Histo-morphometric analysis of kidneys harvested from deceased donors. Important factor for the future outcome

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• gęstość kłębuszków
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• waga nerki
• biopsja przedimplantacyjna nerki

Introduction: Kidney transplantation is recognized as best treatment option for patients with chronic kidney disease stage 5. Long-term outcome of transplanted kidney may be determined among others by graft morphology.

Objectives: The aim of this study was to perform a preimplantation, histomorphometric analysis of glomeruli obtained by needle biopsies of kidneys harvested from deceased donors to assess the relationships between kidney weight , glomerular volume (GV) and glomerular density (GD).

Patients and methods: Of the 28 adult kidney donors, 56 biopsied and weighted at time of transplantation kidneys were included into the analysis. 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. Glomerular passenger cells (neutrophils, monocytes/macrophages and CD-20 positive cells) number per glomerulus were measured. Peritubular capillary density (PTCD) was expressed as the number of capillary profiles per mm² of cortical area. Mean glomerular volumes (MGV) were estimated from the maximal glomerular profile area according to the formula: GV = 4/3 π r³(μm³) and glomerular density (GD) was expressed as the number of non-globally sclerosed glomeruli per mm² of cortical area.

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= 0.001) 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).

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. These pretransplantation histo-morphometric findings may influence the future outcome of transplanted kidney.

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Ocena histomorfometryczna nerek pobranych od dawców zmarłych. Istotny czynnik rokowniczy

Wprowadzenie: Przeszczepianie nerek jest najlepszą metodą leczenia chorych ze schyłkową ich niewydolnością. Odległa czynność wydalnicza nerki przeszczepionej jest między innymi uwarunkowana zmianami morfologicznymi w przeszczepianym narządzie.

Cele: Celem pracy była analiza histo-morfometryczna kłębuszków uzyskanych metodą przedimplantacyjnej biopsji gruboigłowej nerek pobranych od dawców zmarłych i ocena zależności między wagą nerki a objętością (GV) i gęstością kłębuszków (GD).

Pacjenci i metody: Analizie poddano 56 nerek pobranych od 28 dorosłych dawców zmarłych. Przed przeszczepieniem dokonano pomiaru masy nerek oraz wykonano biopsję. Przeanalizowano liczbę kłębuszków kompletnych oraz odsetki kłębuszków prawidłowych (Norm), całkowicie zwłókniałych (GS), zwłókniałych segmentowo (SS), z cechami hiperfiltracji (Hyp) oraz niedokrwienia (Isch). Obliczono liczbę komórek pasażerowych (neutrofile, monocyty/makrofagi oraz CD20 dodatnie) przypadających na kłębuszek, jak również GD i liczbę kapilar okołocewkowych (PTCD) na mm² powierzchni korowej oraz średnią objętość kłębuszków (MGV).

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 wagą nerki a GD (r=

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-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= 0,44) oraz wagą nerki a odsetkiem SS (r= 0,261; p= 0,042).

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. Stwierdzone zmiany histo-morfometryczne w przeszczepianej nerce mogą być czynnikami determinującymi jej dalszą czynność.

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Introduction: Kidney transplantation is recognized as best treatment option for patients with chronic kidney disease stage 5. Both short- and long- term kidney allograft outcomes are influenced by multiple host versus graft interactions which are immune and non-immune in their nature. It was postulated by Seron et al. [1] and Isoniemi et al. [2], that histological changes in post-transplantation protocol biopsies can be useful in predicting long term graft outcome. Other studies [3,4] suggested, that identification of morphological changes detectable at the very early stages after transplantation or even in pre-implantation 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 display acute inflammatory lesions and the range of chronic lesions is even narrower than in protocol biopsies [5]. For these reason, some have proposed to evaluate donor biopsies with a very detailed semi-quantitative scale in order to differentiate groups in this range of lesions [6,7], while others have proposed to employ a morphometric methods [8,9]. Although more laborious and time consuming than conventional histologic evaluation, morphometric biopsy analysis reduces the subjectivity and variability in interpretation. Therefore, due to its laboriousity, it is important to know if histological changes available at transplantation provide only information concerning donor – derived organ injury or which lesions in pre-implantation biopsies could eventually allow the best prediction on outcome as well as whether the evaluation of damage in all renal compartments allow a better prediction of 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 [10,11]. 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 [12]. Glomerular number determine the adoptive capacity of grafted kidney to its new conditions after transplantation. 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 [12,13] 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 [13,14]. Native kidneys are heterogeneous according to glomerular volume and glomerular number. In the biopsy studies glomerular number calculation is not possible. It is thought that in these specimens estimation of glomerular density is a marker of glomerular number. An inverse correlation between GV and total glomerular number noted in autopsied kidneys of people without evidence of renal disease [12,13] and between GV and glomerular density (GD) in IgA Nephropathy (IgAN) [15], Idiopathic Membranous Nephropathy (IMN) [16] and Minimal Change Disease Nephrotic Syndrome (MCDNS) [17]. GV in these 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 at al. [18] documented in children with 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. [17] 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. [19] 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) 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 >60ml/min). Patients with IMN, IgAN and MCDNS and low GD characterized more rapid progression in comparison to patients with high [15-17]. These results identified GD as an important histological predictor of kidney diseases progression. Therefore, the integrated analysis taking into consideration not only global kidney function before its procurement but also its mass, histologic changes and morphometric characteristics at transplantation may allow better prediction of graft outcome.

The objective of this study was to perform a pre-implantation histo-morphometric analysis of glomeruli obtained by needle biopsies of paired kidneys harvested from adult deceased donors.

Patients and methods

To exclude most peri-transplant differences influencing graft function and arising from local practices, only donors whose kidneys did not present any macroscopic deformities (congenital or acquired) harvested 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 measured. Kidney biopsies were performed on the back table after surgical preparation using semiautomatic needle „PRECISA" 16G x 200mm (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 leats 7 glomeruli and 1 arterial section (measurement of six glomeruli provides a reliable estimate of glomerular size by the MPA method [20]) 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" softwere (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 sclerotic glomeruli characterized with no patent capillary loops [21]. Results were expressed as the percentage of complete glomeruli. Numbers of passanger cells (neutrophils, monocytes/macrophages and CD20 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 mm² 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 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-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 sclerotic glomeruli per mm² 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. A p value < 0.05 was considered as statistically significant.

Results

Median of age and 95% CI of the whole donors group was 44.1 and 40.9 – 47.2 years (range 18-64). Last serum creatinine concentration (median and 95% CI) before kidney procurement in the donors was 1.47mg% (1.14 – 1.81) and weight of kidneys between donors ranged two fold, from 248g to 504g. In 16 cases intracranial hemorrhage was cause of death. Remaining twelve donors died due to brain trauma. Significant positive correlation between donor age and kidney weight (r= 0.390; p= 0.001) 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.

Median and 95% CI of complete glomeruli in pre-implantation kidney biopsies for donors was 17.35 (14.95-19.74). The percentage distribution of normal, globally and segmentally sclerosed as well as hyperperfused and ischemic glomeruli in pre-implantation 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).

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 pre-implantation biopsies was 5.15 x 10^6 μm^3 (3.86 – 6.44). The variability in MGV in whole group of donors ranged 3.74 fold, from 1.86 x 10^6 μm^3 to 6.97 x 10^6 μm^3. GD and PTCD both in the pre-implantation biopsies for the whole group of donors were 3.09/mm² (2.79 – 3.38) and 241.27/mm² (226.51 – 256.02), respectively. A significant negative correlation was found between GD and MGV (r= -0.31; p=0.017).

The numbers of neutrophils, monocyte/macrophages and CD20 positive cell numbers per glomerulus were 1.38 (1.14 – 1.61), 1.01 (0.76 – 1.25) and 0.05 (0.02 – 0.07), respectively.

Discussion

A wide diversity of histological damage has been found in studies of subjects older than 50 years without history of renal disease or arterial hypertension and among organ donors with major comorbidities and risk factors [22]. Therefore, histological assessment of pre-implantation biopsies not only permits single recording of chronic lesions but also could help to assess organ quality, properly alocate harvested organs and predict short- and long-term outcomes of renal allografts [23]. It has been shown, that chronic injury observed in pre-implantation biopsies of kidneys from deceased brain-dead, older and extended criteria donors, comprised interstitial [8], vascular [24] and glomerular [25] compartments or combinations of these lesions and correlated with the incidence of DGF, short- and long-term kidney function and survival.

Due to its simplicity for evaluation, global glomerulosclerosis is the most frequent glomerular change used in clinical studies. However its meaning and interpretation is worth of comment. Glomerulosclerosis is not very advanced process in most kidneys hervested from younger donors without significant comorbidities and risk factors. Global glomerulosclerosis is not an active lesion but should be interpreted as a consequence of aging process or pre-existed donor kidney disease, which has no impact

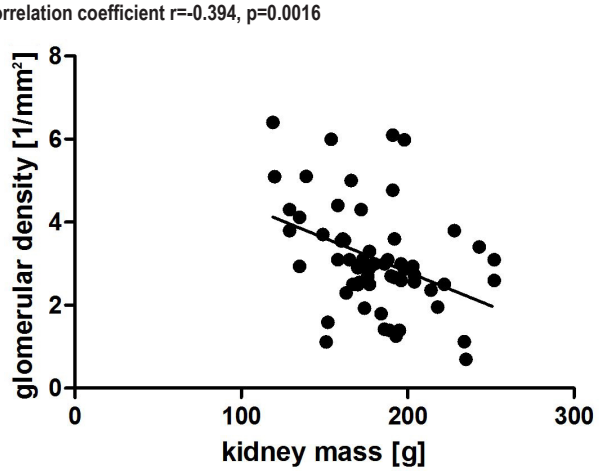

Figure 1
Correlation between kidney mass and glomerular density in donors group.
Korelacja między wagą nerki a gęstością kłębuszków nerkowych w grupie dawców.

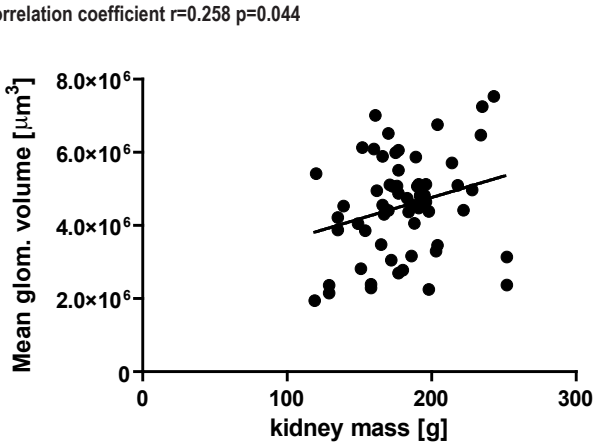

Figure 2
Correlation between kidney and mass mean glomerular volume in donors group.
Korelacja między wagą nerki a średnią objętością kłębuszków nerkowych w grupie dawców.

Correlation coefficient r=0.261, p=0.0421

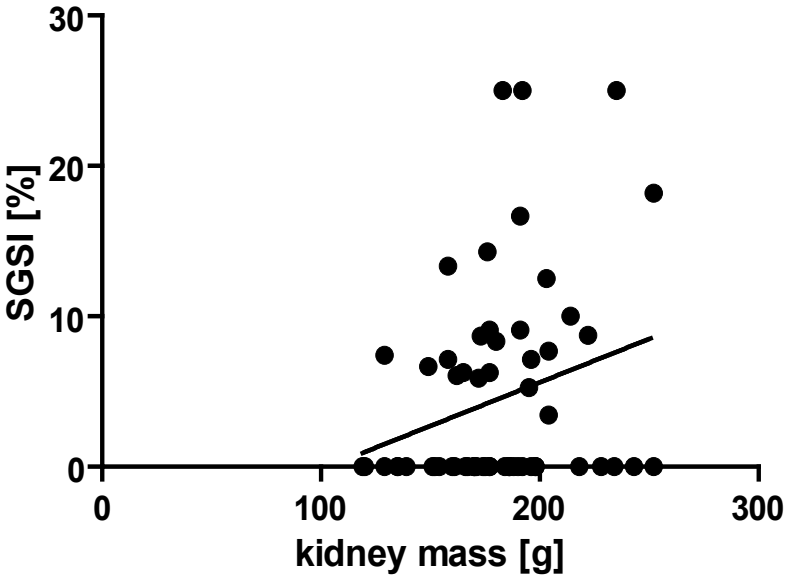

Figure 3
Correlation between kidney mass and percentage of segment glomerulosclerosis in donors group.
Korelacja między wagą nerki a odsetkiem kłębuszków nerkowych z cechami włóknienia segmentowego w grupie dawców.

on kidney functional reserve at the time of transplantation. Additionaly, most of the studies to date uses wedge biopsies to obtain kidney tissue samples for histological assessment . However, superficial wedge biopsies overestimate the percentage of sclerosed glomeruli as these are more frequently found in the subcapsular part of the cortex [26] and may underestimate arterial- and glomerular sclerotic lesions in the cortico-medullary junction of the kidney [27]. Furthermore, the sample size of renal biopsies is an important determinant of accurate assessment of the percentage of glomerulosclerosis in the kidney [28]. So, it seems reasonable, that the quality of kidneys for transplantation and prediction of its long-term outcome should not be assessed based exclusively on glomerulosclerosis index.

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 [29]. 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.

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 [11,10]. 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 [12]. Results of autopsy studies [12,13] 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 [13,14], predictor of long-term graft outcome connecting transplant nephron mass with structural changes [30-32]. In their study, Abdi et al. [33] and Alperovich et al. [19] 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.

In the present study 56 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 [31].

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 [10,34]**.** However, according to Corwin et al. [28] these numbers are somewhat to 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 [20]. Index of globally sclerotic glomeruli in the studied population of donors was 3.84% (1.97–5.71) median nad 95% CI, which is close to the results published by Hoy WE et al. [11].

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 [20]. Due to strong correlation between the method of profile area (MPA) with the Cavalieri method considered the gold standard [20], 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 µm³ (3.86–6.44). The variability in mean glomerular volumes in whole group of donors ranged 3.74. Mean GV correlated positively with kidney weight in the whole group of donors .

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 maxGV 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 [13,14] but also as poor predictor of long-term graft outcome [33]. 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.

In conclusion the presented study revealed that: 1. Mean glomerular volume in kidney biopsy may serve as surrogate marker of glomerular number. 2. Negative relationhsips 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. These pretransplantation histo-morphometric findings may influence the future outcome of transplanted kidney.

References

1. **Seron D, Mordeso F, Bower J, Condom E, Gil-Vernet S. et al**: Early protocol renal allograft biopsies and graft outcome. Kidney Int. 1997; 51: 310-316.
2. **Isoniemi H, Taskinen E, Harry P**: Histological chronic allograft damage index accurately predicts chronic renal allograft rejection. Transplantation 1994; 58: 1195-1198.
3. **Munivenkatappa RB, Schweitzer EJ, Papadimitriou JC, Drachenberg CB, Thom KA. et al**: The Maryland aggregate pathology index: a deceased donor kidney biopsy scoring system for predicting graft failure. Am J Transplant. 2008; 8: 2316-2324.
4. **Snoeijs MG, Buurman WA, Christiaans MHL, van Hoff JP, Goldschmeding R. et al**: Histological assessment of preimplantation biopsies may improve selection of kidneys from old donors after cardiac death. Am J Transplant. 2008; 8: 1844-1851.
5. **Lopes JA, Poreso F, Riera L, Carrera M, Ibernon M. et al**: Evaluation of pre-implantation kidney biop-

sies: Comparison of Banff criteria to a morphometric approach. Kidney Int. 2005; 67: 1595-1600.
6. **Karpinski J, Lajoie G, Cattran D, Fenton S, Zaltzman J. et al**: Outcome of kidney transplantation from high-risk donors is determined by both structure and function. Transplantation 1996; 67: 1162-1167.
7. **Lehtonen SR, Taskinen EI, Isoniemi HM**: Histopathological findings in renal allografts at time of transplantation and correlation with onset of graft function. APMIS 1999; 107: 945-950.
8. **Seron D, Carrera M, Grinyo JM, Castelao AM, Lopez-Costea MA. et al**: Relationship between donor renal interstitial surface and post-transplant function. Nephrol Dial Transplant. 1993; 8: 539-543.
9. **Mingxi Li, Nicholls KM, Becker GJ**: Risk factor for late renal allograft dysfunction: Effects of baseline glomerular size. J Nephrol. 2002; 15: 620-625.
10. **Puelles VG, Hoy WE, Hughson MD, Diouf B, Douglas-Denton R. et al**: Glomerular number and size variability and risk for kidney disease. Curr Opin Nephrol Hypertens. 2011; 20: 7-15.
11. **Hoy WE, Bertram JF, Douglas-Denton R, Zimanyi M, Samuel T. et al**: Nephron number, glomerular volume, renal disease and hypertension. Curr Opin Nephrol Hypertens. 2008: 17: 258-265.
12. **Keller G, Zimmer G, Mall G, Ritz E, Amman K. et al**: Nephron number in patients with primary hypertension. N Engl J Med. 2003; 348: 101-108.
13. **Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K. et al**: A stereological study of glomerular number and volume. Preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int. 2003; 63(Suppl 83): S31-S37.
14. **Fulladosa X, Moreso F, Narvaez JA, Narvaez JA, Grinyo JA. et al**: Estimation of total glomerular number in stable renal transplants. J Am Soc Nephrol. 2003; 14: 2662-2668.
15. **Tsuboi N, Kawamura T, Koike K, Okonogi H, Hirano K. et al**: Glomerular density in renal biopsies specimens predicts the long term prognosis of IgA nephropathy. Clin J Am Nephrol. 2010; 5: 39-44.
16. **Tsuboi N, Kawamura T, Miyazaki Y, Utsunomiya Y, Hosoya T**: Low glomerular density is a risk factor for progression in idiopathic membranous nephropathy. Nephrol Dial transplant. 2011; 26: 3555-3560.
17. **Koike K, Tsuboi N, Utsunomiya Y, Kawamura T, Hosoya T**: Glomerular density-associated changes in clinicopathological features of minimal change nephrotic syndrome in adults. Am J Nephrol. 2011; 34: 542-548.
18. **Fogo A, Hawkins EP, Berry PL, Glick AD, Chiang ML. et al**: Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal segmental glomerular sclerosis. Kidney Int. 1990; 38: 115-123.
19. **Alperovich G, Maldonado R, Moreso F, Fulladosa X, Grinyo JM. et al**: Glomerular enlargement assessed by paired donor and early protocol renal allograft biopsies. Am J Transplant. 2004; 4: 650-654.
20. **Lane PH, Steffes MW, Mauer SM**: Estimation of glomerular volume: a comparison of four methods. Kidney Int. 1992; 41: 1085-1089.
21. **Hill GS, Heudes D, Jacquot C, Gauthier E, Bariety J. et al**: Morphometric evidence for impairment of renal autoregulation in advanced arterial hypertension. Kideny Int. 2006; 69: 823-831.
22. **Kaplan C, Pasternack B, Shah H, Gallo G**: Age-related incidence of sclerotic glomeruli in human kidneys. Am J Pathol. 1975; 80: 227-234.
23. **Mengel M, Sis B**: An appeal for zero-time biopsies in renal transplantation. Am J Transplant. 2008; 8: 2181-2182.
24. **Bosmans JL, Woestenburg A, Ysebaert DK Capelle T, Helbert MJ. et al**: Fibrous intimal thickening at implantation as a risk factor for the outcome of cadaveric renal allografts. Transplantation 2000; 69: 2388-2394.
25. **Gaber LW, Moore LW, Alloway RR, Amiri HH, Vera SR**: Glomerulosclerosis as a determinant of post-transplant function of older donor renal allografts. Transplantation 1995; 60: 334-339.
26. **Muruve NA, Steibecker KM, Luger AM**: Are wedge biopsies of cadaveric kidneys obtained at procurement reliable? Transplantation 2000; 69: 2384-2388.
27. **Nickeleit V**: Donor biopsy evaluation at time of renal grafting. Nat Rev. 2009; 5: 249-251.
28. **Corwin HL, Schwartz MM, Lewis EJ**: The importance of sample size in the interpretation of renal biopsy. Am J Nephrol. 1988; 8: 85-89.
29. **Solez K, Colvin RB, Racusen LC, Hass M, Sis B. et al**: Banff 07 classification of renal allograft pathology: Updates and future directions. Am J Transplant. 2008; 8: 753-760.
30. **Kim YS, Moon JI, Kim DK, Kim SI, Park K**: Ratio of donor kidney weight to recipient body weight as an index of graft function. Lancet 2001; 357: 1180-1181.
31. **Giral M, Nguyen JM, Karam G, Kessler M, Hurault de Ligny B. et al**: Impact of graft mass on the clinical outcome of kidney transplants. J Am Soc Nephrol. 2005; 16: 261-268.
32. **Nankivell BJ, Fenton-Lee CA, Kuypers DR, Cheung J, Allen RD. et al**: Effect of histological damage on long term kidney transplant outcome. Transplantation 2001; 71: 515-523.
33. **Abdi R, Slakey D, Kittur D, Budrick J, Racusen L**: Baseline glomerular size as a predictor of function in human renal transplantation. Transplantation 1998; 66: 329-333.