (44.3-59.7)	43.6 (36.2-56.5)	NS

eGFR – estimated glomerular filtration rate

Significant positive correlation between donor age and 1- and 3-years creatinine concentrations was noted, $r = 0.320$; $p = 0.016$ and $r = 0.311$; $p = 0.036$, respectively.

DISCUSSION

A wide diversity of chronic histological changes in kidneys harvested from optimal deceased and extended criteria donors (34) underscore the significance of histological examination of pre-implantation biopsies in the process of organ acceptation for transplantation but also in proper allocation and prediction of its outcome (35). It has been shown, that chronic injury observed in pre-implantation biopsies of deceased kidney donors comprised all renal compartments (9, 36, 37) and correlates with early and late allograft function. However, most of these studies did not take into consideration the association of chronic changes with donor age, which by itself is associated with graft failure (5).

Global glomerulosclerosis is the most frequent histological change used in clinical studies to date. However, due to its inactive nature and limited advancement observed in most kidneys harvested from optimal donors, has no significant importance on kidney functional reserve at the time of transplantation and future outcome. Additionally, wedge biopsies used in majority of studies overestimate the percentage of sclerosed glomeruli as these are more frequently found in the subcapsular part of the cortex (38) and may underestimate arterial- and glomerular sclerotic lesions in the corticomedullary junction of the kidney (39). Furthermore, the sample size of renal biopsies is an important determinant of accurate assessment of the percentage glomerulosclerosis in the kidney (40).

The Banff 2007 classification recommends routine scoring of zero-time needle biopsies similar to biopsies performed after kidney transplantation, which help to interpret the lesions as a continuum, enables obtaining both cortical and medullary parts of the kidney and allow evaluation particular compartments at different levels of the kidney parenchyma (41). However, for the reasons mentioned earlier, the importance of glomerulosclerosis examined in needle biopsies to predict quality and long-term outcome of transplanted kidney harvested from younger donors, in which advanced chronic changes are scarces, remain very controversial. Therefore, it seems valuable to study early objective histological markers/changes which could serve as better predictors of both, the quality of transplanted kidney and long-term graft function.

Little is known concerning concordance of histological changes in kidneys harvested from the same donor (paired kidneys) and its outcome in this context. Pokorna et al. (42) has been found correlation between left and right kidney for arteriolosclerosis ($r = 0.99$; $p < 0.0001$) and proportion of glomerulosclerosis ($r = 0.88$; $p < 0.0001$) in 29 deceased donors. Based on these findings, only one kidney was biopsied from the remaining 150 donors.

Results of some quantitative histological studies underscore the role of nephron number and glomerular size as well as its variability in the pathogenesis of arterial hypertension and kidney failure (11, 12). It has been found that patients with essential hypertension are characterized by lower nephron number and higher glomerular volume (GV) in comparison to normotensive patients (13). Results of autopsy studies (13, 14) revealed an inverse relationships between kidney mass and glomerular volume as well as between glomerular volume and glomerular (nephron) number, so the GV could serve as surrogate of total glomerular number in clinical studies (14, 15) predictor of long-term graft outcome connecting transplant nephron mass with structural changes (43-45). In their study, Abdi et al. (46) and Alperovich et al. (20) have found that transplanted kidneys with higher baseline glomerular size or GV which already had limited capacity to enlargement after transplantation characterized worse long term outcome in comparison to kidneys with lower GV.

In the present study 56 paired kidneys from 28 donors were evaluated. Significant positive correlation observed between donor age and kidney weight ($r = 0.390$; $p = 0.0011$) confirms the results of other study which comprised both pediatric and adult kidney donors and underscore the presence of physiologic relationship between age and kidney weight in the studied population (44).

In the present study, median and (95% CI) of complete glomeruli obtained in preimplantation kidney

biopsies was 17.35 (14.95-19.74) which is similar to the values obtained in other studies (5, 47) and did not differ significantly between paired kidneys. However, according to Corwin et al. (40) these numbers are somewhat too low for accurate assessment of the percentage glomerulosclerosis in the kidney but completely enough for a reliable estimate of glomerular size by the MPA method (32). Index of globally sclerotic glomeruli in the studied population of donors was 3.84% (1.97-5.71) median and (95% CI), which is close to the results published by Hoy et al. (12) and did not differ significantly between paired kidneys.

For the measurement of the GV, glomerulus was defined as an area inside the intact Bowman's capsule containing tuft, as the strong correlation between glomerular capsular area and glomerular tuft area has been found (32). Due to strong correlation between the method of profile area (MPA) with the Cavalieri method considered the gold standard (32), maximal glomerular volume was estimated from the maximal glomerular profile area. The mean glomerular volume measured for the whole group of donors in preimplantation biopsies was 5.15 x 10^6 μm^3 (3.86-6.44) and no significant difference was found between paired kidneys. The variability in mean glomerular volumes in whole group of donors ranged 3.74 and no significant differences were noted between paired kidneys. Mean GV correlated positively with kidney weight in the whole group of donors.

GD in the preimplantation biopsies for the whole group of donors were 3.09/mm^2 (2.79-3.38) and paired kidneys did not differ significantly concerning this parameter.

Significant negative correlations have been found between donor age and glomerular density, as well as between kidney weight and glomerular density in the whole group of donors. These results confirm age-dependent glomerular number decrease observed by others and underscore, that higher kidney mass in adults is not always related to higher glomerular number. Additionally, significant negative correlation was found between GD and GV in the whole group of donors. This correlation is in line with the previous observations, which also noted an inverse relationship between both parameters and considered GV not only as a surrogate measure of glomerular number/density (14, 15) but also as poor predictor of long-term graft outcome (46). A significant positive correlation observed between kidney weight and percentage of segmental glomerulosclerosis in the whole donors group additionally confirms, that the consequence of the lower glomerular number/density are structural glomerular disturbances like glomerular enlargement and segmental glomerulosclerosis. Examined pre-implantation biopsies of paired kidneys did not differ significantly concerning the percentage of normal, globally, segmentally sclerosed as well as hyperperfused and ischemic glomeruli.

Delayed graft function defined as the need for hemodialysis in the first post-transplant week was observed more frequently in the recipient with longer cold ischemia time (40.3 vs 25.2%; $p < 0.05$). However, one year and three years function of paired kidneys expressed by eGFR values did not reveal significant differences. These results are in concordance with the results of others (21, 22, 25, 28) and confirm, that CIT-induced DGF is fully reversible state which has no impact on graft outcome when is superimposed on scarce histological changes.

Significant positive correlation between donor age and 1- and 3-years recipient serum creatinine concentrations in the context of relationships observed between donor age and GD and kidney mass as well as GV and GD underscore their relevance as predictors of the future graft function.

CONCLUSIONS

In conclusion, the presented study revealed that:

1. Mean glomerular volume in kidney biopsy may serve as surrogate marker of glomerular number.
2. Negative relationships between kidney weight and both glomerular density, and percentage of glomerulosclerosis as well as between mean glomerular volume and glomerular density suggest that higher kidney weight is mainly related to kidney hypertrophy.
3. Needle biopsy of only one of paired kidneys is sufficient to assess the initial quality of the organ and predict its outcome.

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