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IMPORTANCE AND POTENTIAL APPLICATION OF MORPHOMETRIC ANALYSIS OF HUMAN GLOMERULI IN CADAVERIC KIDNEY TRANSPLANTATION

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Cadaveric kidney transplantation is on the constant rise due to decreased mortality of younger individuals. In these transplantations, it is of great importance to determine not only the age limit of recipients, but also the status of donors.

This investigation included 30 tissue samples of human cadaveric kidneys (both genders, aged 20-85). Tissue samples were stained with Mallory's trichrome stain and analyzed by a light microscope. Images were analyzed using ImageJ software. As a result of cluster analysis, 743 glomeruli were classified into 3 groups by morphometric characteristics and into 3 age groups (I with average age of 29, II with 44, III with average of 71 years old). By morphometric characteristics, there were 114 sclerotic glomeruli with the significantly ($p \leq 0.0001$) smallest area and cellularity, and the highest connective tissue percentage in the first group. There were 430 morphologically normal glomeruli with the greatest number of cells/area unit in the second group ($p \leq 0.0001$). In the third group, there were 199 hypertrophic glomeruli with the greatest area, significantly large cellularity and connective tissue area ($p \leq 0.0001$). Out of 114 sclerotic glomeruli, the smallest number belonged to I age group ($p \leq 0.0001$). There were 430 morphologically normal glomeruli in total. Most of them were in II age group ($p \leq 0.0001$). Most of 199 hypertrophic glomeruli were in III age group vs. other two ($p \leq 0.0001$), as well as in II vs. I ($p \leq 0.0001$). Morphometric analysis of morphologically normal glomeruli should be of the greatest importance for transplantation, and not only the assessment of their total number and number of detected manifestly sclerotic glomeruli.

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Key words: *human glomeruli, kidney transplantation, morphometry*

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Introduction

Kidney transplantation in older patients is associated with an array of ethical dilemma. Previous studies have shown that the older patients in the end stage renal disease gain significant benefit from kidney transplantation (1-3). Relative statistical risk of graft rejection is similar either in patients below or over 65 years of age and it mostly depends on the presence of associated diseases which, in the older patients, often may lead to graft rejection and fatal complications (4). Therefore, the older kidney transplant candidates should be carefully screened for can cer, cardiovascular diseases, peripheral vasculopathy, diabetes mellitus, in order to minimize the risk of early post-transplant morbidity and mortality (3). Apart from age limit of the recipient, it is of great importance to determine the status and age limit of the donor as well, particularly in cases of cadaveric transplantation. There are no age limits for the kidney transplant procedure in the USA currently, which is reflected in the fact that the kidneys of donors over 65 years of age make 13% of total cadaveric kidney transplant number. Due to the lower mortality of young indivuduals, the mean age of cadaveric kidney donors is increasing as well (1). Expanded criteria kidney donor include those either from a brain-dead donor over 60 years of age, or a donor 50 to 59 years of age with at least two of the following features: history of hypertension, terminal serum creatinine > 1.5 mg/dL, or cerebrovascular cause of death. In accordance with it, there are two kidney transplant waiting lists in the USA: one with standard and one with expanded criteria donors.

Expanded criteria donor list is recommended for older recipients with renal failure as the main cause of the disease, as well as for those with difficult vascular access. Kidneys donated by expanded criteria make 17% of all donated kidneys in the USA (5). Eurotransplant Senior Program allows kidney transplantation from donors over 65 years of age to a selected group of nonimmunized 65+ patients undergoing their first transplant.

Kidney ageing process is associated with various factors, such as cytokines, growth factors, proliferation, apoptosis, transcription factors, advanced glycation end products (AGEs) (6-8). Genetic mutations, diet, and living conditions appear to have a key role in ageing process as well (9). Age-related alterations in the kidney are similar to those verified in chronic renal diseases and experimental models with chronic renal failure.

With the medulla being relatively spared, loss of renal mass is primarily expressed in the renal cortex, which is confirmed by the studies that found that half of the total nephron number might be lost till ripe old age.

Age-related increase in sclerotic glomeruli number is empirically confirmed by the clinical data and may be found in many studies. Within the glomeruli, mesangial matrix is progressively being expanded, the arterioles are hyalinized, and while the number of renal corpuscles is decreasing, their area is increasing (10, 11).

Changes associated with the ageing of human glomeruli are progressive decrease of the number of glomeruli, which is directly related to the birth weight, existence of shunts between afferent and efferent arterioles, mesangial matrix expansion followed by the onset of glomerusclerosis, and increased number of globally sclerotic glomeruli (12-17). The key question is: what is the nature of glomerular damage mechanisms, are they immunologically (immune complex accumulation) or non-immunologically associated with hemodynamic factors? From the majority of researchers' standpoint, as pathological substrates we may consider mesangial matrix expansion due to the collagen deposits, vascular changes, and glomerular inflammation caused by immunological mechanisms (18-20).

The aim of our research was to differentiate manifestly sclerotic glomeruli from hypertrophic ones and morphologically non-sclerotic ones, to quantify the presence of mesangial connective tissue and number of glomeruli during ageing, and to investigate the importance of these changes in clinical practice.

Materials and methods

The material was human right kidney tissue of 30 cadavers, obtained during routine autopsies at the Institute of Forensic Medicine in Niš. Their age ranged from 20 to 85 years. During autopsy, kidney damage or congenital anomalies were not observed. Cadavers were without previously diagnosed kidney disease, diabetes, hypertension, or any other systemic disease. Tissue specimens were fixed in 10% buffered formalin for 12 hours and then embedded in paraplast. The tissue was then cut into 5 μm thick sections and routinely stained with Mallory's trichrome stain. Histological slices were analyzed under 400x magnification. Images of histological slices were captured with digital camera (5 megapixels resolution).

Glomeruli were analyzed with ImageJ software (http://rsbweb.nih.gov/ij/) which was spatially calibrated with object micrometer (1:100). The glomerular tuft area (A_G), perimeter (B_G), diameter along main (D_M) and secondary axis (D_m), Feret's diameter (D_F), glomerular connective tissue area (A_{CT}), percentage of connective tissue (CT%) and total number of cells per glomerular area unit (N_n) were measured. Glomerular images were additionally processed for connective tissue area measurement. Glomerular tuft image was first manually selected by polygonal selection tool and extracted from the other parts of histological slice image. Selection of its connective tissue, which was green stained on Mallory's trichrome stained sections, was performed by "Color based tresholding" option. Its application was based on green colored sample of glomerular tuft image. Afterwards, only green stained parts of glomerular tuft remained on image, which was further converted into a binary image. The binary image was used for connective tissue area measurement. Green colored samples were taken at three different localizations in each glomerular tuft image. Connective tissue area was measured for each sample. Average connective tissue area was then calculated from three obtained values for each glomerular tuft. Glomerular connective tissue percentage was obtained from the ratio between glomerular connective tissue area and total glomerular area. Seven cortical, seven columnar and seven juxtamedullary glomeruli were analyzed per one case. Additionally, no more than seven globally sclerotic glomeruli were also analyzed per one case. They served as a positive control during morphometric analysis. Totally, 743 (114 sclerotic and 629 morphologically nonsclerosed) glomeruli were analyzed in all 30 cases. Average values of morphometric parameters were calculated for each of all 30 evaluated cases.

Statistical analysis was performed with NCSS-PASS software (http://www.ncss.com/). Cluster analysis by the k-means method was performed for the classification of glomeruli into age groups according to their morphometric characteristics. One-way ANOVA was used for the comparison of more than two groups. In cases where data did not have normal distribution, Kruskal-Wallis One-way ANOVA was used for the comparison of more than two groups. Statistical significance test was performed for $p < 0.05$.

Cluster analysis was performed twice during this study. Firstly, it was used for the classification of glomeruli into types according to their morphometric characteristics and secondly, for the classification of the evaluated human cases into the groups, according to the percentage of obtained types of glomeruli and their age.

Results

After morphometric analysis of a total of 743 glomeruli, three groups of glomeruli were made. The first group included 114 glomeruli with the lowest values of area, cellularity, and greatest percentage of connective tissue. There were 430 morphologically normal glomeruli with the largest number of cells per area unit in the second group. The third group included 199 glomeruli with the largest area, significant cellularity and percentage of connective tissue (Table 1). All investigated morphometric parameters of glomeruli showed statistically significant alterations (Table 2).

Table 1. Morphometric characteristics of glomeruli groups classified by the cluster analysis

	A_G (μm²)		B_G (μm)		D_M (μm)		D_m (μm)	
Cluster	I (n = 114)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	6712.36	6520.95	302.39	302.31	102.73	100.87	81.97	82.38
SE	173.86	/	3.85	/	1.41	/	1.20	/
95% LCL	6367.90	6148.01	294.76	289.59	99.93	98.80	79.60	78.91
95% UCL	7056.82	7013.99	310.01	314.58	105.53	106.53	84.35	85.87
Cluster	II (n = 430)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	14618.41	14154.06	453.37	450.69	152.07	150.43	121.43	120.54
SE	154.99	/	2.80	/	0.95	/	0.74	/
95% LCL	14313.77	13825.43	447.86	442.54	150.19	148.05	119.99	118.65
95% UCL	14923.05	14697.38	458.87	457.68	153.95	152.03	122.88	121.84
Cluster	III (n = 199)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	22478.81	21894.61	564.24	556.76	189.36	185.69	149.84	149.53
SE	314.27	/	4.69	/	1.53	/	1.30	/
95% LCL	21859.05	21188.06	554.99	546.10	186.36	183.13	147.27	146.19
95% UCL	23098.56	22664.15	573.50	568.77	192.37	189.62	152.40	151.94

	D_F (μm)		A_{CT} (μm²)		CT%		N_n (1/μm²) x 10^{-3}	
Cluster	I (n = 114)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	107.03	105.17	4761.58	4774.59	71.10	71.77	1.7	1.6
SE	1.44	/	128.66	/	0.53	/	0.1	/
95% LCL	104.18	102.06	4506.69	4273.92	70.04	69.86	1.6	1.5
95% UCL	109.87	110.44	5019.23	5019.23	72.16	73.24	1.8	1.8
Cluster	II (n = 430)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	158.87	157.14	3576.19	3591.79	25.26	25.70	6.8	6.9
SE	0.96	/	47.80	/	0.37	/	0.1	/
95% LCL	156.98	154.64	3482.23	3437.98	24.52	24.76	6.7	6.7
95% UCL	160.75	160.00	3670.15	3739.07	25.99	26.11	6.9	7.0
Cluster	III (n = 199)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	197.22	193.01	6774.02	6406.87	30.66	31.49	5.9	5.9
SE	1.58	/	115.15	/	0.45	/	0.1	/
95% LCL	194.11	189.62	6546.94	6207.42	29.76	30.54	5.7	5.8
95% UCL	200.33	197.60	7001.10	6588.96	31.55	32.46	6.0	6.1

Md – median,
SE – standard error,
95% LCL – lower limit of confidence interval,
95% UCL – upper limit of confidence interval

Table 2. Results of One Way ANOVA test of morphometric characteristics of the glomeruli classified into groups

Parameter	One-Way ANOVA			Kruskal-Wallis One-Way ANOVA	
	F	p	Power	H	p
A_G *	799.64	≤ 0.0001	1.00	503.66	≤ 0.0001
B_G *	740.03	≤ 0.0001	1.00	478.13	≤ 0.0001
D_F *	748.78	≤ 0.0001	1.00	469.26	≤ 0.0001
D_M *	712.63	≤ 0.0001	1.00	464.30	≤ 0.0001
D_m *	673.00	≤ 0.0001	1.00	449.39	≤ 0.0001
A_{CT} *	445.72	≤ 0.0001	1.00	416.66	≤ 0.0001
CT% *	1886.46	≤ 0.0001	1.00	337.67	≤ 0.0001
N_n *	1345.94	≤ 0.0001	1.00	365.70	≤ 0.0001

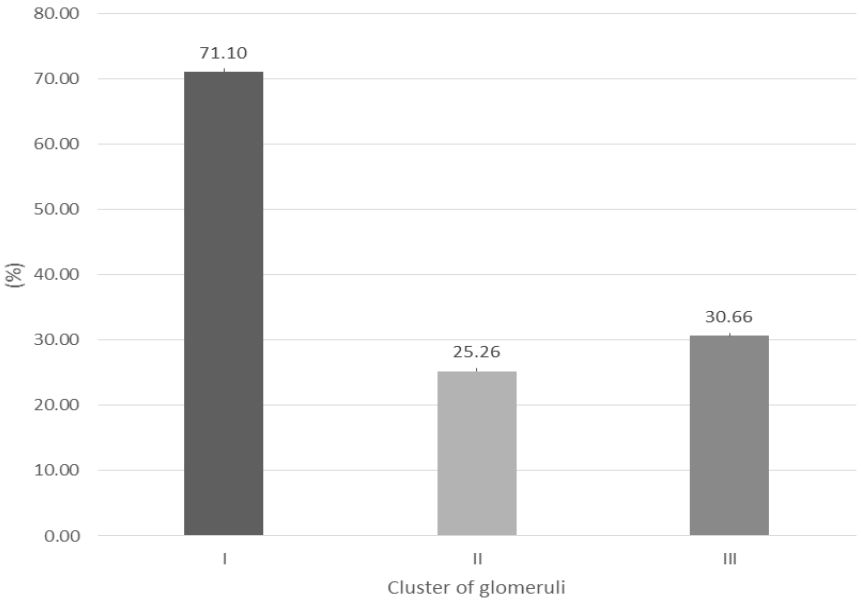
* $p < 0.05$ – positive D'Agostino-Pearson Omnibus normality test

The average values of area, perimeter, diameter along main and secondary glomerular axis, Feret's diameter are considered as morphometric parameters which describe the size and form of the glomerulus.

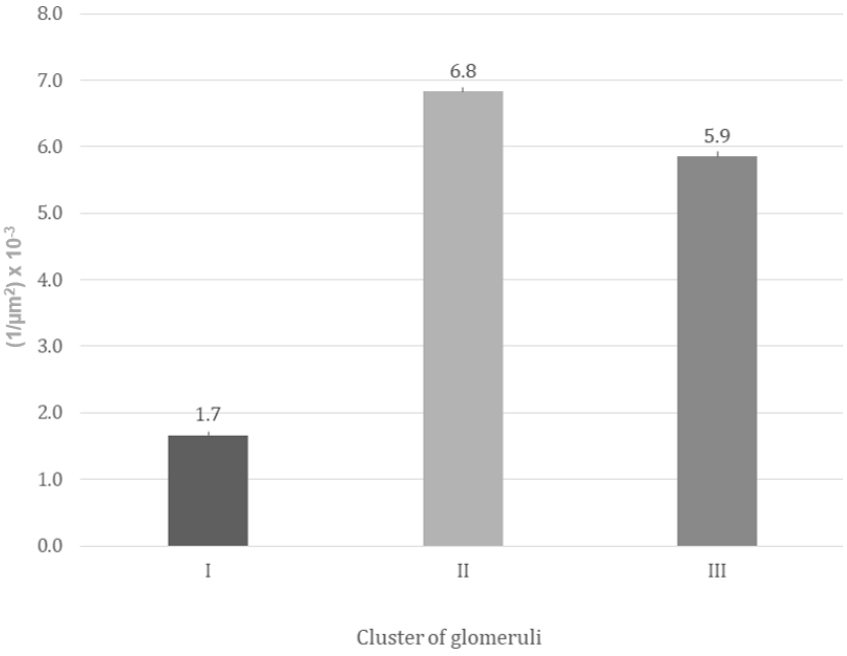
The average area of glomeruli in the first group was 6712.36 μm^2 and it was significantly ($p \leq 0.0001$) lower compared to the second and third group. The mean area in the third group was 22478.81 μm^2, which was a statistically significant increase in comparison with the first and second groups of glomeruli (Table 1; Table 2). The average area (4761.58 μm^2) and percentage (71.1%) of connective tissue within glomeruli had the greatest values in the first group. There was a statistically significant decrease in connective tissue area in the second group vs. the first group (3576.19 μm^2, $p \leq 0.0001$), which made 25.26% of the total glomerular connective tissue in group II. In the third group of glomeruli, the mean connective tissue area was 6406.87 μm^2, or 30.66% of the total glomerular connective tissue, which was significantly lower ($p \leq 0.0001$) vs. the first group (Table 1, Graph 1).

Mean perimeter, diameter along main and secondary glomerular axis, Feret's diameter were statistically significantly lowest in group I of glomeruli ($p \leq 0.0001$). Their values were statistically significantly increasing towards the third group (Table 1, 2).

The average cell nuclei number per area unit of the investigated glomeruli showed the largest increase in group II of glomeruli. It was significantly higher ($p \leq 0.0001$) vs. the first group, where it had the lowest values, as well as vs. the third group of glomeruli (Table 1, Graph 2).



Graph 1. Mean glomerular connective tissue percentage in the group of glomeruli classified by the cluster analysis



Graph 2. Mean cellularity in the group of glomeruli classified by the cluster analysis

According to the changes in morphometric parameters, it is evident that morphologically sclerotic glomeruli, which are the smallest and with highest connective tissue percentage, are present in the first group. Morphologically normal glomeruli in the second group are characterized by a higher cell number and the lowest connective tissue percentage. In the third group, there are morphologically normal glomeruli with the largest area, decreased cell number per are unit, and increased connective tissue percentage, which suggests the presence of hypertrophy.

Further analysis was performed to investigate the representation of the aforesaid group of glomeruli within the age groups. The first group consisted of the youngest cases, six in total, who were aged 24-33 years, average 29. Eleven older cases, aged 40-49 years, average 44, were in the second group. In the third group there were 13 oldest cases aged 65-76 years, average 71 (Table 3). Out of 114 sclerotic glomeruli only two were identified in the first age group, which was significantly lower ($p \leq 0.0001$) than in the second (37 glomeruli) and the third age group (75 glomeruli). There were 430 morphologically normal glomeruli, most of them being in the second age group (162), which was significantly higher number of glomeruli ($p \leq 0.0001$) than in the first (124) and third age groups (144). We found 199 hypertrophic glomeruli, only 2 of them classified in first age group, 69 of them in the second age group, which was significant increase vs. the first group ($p \leq 0.0001$), and 128 glomeruli in the third age group, being statistically significantly higher vs. both younger groups ($p \leq 0.0001$) (Table 4).

These data show a significant increase in the number of sclerotic and hypertrophic glomeruli during ageing, particularly in age group II and III, while morphologically normal glomeruli are most frequent in the first and second age groups. It is at the age of 40-49 when the first changes appear, followed by hypertrophy and glomerulosclerosis, intensifying with age and being the most prominent in the oldest ones.

Gained and expected distribution of clusters of glomeruli within age groups classified by cluster analysis are statistically significantly different ($\chi^2 = 118.91$, d.f. = 4, $p \leq 0.0001$).

Table 3. Age groups of the investigated cases

	Age		**A_G (μm²)**		**B_G (μm)**		**D_M (μm)**		**D_m (μm)**	
Cluster	I (n = 6)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	29	27	15605.61	15693.20	463.64	465.11	154.99	157.53	126.43	127.08
SE	2	/	1157.63	/	17.16	/	6.32	/	4.61	/
95% LCL	24	25	12629.84	11055.55	419.53	393.21	138.73	128.21	114.57	108.91
95% UCL	33	34	18581.39	19991.87	507.76	521.23	171.25	176.25	138.28	144.30
Cluster	II (n = 11)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	44	45	15982.37	14524.17	466.58	439.88	157.16	148.42	125.03	122.35
SE	2	/	1299.80	/	18.04	/	6.64	/	4.61	/
95% LCL	40	38	13086.23	12873.85	426.38	416.36	142.37	137.36	114.75	112.19
95% UCL	49	50	18878.51	17306.07	506.79	490.13	171.95	166.27	135.30	129.78
Cluster	III (n = 13)									
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	71	71	15340.53	14772.57	456.81	462.46	153.44	152.87	121.01	119.92
SE	2	/	1071.91	/	15.08	/	4.51	/	4.31	/
95% LCL	65	63	13122.69	12551.79	423.97	414.25	143.61	140.12	111.61	109.28
95% UCL	76	78	17558.37	19091.36	489.66	516.56	163.27	170.55	130.40	138.91

	D_F (μm)		**A_{CT} (μm²)**		**CT%**		**N_n (1/μm²) x 10^{-3}**	
Cluster	I (n = 6)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	161.47	164.43	2601.18	2451.77	17.53	15.16	6.9	7.1
SE	6.24	/	133.16	/	2.28	/	0.2	/
95% LCL	145.41	134.60	2258.87	2336.03	11.67	14.69	6.4	6.2
95% UCL	177.52	181.76	2943.48	3012.00	23.38	28.86	7.5	7.4
Cluster	II (n = 11)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	163.44	155.02	4468.52	4280.17	32.11	33.48	6.1	6.0
SE	6.72	/	213.61	/	1.42	/	0.2	/
95% LCL	148.46	144.63	3992.57	3761.00	28.96	30.41	5.7	5.6
95% UCL	178.41	172.25	4944.48	5245.75	35.27	34.91	6.4	6.5
Cluster	III (n = 13)							
Parameter	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md	$\bar{X}$	Md
Value	160.53	160.78	5496.72	5507.65	40.66	41.58	5.1	5.0
SE	4.56	/	364.47	/	1.19	/	0.2	/
95% LCL	150.61	147.03	4702.62	4347.51	38.08	35.96	4.7	4.5
95% UCL	170.46	176.88	6290.83	6600.06	43.25	44.32	5.6	5.9

Md – median,
SE – standard error,
95% LCL – lower limit of confidence interval,
95% UCL – upper limit of confidence interval

Table 4. Distribution of clusters of glomeruli within the age groups classified by the cluster analysis

Cluster of glomeruli	Age group			Σ
	I	II	III	
I	2	37	75	114
II	124	162	144	430
III	2	69	128	199
Σ	128	268	347	743

Discussion

Structural changes found during normal ageing of the kidney support the concept of decline of renal function along with ageing. Some authors do not consider this as a rule, due to a phenomenon of vascular adaptation which may preserve glomerular filtration by provoking hyperperfusion and hyperfiltration in healthy glomeruli (21-23). Functional alterations are reflected as a decrease of renal functional reserve, associated with limits in renal capacity to adequately answer the challenges of excess or deficit.

Kidney has an ability for adaptation and regeneration. Temporary increase of load is being compensated in kidney by turning on its reserve functional units which are out of function occasionally. If this functional load increase lasts for a long time, it leads to the onset of renal hypertrophy. The best example is compensatory renal hypertrophy, where there are no new nephrons produced, but the diameter and epithelial cell number are being increased, mostly within the wall of the proximal segment of nephron. It is confirmed that, along with increase in kidney size, there is an enlargement of glomeruli as well, whose number stays the same or even get lower (8, 9).

Histological assessment of preimplantation kidney biopsies gained on the basis of expanded criteria donors enables their further use according to the scale for histological kidney sustainability scale (24, 25). In humans, the number of nephrons is determined by genetic and environmental factors and does not increase after birth. Therefore, glomerular adaptation on the higher metabolic demand or decrease in renal mass is associated only with a change in glomerular size (26). So far, studies have shown that "physiological" decline in glomeruli normally begins *in utero* and continues during child's growth and development. In adults aged between 20 and 33, the process may be repaired, therefore 95% of population under 40 have less than 10% of sclerotic glomeruli, while its percentage starts increasing after the age of 50, being 12.5% in average, whilst in patients older than 70 years of age the percentage may reach 30% (9, 17). Most authors consider the presence of glomerulosclerosis as a consequence of a renal failure, if there are more than 10% of sclerotic glomeruli in persons younger than 40 years of age (12, 14, 19). Contrary to that, we cannot precisely define clear boundary between abiotrophic involutional sclerosis caused by ageing from that caused by a renal disease, in persons aged over 40 (18, 23). However, some studies show that the ability of glomeruli to grow in size without any consequent damage progressively decrease during ageing (27, 28).

It is assumed that the implantation of one kidney may be in favor of the onset of hyperfiltration-mediated damage of glomeruli due to the imbalance between mass of the nephron and size of the recipient. Further, it leads to glomerular growth, then to glomerulosclerosis and eventually to progressive renal insufficiency (29). Glomerular adaptation after renal transplantation may be influenced also by present chronic renal failures, post-transplantation injuries, vascular lesions (30), glomerulosclerosis diagnosed after donor kidney biopsies (31), which altogether may affect the outcome of the kidney transplantation. Larger glomerular volume in donor biopsies is related to allograft malfunction (32) and it is suggested that extreme post-transplantation glomerular size is associated with glomerulosclerosis (33). In morphometric study of the kidney tissue obtained from older and young donors by Tan et al. (34), a significant increase of globally sclerotic glomeruli percentage was observed in the older donor group versus the younger donors group. According to Tracy et al. (15) nonsclerosed glomeruli showed increasing volume in the older group compared to the young donors. This resulted in a significant increase of the filtration surface area and single nephron ultrafiltration coefficient. Sclerosing glomerulopathy led to consequent glomerulopenia and compensatory hypertrophy with adaptive hyperfiltration of nonsclerosed glomeruli.

The method for quantification of the glomerular size, area of connective tissue, and cell number within glomeruli that we used might be useful to evaluate donor kidney tissue intended for transplantation. It is known that significant alterations of glomerular morphology are present in older donors. Anyway, similar data about potential donors aged 40-49 years old are very scarce. Our results showed that these examinees might possess a significantly higher number of morphologically normal glomeruli at the first sight, but morphometrically they might belong to the group of hypertrophic glomeruli which are in the initial phase of sclerosis. In our former study we predominantly found the presence of hypertrophic glomeruli in cases over 55 years of age, while those morphologically normal were prevailing in younger ones (28). Such glomeruli are probably in the initial phase of glomerulosclerosis and their predomination in older cases might affect the impairment of their renal functional reserve, as well as the success of the renal transplantation in cases

where renal allograft originated from such older individuals (34-37).

It might be suggested that morphometric assessment of morphologically normal glomeruli should be of greatest importance transplantation-wise, and not only estimation of its total number and number of detected manifestly sclerotic glomeruli (26). Alperovich et al. (38) evaluated mean glomerular volume before and after transplantation using paired pre-implantation and protocol biopsies performed in stable renal allografts. Multiple regression analysis confirmed that glomerular volumes in donors and allografts with chronic nephropathy are independent indicators of glomerular size after transplantation. They detected the increase in glomerular volume after four months, which correlated with creatinine values and indirectly points out that glomerular enlargement is a necessary condition for glomerular adaptation after transplantation.

Glomerular enlargement is reduced in patients with chronic allograft nephropathy. Data from studies on donor biopsies suggested inversely proportional relationship between age and glomerular volume (37). Abdi et al. (32) showed that the increase in glomerular volume in donor biopsies correlated with renal allograft dysfunction. They noticed that the larger glomerular volume in donor biopsies, the smaller post-transplant glomerular size. This result suggests that kidneys with larger glomeruli are already adapted to metabolic needs of a donor and therefore their potential for further adaptation is limited. In stereological studies on a cadaveric material, the inverse ratio between the number and the perimeter of the glomeruli is often described (26). Thus, greater glomerular volume may be considered as a consequence of a decrease in the total number of glomeruli and as such may be predisposing factor for the development of renal disease (39).

Our results suggest that glomerular enlargement may be a necessary precondition for the transplanted kidney to reach adequate renal function, and that higher values of the glomerular area in donors indicate depleted capacity for further functional adaptation of the kidney after transplantation.

Adaptation of glomerular volume either to renal mass loss or increased metabolic needs has been investigated in various experimental and clinical studies. In adults, glomerular volume was doubled after a kidney removal, but mostly without the onset of sclerosis, while glomerular hypertrophy and intraglomerular hypertension developed after a decrease in the number of nephrons may initiate and accelerate the onset of hypertension and progressive renal insufficiency (39). On the contrary, in patients with unilateral renal agenesis, conditions characterized by lower number of nephrons, or oligomeganephronia, the glomerular volume increases five - to eightfold and is associated with glomerulosclerosis (33). This indicates that the capacity for glomerular enlargement in the old age depends on nephron loss, whereby the onset of compensatory glomerular hypertrophy is related to the loss below critical threshold. Our results of increased number of hypertrophic glomeruli in the oldest group supports this assumption.

Conclusion

In accordance with the aforementioned data and our results on cadaveric material, it may be concluded that the donor age alone should not be a problem if a histological analysis of the kidney finds it adequate. Also, if there is a great number of enlarged glomeruli in young donors before transplantation, it may be a reason for possible graft rejection. It is for sure that renal vascular diseases, acute renal failure, obstructive nephropathy, and some systemic diseases are more frequent in the older population. Such conditions may lead to the damage of glomeruli accelerating age-related physiological alterations in the kidney.

Using the new methods for the investigation and classification of glomeruli which morphologically have not shown signs of sclerosis on the basis of area, presence of connective tissue, and cell number within them, the given results may be useful to assess renal function after transplantation.

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ZNAČAJ I POTENCIJALNA PRIMENA MORFOMETRIJSKE ANALIZE HUMANIH GLOMERULA U KADAVERIČNOJ TRANSPLANTACIJI BUBREGA

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Kadaverična transplantacija bubrega u stalnom je porastu, zbog smanjenog mortaliteta mlađih pojedinaca. Tokom procesa ovih transplantacija, od velike je važnosti odrediti ne samo starosnu granicu primaoca, već i status davaoca.

Istraživanje je obuhvatilo 30 uzoraka tkiva ljudskih kadaveričnih bubrega (oba pola, starosti od 20 do 85 godina). Uzorci tkiva obojeni su Mallori trihromskim bojenjem i analizirani pod svetlosnim mikroskopom. Slike su analizirane pomoću softvera ImageJ. Kao rezultat klaster analize, 743 glomerula klasifikovana su u 3 grupe prema morfometrijskim karakteristikama i u 3 starosne grupe (I grupa prosečne starosti 29 godina, II grupa 44 godine, III grupa sa prosekom starosti 71 godina). U prvoj grupi po morfometrijskim karakteristikama bilo je 114 sklerotičnih glomerula sa značajno ($p \leq 0,0001$) najmanjom površinom i calularnošću, a najvećim procentom vezivnog tkiva. U drugoj grupi bilo je 430 morfološki normalnih glomerula sa najvećim brojem ćelija po jedinici površine ($p \leq 0,0001$). U trećoj grupi bilo je 199 hipertrofičnih glomerula sa najvećom površinom, značajno velikom calularnošću i površinom vezivnog tkiva ($p \leq 0,0001$). Od 114 sklerotičnih glomerula najmanji broj pripada I starosnoj grupi ($p \leq 0,0001$). Ukupno je bilo 430 morfološki normalnih glomerula. Većina morfološki normlanih glomerula bila je u II starosnoj grupi ($p \leq 0,0001$). Većina od 199 hipertrofičnih glomerula bila je u III starosnoj grupi, naspram druge dve grupe ($p \leq 0,0001$), kao i u II grupi u odnosu na I grupu ($p \leq 0,0001$). Morfometrijska analiza morfološki normalnih glomerula treba da bude od najveće važnosti za transplantaciju, a ne samo procena njihovog ukupnog broja i broja detektovanih manifestno sklerotičnih glomerula.

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Ključne reči: *ljudski glomeruli, transplantacija bubrega, morfometrija*