Do exist histo-morphometric differences of glomeruli obtained by pre-implantation needle biopsies of both kidneys obtained from the same donor?

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• kidney morphology
• paired kidneys
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• kidney function
• different recipient

Słowa kluczowe:
• morfologia nerek
• jeden dawca
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• czynność nerek
• różny biorca

Introduction: Kidney transplantation is regarded as treatment of choice for most patients with end stage renal disease. Among others, morphology of transplanted kidney determine allograft outcome. However, in case of paired kidneys (kidneys obtained from the same donor) it is not known whether it is necessary to perform biopsy of both kidneys to establish its basic status or it will be enough to biopsy only one kidney.

Objectives: This study aimed to assess: 1. Histo-morphometric differences of glomeruli obtained by pre-implantation needle biopsies between both kidneys obtained from the same donor. 2. 1- and 3-years graft outcome of paired kidneys transplanted into different recipients in context of histo-morphometric analysis, influence of cold ischemia time and donor-recipient HLA mismatch.

Patients and methods: 33 deceased adults (13 F/20 M) from which paired kidneys were procured, biopsied and weighted at time of transplantation were included into the analysis. CIT, incidence of IGF/DGF, KW/RBW ratio and HLA-mismatches were assessed. Total number of complete glomeruli, as well as percentage of normal, globally and segmentally sclerosed, hyperperfused and ischemic glomeruli were calculated. Glomerular passenger cells number per glomerulus were measured. Peritubular capillary density was expressed as the number of capillary profiles per mm^2 of cortical area. Mean glomerular volumes were estimated from the maximal glomerular profile area and glomerular density was expressed as the number of non-globally sclerotic glomeruli per mm^2 of cortical area.

Results: DGF was observed with significantly higher frequency in recipients with longer CIT in comparison to shorter CIT recipients (42.4% vs 27.3%; p<0.05). Paired kidneys did not revealed significant differences in percentage distribution of normal, globally and segmentally sclerosed as well as hyperperfused and ischemic glomeruli, number of glomerular passenger cells and did not differ significantly according to weight, glomerular density, mean glomerular volume and peritubular capillary density. Significant negative correlations have been observed between donors age and glomerular density as well as mean GV and GD, r= -0.269; p= 0.02 and r= - 0.274; p= 0.02, respectively. Significant positive correlation between donor age and kidney weight (r= 0.380; p= 0.002) was noted as well as donor age and 1 – and 3-years creatinine concentrations, r= 0.323; p= 0.015 and r= 0.299; p= 0.038, respectively.

Conclusions: 1. Histo-morphometric changes of glomeruli observed in needle biopsies of paired kidneys harvested from optimal donors are equally advanced. 2. Needle biopsy of only one of paired kidneys is sufficient to assess the basal status of the organ and predict its outcome.

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Czy kłębuszki uzyskane metodą przedimplantacyjnej biopsji igłowej nerek pobranych od jednego dawcy różnią się w ocenie histo-morfometrycznej?

Wprowadzenie: Przeszczepianie nerek jest metodą z wyboru w leczeniu chorych ze schyłkową ich niewydolnością. Czynność nerki przeszczepionej jest warunkowana między innymi zmianami morfologicznymi w przeszczepianym narządzie. Dotąd nie poznano jednak czy charakter i zaawansowanie zmian histo-morfometrycznych w jednej nerce odpowiada zmianom w drugiej nerce pobranej od tego samego dawcy.

Cele: Celem pracy była ocena: 1. różnic histo-morfometrycznych kłębuszków uzyskanych metodą przedimplantacyjnej biopsji nerek pobranych od jednego dawcy. 2. Pierwszo-rocznej i 3-letniej czynności nerek pobranych od jednego dawcy i przeszczepionych różnym biorcom w kontekście zmian histo-morfometrycznych kłębuszków, wpływu czasu niedokrwienia zimnego oraz niezgodności w układzie HLA między dawcą a biorcą.

Pacjenci i metody: 33 zmarłych dawców (13 K/20 M), od których pobrano,

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wykonano biopsję i zważono przed przeszczepieniem obie nerki zostało włączonych do badania. Mierzono czas niedokrwienia zimnego, obliczono częstotliwość natychmiastowego i opóźnionego podjęcia czynności przez nerkę przeszczepioną, współczynnik wagi nerki do wagi ciała biorcy oraz niezgodność w zakresie układu HLA między dawcą a biorcą. Analizie poddano liczbę kłębuszków kompletnych oraz odsetki kłębuszków prawidłowych, zwłókniałych całkowicie i segmentowo, z cechami hiperfiltracji oraz niedokrwienia. Obliczono liczbę komórek pasażerowych przypadających na kłębuszek, jak również gęstość kłębuszków i kapilar okołocewkowych na mm^2 powierzchni korowej oraz średnią objętość kłębuszków.

Wyniki Opóźnione podjęcie czynności przez nerkę przeszczepiono stwierdzono znamiennie częściej w grupie biorców z dłuższym czasem niedokrwienia zimnego (42,4% vs 27,3%; p<0,05). Nie stwierdzono istotnych różnic w zakresie masy oraz ocenianych zmian histo-morfometrycznych w kłębuszkach między obu nerkami pobranymi od jednego dawcy. Wykazano znamienną, ujemną korelację pomiędzy wiekiem dawcy a gęstością kłębuszków jak również między średnią objętością kłębuszka a gęstością kłębuszków, odpowiednio r= -0,269; p= 0,02 i r= - 0,274; p= 0,02. Stwierdzono dodatnią korelację między wiekiem dawcy a masą nerki (r= 0,380; p= 0,002) oraz między wiekiem dawcy a stężeniem kreatyniny w surowicy krwi po upływie pierwszego i trzeciego roku po przeszczepieniu, odpowiednio r= 0,323; p= 0,015 i r= 0,299; p= 0,038.

Wnioski: 1. Zmiany histo-morfometryczne w kłębuszkach uzyskanych metodą przedimplantacyjnej biopsji igłowej nerek pobranych od jednego dawcy "optymalnego" wykazują podobny charakter i zaawansowanie.

2. 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.

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Introduction

Kidney transplantation is regarded as treatment of choice for most patients with end stage renal disease. However, despite substantial progress in field of transplantology, long- term graft outcome is still unsatisfactory [1]. Kidney allograft outcome is influenced by the baseline status of transplanted organ as well as multiple immune and non-immune host versus graft interactions. Relationship between duration of cold ischemia time (CIT) and the incidence of DGF has been well established [2-5]. However, there is still no clear consensus concerning the influence of CIT-induced delayed graft function (DGF) on long- term graft function and outcome [2,3,6-8]. Recent studies of Kayler et al. [3] and summary of data by Terasaki [9] on paired kidneys (kidneys obtained from the same donor) have shown, that the differences in CIT (if CIT is shorter than 15h) have no influence on long-term graft outcome between longer- and shorter-CIT groups of paired kidneys. They conclude 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. [10] 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 the same 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 [11] and analysis of US Renal Data System [12] have found greater risk of graft failure in recipients with CITs>18h and continuous worsening of graft outcome associated with each 6 h 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 partially explain the differences in published results [3,9]. It is suggested that identification of objective morphological changes not only in post-transplant biopsies [13,14] but also in preimplantation biopsies [15,16] is useful to predict graft outcome. Because donor kidneys, usually do not display advanced chronic lesions [17], some have proposed to evaluate donor biopsies not only qualitatively but also quantitatively [18,19] or to employ a morphometric approach [20,21] which reduces the observer's interpretation variability. It is still the matter of debate, which lesions detected in pre-implantation biopsies could serve as the best predictors of graft outcome. 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 [22,23]. 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 [24]. Results of autopsy studies [24,25] 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 [25,26]. Calculation of glomerular number from biopsy material is not possible. However, it is though, that in these specimens estimation of glomerular density (GD) 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 autopsy kidneys of people without evidence of renal disease [24,25] and between GV and GD in patients with various forms of primary glomerulonephritis [27-29] suggest that glomerular hypertrophy is structural adaptation of glomeruli to its lower number. Signs of glomerular hypertrophy may also be a marker of subsequent renal injury and faster loss of function as well as worse response to therapy. Fogo at al. [30] documented in children with minimal change disease nephrotic syndrome (MCDNS), that the presence of glomerular hypertrophy in a biopsy obtained at diagnosis is an indicator of an increased risk of progression to focal segmental glomerulosclerosis (FSGS). Koike et al. [29] 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. [31] has been found that transplanted kidneys with higher GV characterized worse long term outcome in comparison to kidneys with lower GV. Therefore, variations in glomerular volume (size) 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) 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 idiopathic membranous nephropathy (IMN), IgA

nephropathy (IgAN) and MCDNS and low GD characterized more rapid progression in comparison to patients with high GD [27-29]. These results identified GD as an important histological predictor of kidney diseases progression. Therefore, the integrated analysis taking into consideration not only kidney function before its procurement but also its mass, histologic changes and morphometric characteristics at transplantation seems to be the optimal method for assessment of the basic status of transplanted kidney and should allow better prediction of graft outcome.

Taking into account the above mentioned facts, the value of pre-implantation kidney biopsy seems to be unquestionable. It is not known however, whether is it necessary to perform of pre-implantation biopsies in both kidneys of the same donor, or it will be enough to biopsy only one kidney.

This study aimed to assess: 1. Histomorphometric differences of glomeruli obtained by pre-implantation needle biopsies between kidneys obtained from the same donor. 2. 1- and 3-years graft outcome of paired kidneys transplanted into different recipients in context of histo-morphometric analysis, influence of cold ischemia time and donor-recipient HLA mismatch.

Patients and methods

To minimize peri-transplant factors influencing graft function and arising from local practices, only paired kidneys harvested and transplanted in a single center between January 1, 2005 and December 31, 2007 were included into the analysis. The kidneys with any macroscopic deformities (congenital or acquired) were excluded. After procurement, all the kidneys were stored in „Viaspam" solution until implantation, without machine perfusion. In 33 deceased donors with adequate biopsy material (according to Banff criteria - at least 7 glomeruli and 1 artery section), weight of both kidneys (KW) were measured. Kidney biopsies were performed on the back table after surgical preparation using semiautomatic needle „PRECISA" 16G x 200 mm (Italy). For measurement of kidney weight an electronic scale was used. Kidney weight measurements were performed with precision of 1g.

Kidney biopsies were fixed in 4% buffered formalin, processed and embedded in paraffin, then cut on 2-3 μm thick sections, and stained with hematoxillin and eosin (H&E), periodic acid-Schiff (PAS), Masson's trichrome, periodic acid silver metchenamine 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 [32] as well as cortico-medullary junction and negative or unspecific immunostaining underwent subsequent histo-morphometric analyses using „OLYMPUS BX51" microscope connected with „OLYMPUS CX50" camera (Olympus, Japan) and „cellSense Standard" software (Olympus, Japan).

Total number of complete (being encircled by non-interrupted Bowman's capsule except vascular and tubular poles) glomeruli, as well as numbers of normal (Norm), globally sclerosed (GS), segmentally sclerosed (SS), hyperperfused (Hyp) and ischemic (Isch) 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: i) evident increase in size in comparison to most surrounding glomeruli, ii) a dilated hilar capillary region in comparison to most surrounding glomeruli, substantialy wider than the lumen of it feeding afferent arteriole, usually with evident dilatation of the primary branches, iii) widely patent, often distended capillary loops, in the absence of increase in mesangium or any focal adhesions, sclerosis or podocyte alterations, iv) 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 sclerosed glomeruli characterized with no patent capillary loops [33]. Results were expressed as the percentage of complete glomeruli.

Numbers of passanger cells (neutrophils, monocytes/macrophages and CD-20 positive cells) were counted in each complete glomerulus and expressed as mean number per glomerulus.

Peritubular capillary density (PTCD) was assessed in the cortical part of biopsy after staining tissue sections with anti-CD34 antibody (DAKO; Germany). Results were expressed as the number of open peritubular capillary profiles per mm^2 of cortical area.

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

GV – glomerular volume (assuming that its 3-dimentional 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 sclerosed glomeruli per mm^2 of renal cortical area of needle biopsy.

At the time of kidney transplantation an information concerning age, gender, cause of death and the last eGFR of the donor as well as recipient age, gender, body weight (RBW) and cause of end stage renal failure, cold ischemia time (min), number of human leukocyte antygen (HLA) – A-B-Dr incompatibilities, immediate post-transplant graft function, initial immunosuppression protocol were collected. At the time of each follow-up (12-months and 36-months) graft function (eGFR) was evaluated. Recipients who lost their graft or died before the expiry of twelve months were not considered for the analysis.

Statistical analysis

Results were expressed as the median and 95% confidence interval (CI). 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

Donors population consisted of 33 deceased adults (13 F/20 M) from which paired kidneys were procured. In 16 cases intracranial hemorrhage was cause of death. Twelve donors died due to brain trauma and remaining five due to other reasons. Age of the whole donors group was 48 and 40.9–47.2 years (range 18-64). Last eGFR before kidney procurement in the whole group of donors was 69.7 ml/min (62.9 – 87.0) and combined weight of both kidneys between donors ranged two fold, from 248 g to 504 g. No correlation was observed between last serum creatinine concentration and donor age, kidney weight and histo-morphometric changes, respectively.

66 recipients (31 F/35 M) aged 45 (41.5–47.2) 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 33 patients (17 F/16 M) who received single of the paired kidneys with shorter CIT, and the second one consisted also of 33 recipients (13 F/20 M) of the remaining paired kidney's 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 (42.4% vs 27.3%; $p<0.05$). Recipients of paired kidneys did not differ significantly neither for KW/RBW ratio nor for HLA-incompatibility. Clinical characteristic of both groups summarizes table I.

Paired kidneys did not differ significantly according to weight, glomerular density, mean glomerular volume and peritubular capillary density (Table II).

Table III summarizes histological assessment of paired kidneys which did not revealed significant differences in percentage distribution of normal, globally and segmentally sclerosed as well as hyperperfused and ischemic glomeruli.

Median and 95% CI of glomerular neutrophils, monocyte/macrophages and CD 20 positive cells for the whole group of donors were 1.14 (1.14–1.61), 0.67 (0.76–1.25) and 0.00 (0.02–0.07), respectively and not significant differences were found between both of paired kidneys (Table IV).

At the end of the first year after trans-

plantation kidney graft function was assessed in 24 of the 33 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 theraphy, one due to surgical complications, two recipients lost to follow up due to transfer to other centers and in one case primary graft non-function was noted. 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 V, 1- and 3 – years eGFR did not differ significantly between paired kidneys.

Significant negative correlations have been observed between donors age and glomerular density as well as mean GV and GD, r= -0.269; p= 0.02 and r= - 0.274; p= 0.02, respectively.

Significant positive correlation between donor age and kidney weight (r= 0.380; p= 0.002) was noted as well as donor age and 1 – and 3-years creatinine concentrations, r= 0.323; p= 0.015 and r= 0.299; p= 0.038, 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 [20,36,37] 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 [16].

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 coretx [38] and may underestimate arterial- and glomerular sclerotic lesions in the cortico-medullary 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]. So, assessment of kidney quality and prediction of its outcome should not be based exclusively on glomerulosclerosis index.

Table I

Clinical characteristic of recipients of paired kidneys. Median and 95% CI.

Charakterystyka kliniczna biorców nerek pobranych od jednego dawcy. Mediana i 95% CI.

	Group I	Group II	P
Recipient age (years)	44 (41.5 – 49.5)	46 (38.9 – 47.4)	NS
CIT (min)	735 (674 – 945)	1095 (1020 – 1313)	p<0.05 (0.00065)
IGF/DGF	24/9 (72.7%/27.3%)	19/14 (57.6%/42.4%)	p<0.05
KW/RBW (g/kg)	2.47 (2.4 – 2.76)	2.77 (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

Abbreviations: CIT – Cold Ischemia Time, IGF – Immediate Graft Function, DGF – Delayed Graft Function, KW – Kidney Weight, RBW – Recipient Body Weight, HLA – Human Leukocyte Antigen

Table II

Weight, glomerular density, mean glomerular volume and peritubular capillary density of paired kidneys. Median and 95% CI.

Waga, gęstość kłębuszków nerkowych, średnia objętość kłębuszków nerkowych oraz gęstość kapilar okołocewkowych w nerkach pobranych od jednego dawcy. Mediana i 95% CI.

	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 (x10^{6}µm^3)	4.56 (4.16 – 5.22)	4.28 (3.02 – 8.19)	NS
PTCD (1/mm^2)	257.4 (223.4 – 270.8)	237.3 (216.8 – 254.1)	NS

Abbreviations: GD – Glomerular density, GV – Glomerular Volume, PTCD – Peritubular Capillary Density.

Table III

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

Procentowy rozkład kłębuszków prawidłowych, całkowicie i segmentowo zwłókniałych oraz z cechami hiperfiltracji i niedokrwienia w nerkach pobranych od jednego dawcy. Mediana i 95% CI.

	Kidney 1	Kidney 2	p
N. glom.	16.0 (15.1 – 23.9)	15.0 (13.2 – 17.2	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

Abbreviations: N. glom. – Number of complete glomeruli per biopsy, GGS – Global Glomerulosclerosis, SGS – Segmental Glomerulosclerosis, Hyp – Hyperperfused glomeruli, Isch – Ischemic glomeruli

Table IV

The numbers of neutrophils, monocyte/macrophages and CD20 positive cells per glomerulus in paired kidneys. Median and 95% CI.

Liczby neutrofili, monocytów/makrofagów oraz komórek CD20 dodatnich na kłębuszek w nerkach pobranych od jednego dawcy. Mediana i 95% CI.

	Kidney 1	Kidney 2	p
Neutrophils/glom.	1.00 (1.00 – 1.78)	1.22 (1.08 – 1.66)	NS
Monocyte/macrophages/glom.	0.87 (0.77 – 1.49)	0.60 (0.54 – 1.22)	NS
CD20 positive/glom.	0.00 (0.02 – 0.09)	0.00 (0.00 – 0.06)	NS

Table V

1- and 3-years graft function of paired kidneys. Median and 95% CI.

Pierwszoroczna i trzyletnia czynność nerek pobranych od jednego dawcy. Mediana i 95% CI.

	Kidney 1	Kidney 2	p
1-year eGFR ml/min (N=24)	51.7 (44.5 – 56.3)	37.0 (36.2 – 49.8)	NS
3-years eGFR ml/min (N=21)	52.84 (44.3 – 60.9)	44.8 (37.8 – 57.4)	NS

Abbreviations: eGFR – estimated Glomerular Filtration Rate.

Little is known concerning concordance of histological changes in kidneys harvested from the same donor and its outcome in this context. Pokorna et al. [41] 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.

For the reasons mentioned above, the importance of glomerulosclerosis examined in needle biopsies to assess basal status and predict long-term outcome of transplanted kidney harvested from younger donors remain very controversial. Therefore, it seems valuable to looking for early objective histological markers/changes which could serve as better predictors of both, the quality of transplanted kidney and long-term graft function.

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 [23,42]. 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 [24]. Results of autopsy studies [24,25] 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 [25,26], predictor of long-term gaft outcome connecting transplant nephron mass with structural changes [43-45]. In their study, Abdi et al. [46] and Alperovich et al. [31] have been 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.

The present study comprised 33 deceased donors from which 66 kidneys were evaluated. Significant positive correlation observed between donor age and kidney weight (r= 0.380; p= 0.002) confirms the results of other studies which comprised both pediatric and adult kidney donors and underscore the presence of physiologic relationship between age and kidney weight in studied population [44,47].

Median of complete glomeruli obtained in preimplantation kidney biopsies was 15.0 (14.25-18.45) which is similar to the values obtained in other studies [16,48] and did not differ significantly between paired kidneys**.** However, it should be stress, that these numbers are too low for accurate assessment of the percentage glomerulosclerosis [40] but sufficient for a reliable estimate of glomerular size by the MPA method [32].

Maximal glomerular volume was estimated from the maximal glomerular profile area (area inside the intact Bowman's Capsule containing tuft). The maximal glomerular volume measured for all pre-implantation biopsies was 4.51 μm³ (3.86–6.44) and no significant difference was found between paired kidneys. The variability in maximal glomerular volumes for all examined biopsies is high and ranged 3.44 fold. There were no significant differences concerning maximal glomerular volumes variability between paired kidneys.

Glomerular density for all examined pre-implantation biopsies was 2.92mm^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 mass and glomerular density in the whole group of donors (r= - 0.269; p= 0.02) and (r= - 0.386; p= 0.002), respective. These results confirm age-dependent glomerular number decrease observed by others and underscore the lack of universal positive correlation between kidney mass and glomerular density/number in adult patients. Additional, significant negative correlation observed between GD and GV in the whole group of preimplantation biopsies (r= -0.274; p=0.025) is in line with the previous observations, which considered GV not only as a surrogate measure of glomerular number/density [25,26] but also as poor predictor of long-term graft outcome [46]. Examined pre-implantation biopsies of paired kidneys did not differ significantly concerning the percentage of normal, globally and segmentally sclerosed as well as hyperperfused and ischemic glomeruli. There were also no significant differences in the number of glomerular passenger cells between biopsies of paired kidneys.

Delayed graft function defined as the need for hemodialysis in the first post-transplant week was observed in 33% of recipients and was more frequent in the recipients with longer cold ischemia time (27.3% vs 42.4%; 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 [2,3,6,9] 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.

In conclusion the present study revealed that:

1. Histo-morphometric glomerular changes observed in needle biopsies of paired kidneys harvested from optimal donors are equally advanced.

2. Needle biopsy of only one of paired kidneys is sufficient to assess the basal status of the organ and predict its outcome.

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