RIGA STRADINS UNIVERSITY

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THE ROLE OF CORE BIOPSY IN KIDNEY GRAFT PATHOLOGY DIAGNOSTICS

(SPECIALITY – TRANSPLANTATION)

Summary of Doctoral Thesis

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1. INTRODUCTION

Kidney transplantation is recognized as the “gold standard” for the treatment of chronic renal failure. Recently, due to introduction of new immunosuppressive drugs kidney transplantation results have noticeably improved. However, despite the fact that the number of acute renal graft rejections and graft losses in the early postoperative period has significantly reduced, adequate long-term outcomes are not observed, and the treatment of chronic renal graft dysfunction in the late postoperative period is still problematic. Interstitial fibrosis, tubular atrophy, vascular damages, glomerulosclerosis characterizing chronic renal graft dysfunction is largely related to the course of the early postoperative period. One of the possible factors establishing the abovementioned trend are rather similar clinical symptoms characteristic to many pathological changes in renal grafts (rise in serum creatinine levels, rise in body temperature, decrease in diuresis, etc.). Therefore, it is not always possible to estimate the renal graft condition and to make a differential diagnosis based only on clinical symptoms and the laboratory finding. Thus, renal graft core biopsy followed by a pathohistological examination of the material is also becoming important. The finding may help make an accurate diagnosis and to choose the most appropriate type of therapy. An early correct diagnosis is very important, because the graft might be lost as a result of an incorrect or delayed treatment. It is especially topical, if acute renal failure is developing and renal graft function is reduced or absent in the early postoperative period. The absence of such functions immediately after the surgery is called delayed graft function (DGF). Literature data evidence that renal function in the late period is the worst in patients having DGF in the early postoperative period. The risk of acute rejection in these patients is higher than with primary graft function, and its diagnostics is mainly based on protocol biopsy data, as there is no clinical presentation of a typical rejection. The influence of this “hidden” rejection on further graft function has not been sufficiently studied.

Kidney graft biopsy is especially important in the diagnostics of subclinical rejection, when neither rise in creatinine levels nor other known signs are present. Theoretically, non-diagnosed and untreated subclinical rejections may provoke the development of chronic renal graft dysfunction in the late period, though there is still no common opinion about the influence of this acute rejection and its treatment on further graft function. One of the main diagnostic methods for subclinical rejection is the so called protocol biopsy which is made

according to the schedule irrespective of the renal graft condition. Essential information might be obtained through time-zero biopsy, a donor kidney biopsy before explantation, because after transplantation problems might be related to the previous pathology in the donor kidney.

In view of this, to improve the outcome of kidney transplantation a task was set to analyze the results of core biopsy in the early postoperative period and to study the influence of pathological conditions of the renal graft on further function of the kidney.

2. AIM, OBJECTIVES AND HYPOTHESES OF THE STUDY

Aim of the study:

To investigate the role of core biopsy in renal graft pathology diagnostics in order to improve outcome of kidney transplantation.

Objectives of the study:

1. Based on results of time-zero biopsy to determine the impact of the initial condition of the donor kidney to further graft function.
2. To analyze the results of protocol biopsy in case of primary and delayed graft function and to determine the incidence of subclinical and "hidden" rejections and their influence on outcomes of kidney transplantation.
3. To analyze the results of acute biopsy and to study the influence of clinical rejection on outcomes of kidney transplantation.
4. To make up the type and the incidence of core biopsy-related complications.
5. To develop an algorithm for use of core biopsy to optimize the diagnostics of pathological conditions of renal grafts in the early postoperative period.

Working hypotheses:

1. Renal graft dysfunction in the early and late postoperative period may be related to the previous (pre-transplant) pathology of the donor kidney.
2. Non-diagnosed subclinical renal graft rejection may provoke the development of dysfunction in the late postoperative period.
3. The influence of delayed graft function on the transplant kidney function in the late postoperative period may be reduced by duly diagnosing and treating "hidden" rejection.

4. A due diagnosis and treatment of pathological conditions of a renal graft in the early postoperative period may help to reduce the number of renal graft dysfunctions in the late postoperative period and to improve outcomes of kidney transplantation.

3. NOVELTY OF THE STUDY

1. It is proved that subclinical rejections, though untreated, do not affect further function of the graft in patients with primary and stable renal graft function.
2. It is proved that due diagnostics and treatment of "hidden" rejection improves prognosis for patients with delayed graft function.
3. An algorithm for core biopsy of kidney grafts to diagnose pathological conditions in the early postoperative period was developed.

4. MATERIALS AND METHODS

Definitions

Early postoperative period – first three months after kidney transplantation.

Late postoperative period – period starting from the fourth month after kidney transplantation.

Primary renal graft function – the serum creatinine level at adequate diuresis and without renal replacement therapy (RRT) during 3-5 days after surgery has reduced to 0.3 mmol/l or lower.

Delayed renal graft function – the need to continue RRT in the post-transplant period due to the lack of graft function.

Acute renal graft dysfunction – sudden deterioration in renal graft function in the form of a rapid rise in serum creatinine level.

Acute renal graft rejection – acute renal graft dysfunction of immunological origin in the form of a decrease in diuresis, a rise in serum creatinine level, sometimes a rise in body temperature and pain in the area of the renal graft.

Subclinical renal graft rejection – occurs without usual signs of rejection and may be diagnosed only with the help of puncture biopsy.

"Hidden" rejection – a type of subclinical rejection in case of delayed graft function.

4.1. Study population

Within the framework of the research 274 biopsies were performed in the Latvian Center of Transplantation in the time period from 01.01.2004 to 28.02.2009: 65 time-zero biopsies, 101 protocol biopsies and 108 biopsies were performed due to deterioration in graft function in the early postoperative period.

The research was divided into 3 parts:

I. analysis of time-zero biopsy results;

II. analysis of protocol biopsy results;

III. analysis of acute biopsy results.

4.2. Patient examination methods

Renal graft core biopsy.

Renal graft core biopsy is usually performed with the patient positioned lying on his back or side under ultrasonic control to avoid damages to the surrounding structure – the ureter, blood-vessels, the bowels. The surgery area is cleansed with a disinfectant and delimited by sterile linen.

The biopsy area is infiltrated with S.Lidocaine 2% (5-10 ml). Tissues are punctured with a biopsy needle up to the renal graft and a renal tissue fragment is taken.

Two renal tissue specimens from different places are usually taken to increase sensitivity of the method. High accuracy of the method is ensured, if at least 10 glomeruli and 2 arteries are obtained. After the procedure the patient is indicated bed rest for 6-8 hours.

The material obtained is fixed in 4% formalin (pH-7.4). Hematoxiline-eozine, PAS-reaction, Masson method are used to stain histological specimens. In case of steroid-resistant rejection renal tissues are examined also for C4D deposits in peritubular capillaries, applying one of immunohistochemistry methods – a immunoperoxidase test. The principle of the method is the treatment of the research specimen with peroxidase-labeled antibodies with subsequent visualization with the help of histochemical staining reaction. Optical microscopy is used to examine the specimen. Morphological changes are evaluated according to Banff'05 classification (Table1).

Table 1. Banff classification.

1. Normal
2. Antibody-mediated rejection.

Acute antibody-mediated rejection:

I. C4d+; minimal inflammation.

II. C4d+; capillary thromboses.

III. C4d+; arterial - v3.

Chronic active antibody-mediated rejection – C4d+, interstitial fibrosis, tubular atrophy, fibrous intimal thickening in arteries.

3. Boderline changes. „Suspicious" for acute T-cell-mediated rejection. No intimal arteritis is present, but there are signs of mild tubulitis (t1, t2 or t3 with i0 or i1).
4. T-cell mediated rejection.

Acute T-cell mediated rejection.

IA. Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (t2).

IB. Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of severe tubulitis (t3).

IIA. Cases with mild to moderate intimal arteritis (v1).

IIB. Cases with severe intimal arteritis comprising > 25% of the luminal area (v2)

III. Cases with "transmural" arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)

Chronic active T-cell mediated rejection – „chronic allograft arteriopathy" (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima).

5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology.

I. Mild interstitial fibrosis and tubular atrophy (< 25% of cortical area).

II. Moderate interstitial fibrosis and tubular atrophy (26 - 50% of cortical area).

III. Severe interstitial fibrosis and tubular atrophy (> 50% cortex).

<u>Quantitative criteria for tubulitis.</u>

t0 – no mononuclear inflammatory cell in tubules.

t1 – foci with 1 to 4 mononuclear cell per tubular cross section

t2 - foci with 5 to 10 mononuclear cell per tubular cross section

t3 - foci with > 10 mononuclear cell per tubular cross section

Quantitative criteria for interstitial mononuclear cell inflammation.

i0 – no trivial interstitial inflammation.

i1 – up to 25% of parenchyma inflamed.

i2 – 26-50% of parenchyma inflamed.

i3 – >50% of parenchyma inflamed.

Quantitative criteria for intimal arteritis.

v0 – no arteritis.

v1 – mild-to-moderate intimal arteritis in at least one arteral cross section.

v2 – moderate-to severe intimal arteritis in more than one arterial cross section

v3 - "transmural" arteritis, fibrinoid change and medial smooth muscle necrosis.

Renal graft function will be evaluated based on serum creatinine levels determined with a blood biochemistry analyzer Abbott Spectrum Series II (Abbott, USA) and glomerular filtration rate (GFR). The estimation of the degree of light absorption of different length of waves was used to quantitatively determine plasma substances. GFR was estimated using Cockroft-Gault formulas:

$$\frac{(140 - \text{age}) \times \text{body weight (kg)}}{0.81 \times \text{serum creatinine } (\mu\text{mol/l})}$$

For women the acquired result is multiplied by 0.85.

To find out the influence of different factors on the transplantation outcome groups of patients were compared according to the following parameters:

- renal graft function during the observation period;
- incidence of acute rejections during the observation period;
- incidence of delayed graft functions;
- graft survival.

4.3. Kidney allotransplantation.

Taking and conservation of a donor kidney. Donor kidney explantation takes places after the donor's biological or brain death has been stated. Median laparotomy is used to explant the donor kidney. *Colon ascendens* and the small intestine are mobilized to provide

access to the great vessels. The aorta and *v. cava inferior* are separated from surrounding tissues from the bifurcation area and proximally 2-3 cm above renal blood vessels. The aorta and *v. cava inferior* are canuled and a renal perfusion is started with 3-4 liters of preservative solution. When the perfusion is complete, kidneys are separated from surrounding tissues, *a.renalis, v.renalis* and the ureter are divided, kidneys are taken and placed in a special container with preservative solution.

The kidney transplantation surgery is performed under general anesthesia with the patient positioned lying on his back. When the surgery area has been treated with a disinfectant, a cut is made in the right or left hypogastrium. The access to pelvis vessels is provided retroperitoneally. The venous anastomosis is performed usually first to the external iliac vein. External iliac artery is used for the anastomosis of the renal artery. Anastomoses are performed with a continuous suture of the vessel with Prolene 6-0 suture. When connections have been established, they are inspected for leaks and additional sutures are placed, if required. Blood circulation in the kidney is restored and hemostasis is performed. When hemostasis has been ensured the kidney is placed into the retroperitoneal space. The urinary bladder is separated from surrounding tissues and cystoureteroneostomy is created with 4-0 PDS suture. 3-4 antireflux sutures are placed above anastomosis. The peritoneal cavity is drained with a soft plastic pipe drain.

4.4. Post-transplant immunosuppression.

The immunosuppressive therapy consists of two parts: induction and maintenance. One of drugs containing anti-interleykin-2 receptor monoclonal antibodies – Basiliximab (Simulect, Novartis) or Daclizumab (Zenapax, F.Hoffmann-La Roche), or polyclonal anti-T-lymphocyte globulin antibodies (ATG, Fresenius Biotech) were used for induction immunosuppression.

During the maintenance immunosuppression patients received metilprednizolon (Solu-Medrol, Pfizer) at the beginning and then prednizolon (Prednisolon, Gedeon Richter). Patients received cyclosporine A as a calcineurine inhibitor, micophenolat-mofetil as an antiproliferative drug.

For acute rejection therapy patients received 500 mg metilprednizolon iv for 3-5 days in sequence, and, if the rejection was steroid-resistant, its treatment continued with anti-T-lymphocyte globulin 1.5-3 mg/kg per day i/v for 10-14 days.

4.5. Time-zero biopsies.

Pre-transplant donor kidney biopsy – time-zero biopsy - is performed during explantation of a kidney before the interruption of blood circulation for the purpose of obtaining information about the quality of the organ, and possibly to determine pathologies not diagnosed while the donor was alive.

Within the framework of the research 65 time-zero biopsies were performed in the time period from 01.01.2004 to 31.12.2007. Time-zero biopsy is usually performed on the right kidney, because it can be better visualized during explantation and is better available for puncture.

Recipients who received their kidney after time-zero biopsy were selected with aim to detect the relationship between the initial condition of the donor kidney and the outcome of transplantation. The patients were divided into groups depending on the degree of pathological changes.

The influence of glomerular, interstitial and arterial sclerosis on postoperative renal function was studied individually.

2 groups of recipients were compared to estimate the influence of glomerular sclerosis on the outcome of transplantation:

- Group 1 – patients without signs of glomerulosclerosis in the donor kidney according to the data of time-zero biopsy;
- Group 2 – patients with signs of glomerulosclerosis in the donor kidney according to the data of time-zero biopsy.

3 groups of recipients were compared to estimate the influence of interstitial sclerosis on the outcome of transplantation:

- Group 1 – patients with a degree of interstitial sclerosis in the donor kidney from 0 to 10%
- Group 2 – patients with a degree of interstitial sclerosis in the donor kidney from 11 to 20%
- Group 3 – patients with a degree of interstitial sclerosis in the donor kidney above 20%

2 groups of recipients were compared to estimate the influence of arterial sclerosis of the donor kidney on the outcome of transplantation:

- Group 1 – patients without signs of arterial sclerosis in the donor kidney according to the data of time-zero biopsy;
- Group 2 – patients with signs of arterial sclerosis in the donor kidney according to the data of time-zero biopsy.

4.6. Protocol biopsies

Protocol biopsies are performed according to the schedule (irrespective of renal graft function) to monitor the condition of the graft and to diagnose subclinical and hidden pathologies.

Protocol biopsies in case of delayed graft function.

61 protocol biopsies were performed to 34 patients with delayed graft function in the time period from 01.01.2004 to 31.12.2007. The biopsy was usually performed on the 3rd to 5th day after surgery and repeated each 6-7 days, if possible, until function was restored.

The patients were observed for 3 years. Two groups of recipients were compared to study the possibility to improve prognosis for patients with delayed graft function.

1. Group 1 – patients with primary graft function;
2. Group 2 – patients with DGF and rejection discovered during biopsies.

Protocol biopsies in case of primary graft function.

Within the framework of the research 40 protocol biopsies were performed to 26 patients with primary and stable renal graft function in the time period from 01.01.2007 to 28.02.2009.

Protocol biopsies were usually performed on week 3 to 4 after transplantation. Biopsy performance conditions were as follows:

- serum creatinine level < 0.2 mmol/l;
- adequate diuresis;
- standard body temperature;
- stable renal graft function signifying fluctuations in serum creatinine level during the last 7 days not exceeding 20%.

Four groups of recipients were selected and compared to estimate the influence of subclinical rejection on further renal graft function:

- Group A – patients with primary and stable renal graft function without signs of rejection in the biopsy specimen.

- Group B – patients with primary and stable renal graft function with morphological signs of rejection, who were treated subclinical rejection (SR).
- Group C – patients with primary and stable renal graft function with signs of rejection in the biopsy specimen, who were not treated SR.
- Group D (reference group) – patients with primary and stable renal graft function without clinical signs of rejection, who were not performed protocol biopsy.

4.7. Acute biopsies.

The indication for acute renal graft biopsy is sudden deterioration in renal graft function.
108 acute renal graft puncture biopsies were performed to 77 patients in the early postoperative period in the time period from 01.01.2004 to 31.12.2007:
The patients were observed for 3 years. Two groups of recipients were compared to precise the influence of early acute rejection on further renal graft function:

Group 1 – patients with acute rejection (proved by puncture biopsy).

Group 2 – patients without acute rejection.

Two groups of recipients were compared to estimate the influence of the degree of rejection on further renal graft function:

Group 1 – patients with acute rejection grade I;

Group 2 – patients with acute rejection grade II and III and antibody-mediated rejection.

4.8. Data acquisition and statistical analysis

Microsoft Excel 2003 was used to keep the collected data, Microsoft Word 2003 – to frame up the text and tables, SPSS 13.0 for Windows – to perform statistical analysis of the data. All figures are shown as the arithmetic mean ± standard deviation (SD) or in numbers and percentage.

One-way ANOVA (General Linear Model, Univariate) test was used to analyze parametric data (demographic data of patients, serum creatinine level, glomerular filtration rate). The statistical credibility of results was established with $p< 0.05$.

Chi-square test and Fisher's exact test were used to analyze non-parametric data (acute rejections and the incidence of delayed renal graft function). The statistical credibility of results was established with $p< 0.05$.

Odds ratio (OR), the probability of the occurrence of an event in one group compared to the probability of occurrence of the same event in the second group (the probability of acute rejection and delayed renal graft function)), was analyzed during the work. The confidence interval (CI) was 95%.

Graft survival was analyzed using Kaplan-Meier surveillance test. Log Rank (Mantel-Cox) criterion was used to compare the two groups. The data were censored when the patient had reached the end of the research period.

5. RESULTS

In total, 274 biopsies were performed within the framework of the research, 209 of which were performed to 137 patients. After the manipulation complications were detected in 7 cases (3.3%). 6 patients showed macrohematuria which spontaneously disappeared within 24-72 hours. One patient had a clot in the urinary bladder after biopsy with clinical manifestations of acute cystitis. The clot was washed out retrogradely using Foley catheter.

There were no complications in other biopsies.

5.1. Analysis of time-zero biopsy results.

Time-zero biopsies results were as follows (Table 2.):

Table 2. Time-zero biopsies results.

Finding	The number of cases (%)
No pathological changes	15,4% (10)
Only interstitial sclerosis	38,5% (25)
Interstitial and glomerular sclerosis	13,8% (9)
Interstitial and arterial sclerosis	15,4% (10)
Interstitial, arterial and glomerular sclerosis	16,9% (11)
Interstitial nephritis	9,2% (6)
Glomerulonephritis	4,6% (3)

Analyzing the influence of glomerular sclerosis on the outcome of transplantation, it was stated that both donors and recipients in the second group were older compared to the first group. (Table 3):

Table 3. Comparison of renal graft function and clinical details in patients with donor kidney glomerular sclerosis (group 2) and without it (group 1)

	Group 1	Group 2	Fisher's Exact
Patients (n)	45	20	
Recipient age (years)	44,1,0±14,6	53,8±13,6	p=0,02
Donor age (years)	43,2±14,5	52,0±9,5	p=0,02
Cold ischemia time (hours)	16,6±3,6	17,1±4,9	p=NS
Sr cr after 3 months (mmol/l)	0.118±0,032	0.150±0,045	p=0,002
Sr cr after 36 months (mmol/l)	0,149±0,058	0.181±0,063	p=0,092
GFR after 3 months (ml/min)	73,2±17,1	57,5±15,7	p=0,001
GFR after 36 months (ml/min)	57,2±16,2	46,9±16,2	p=0,07

The analysis of the comparison results demonstrated that in patients with signs of glomerulosclerosis in the donor kidney:

- graft function was worse in the early postoperative period and there were trends for worse function in the late period (Figure 1)
- delayed graft functions (DGF) developed more frequently and there was a trend for more frequent acute rejection (AR) (Figure 2)
- there was a trend for shorter graft survival (Figure 3)

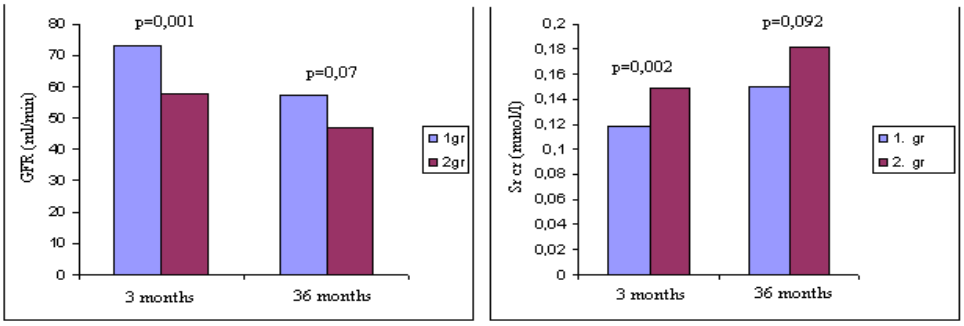


Figure 1. Level of Sr cr and GFR after 3 and 36 months in patients with donor kidney glomerular sclerosis (group 2) and without it (group 1).

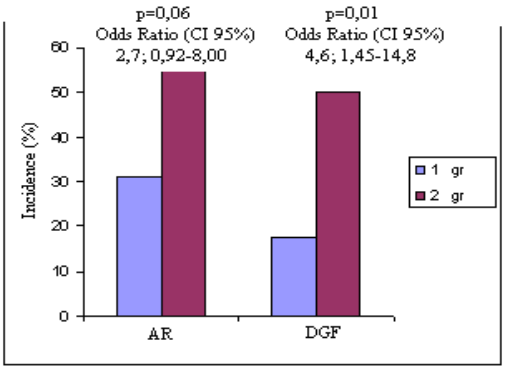


Figure 2. Incidence of DGF and AR in patients with donor kidney glomerular sclerosis (group 2) and without it (group 1).

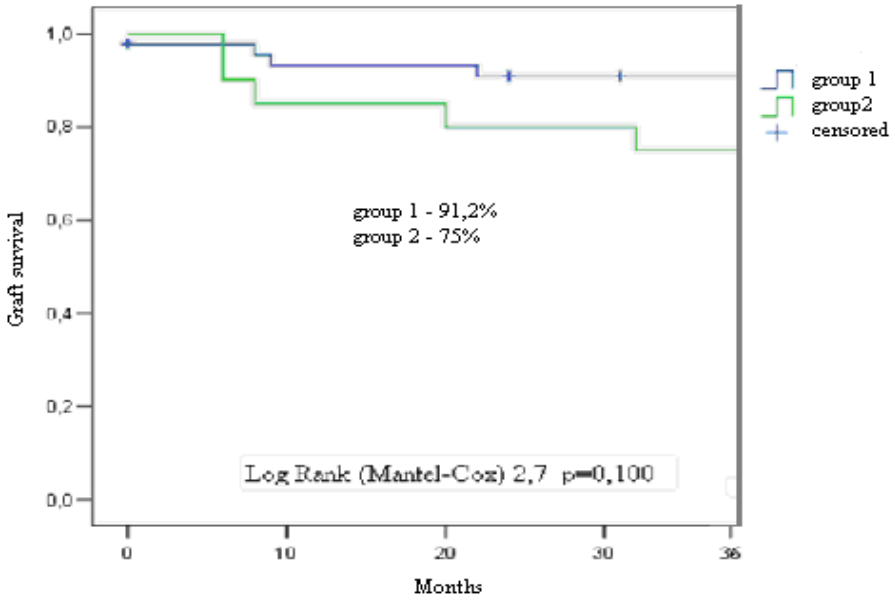


Figure 3. Three years graft survival (group 1 - patients without donor kidney glomerular sclerosis; group 2 - patients with donor kidney glomerular sclerosis).

Analyzing the influence of interstitial sclerosis on the outcome of transplantation (Table 4), it was stated that donors in the third group were older compared to the first group ($p = 0.013$) and the second group ($p = 0.084$).

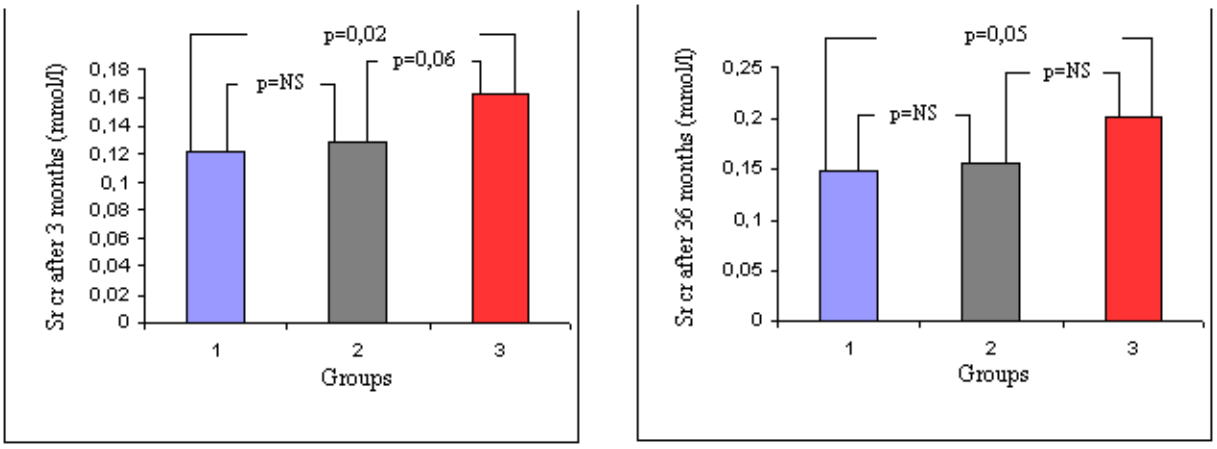
Table 4. Comparison of renal graft function and clinical details in patients with different stage of donor kidney interstitial sclerosis.

	Group 1 (0%-10%)	Group 2 (11%-20%)	Group 3 (>20%)
Patients (n)	37	21	7
Recipient age (years)	45,0±16,0	47,0±10,4	57,6±9,9
Donor age (years)	42,7±13,0	47,8±12,8	57,0±5,5
Cold ischemia time (hours)	17,6±3,3	15,8±4,4	16,5±5,9
Sr cr after 3 months (mmol/l)	0.121±0,035	0,128±0,032	0.163±0,062
Sr cr after 36 months (mmol/l)	0,148±0,045	0,155±0,061	0.202±0,089
GFR after 3 months (ml/min)	68,8±12,1	67,9±14,6	59,9±11,8
GFR after 36 months (ml/min)	57,0±12,3	54,0±20,4	43,0±14,6

The age of recipients in the third group was also older. The cold ischemia time was similar in all groups.

The analysis of results proved that patients with higher degree of interstitial sclerosis (especially > 20%) trend to have:

- worse renal function both in the early and late postoperative period (Figure 4);



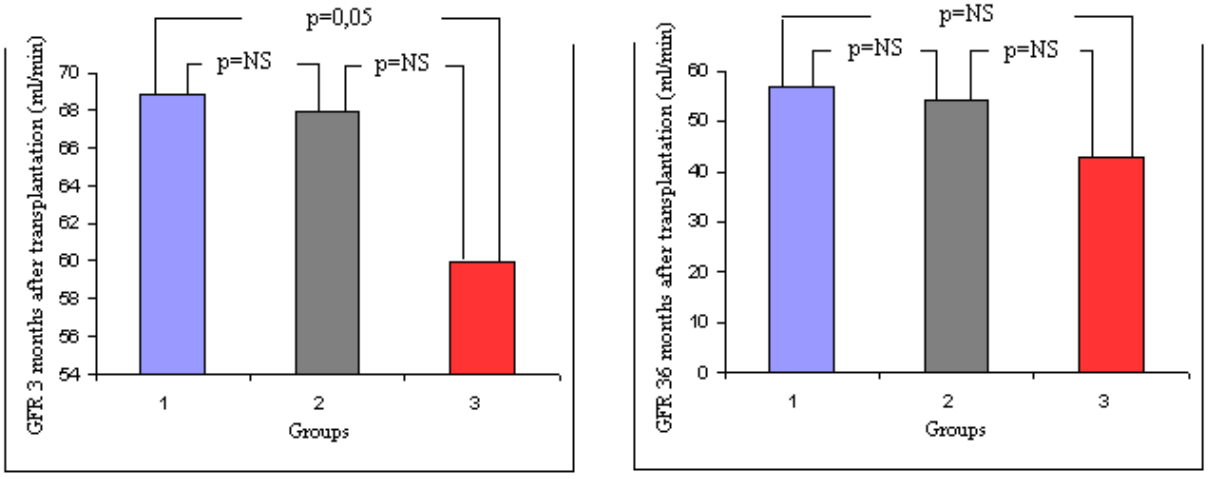


Figure 4. Level of Sr cr and GFR after 3 and 36 months in patients with different stage of donor kidney interstitial sclerosis

- more frequent development of delayed graft function (fig.5);

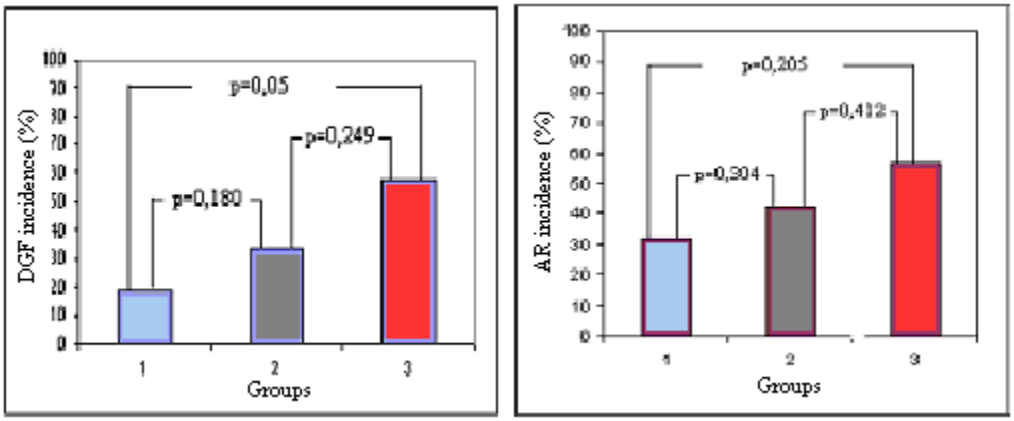


Figure 5. Incidence of DGF and AR in patients with different stage of donor kidney interstitial sclerosis.

- shorter graft survival (fig.6).

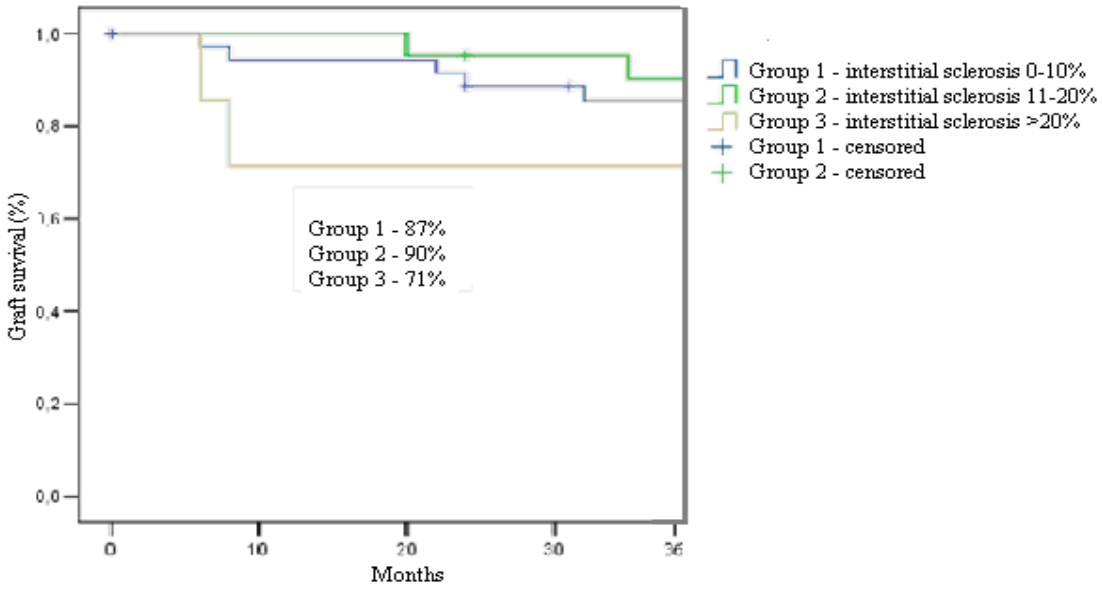


Figure 6. Three years graft survival in patients with different stage of donor kidney interstitial sclerosis.

Analyzing the influence of arterial sclerosis on renal function in the post-transplant period, it was stated that both donors and recipients in the second group (with sclerotic changes in arteries) were older compared to the first group (with signs of arteriosclerosis) (Table 5).

Table 5. Comparison of renal graft function and clinical details in patients with donor kidney arterial sclerosis (group 2) and without it (group 1)

	Group 1 (n=30)	Group 2 (n=21)	Fisher's Exact
Patients (n)	44,4,0±15,7	55,7±9,7	p=0,01
Recipient age (years)	41,4±12,9	54,3±11,5	p=0,002
Donor age (years)	17,0±3,3	17,5±4,8	p=NS
Cold ischemia time (hours)	0.123±0,029	0.138±0,049	p=NS
Sr cr after 3 months (mmol/l)	0,155±0,064	0.152±0,039	p=NS
Sr cr after 36 months (mmol/l)	70,1±12,7	64,0±13,0	p=NS
GFR after 3 months (ml/min)	59,2,0±18,5	59,8±13,6	p=NS

The analysis of the comparison results proved that

- serum creatinine level and GFR both in the early postoperative period and after 3 years is similar in both groups (figure 7);
- patients with sclerotic changes in arteries of the donor kidney tend to have more frequent development of delayed graft function (figure 8);
- three years' graft survival statistically does not differ in both groups (figure 9):

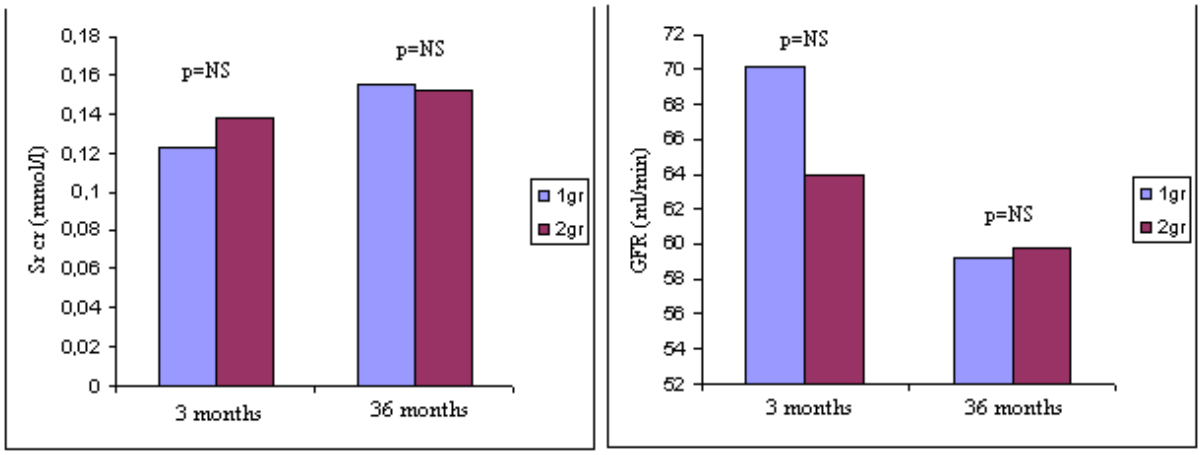


Figure 7. Level of Sr cr and GFR after 3 and 36 months in patients with donor kidney arterial sclerosis (group 2) and without it (group 1).

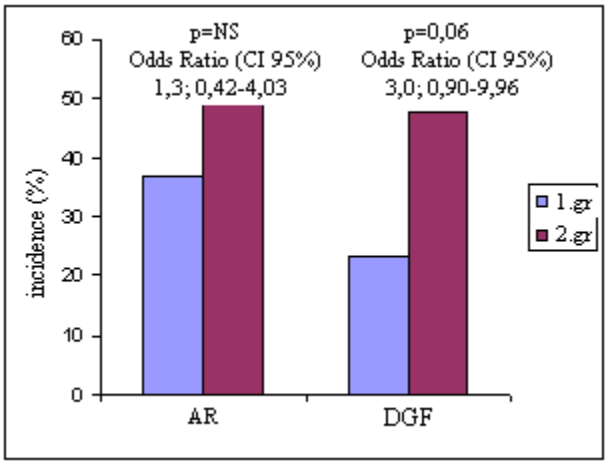


Figure 8. Incidence of DGF and AR in patients with donor kidney arterial sclerosis (group 2) and without it (group 1).

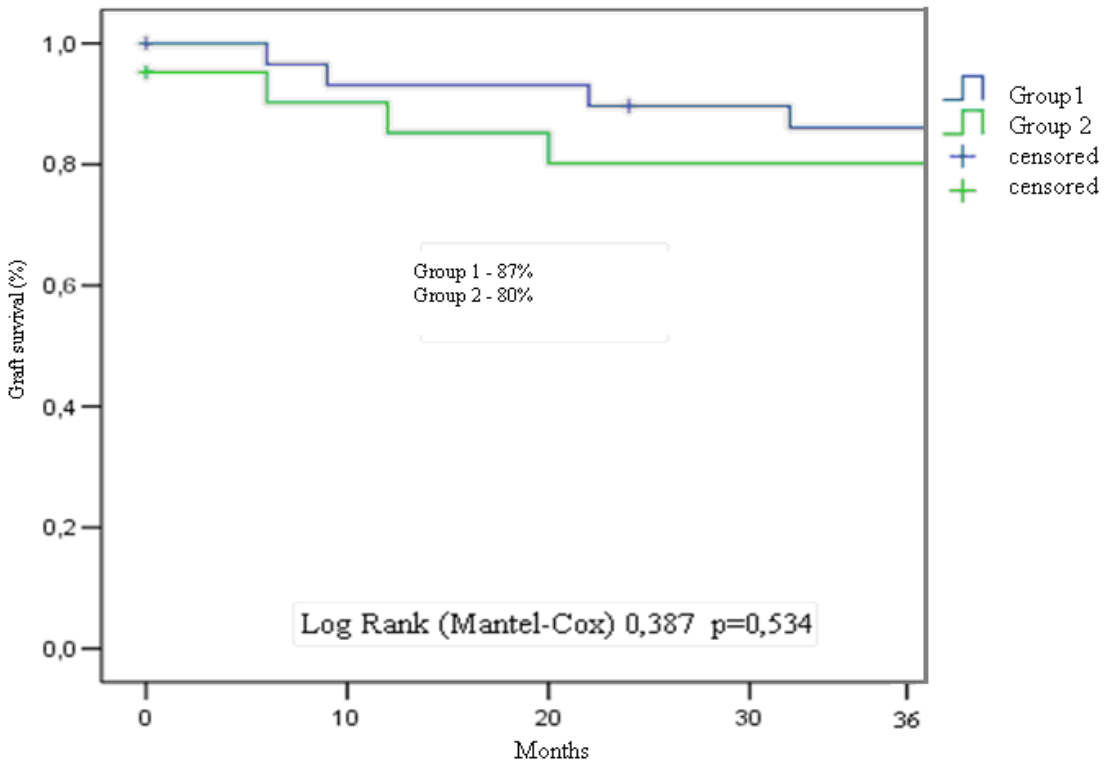


Figure 9. Three years graft survival (group 1 - patients without donor kidney arterial sclerosis; group 2 - patients with donor kidney arterial sclerosis).

Summary.

1. The analysis of time-zero biopsy results demonstrated that sclerotic changes in both glomeruli and interstitium of the donor kidneys are closely related to further graft function, but the influence of arterial sclerosis is not so significant.
2. Time-zero biopsy helped to discover 3 previously unknown cases of donor kidney glomerulonephritis and 6 cases of interstitial nephritis.

5.2. Analysis of protocol biopsy results

5.2.1. Protocol biopsy in case of delayed graft function (DGF)

Histological investigation results were as follows (figure 10):

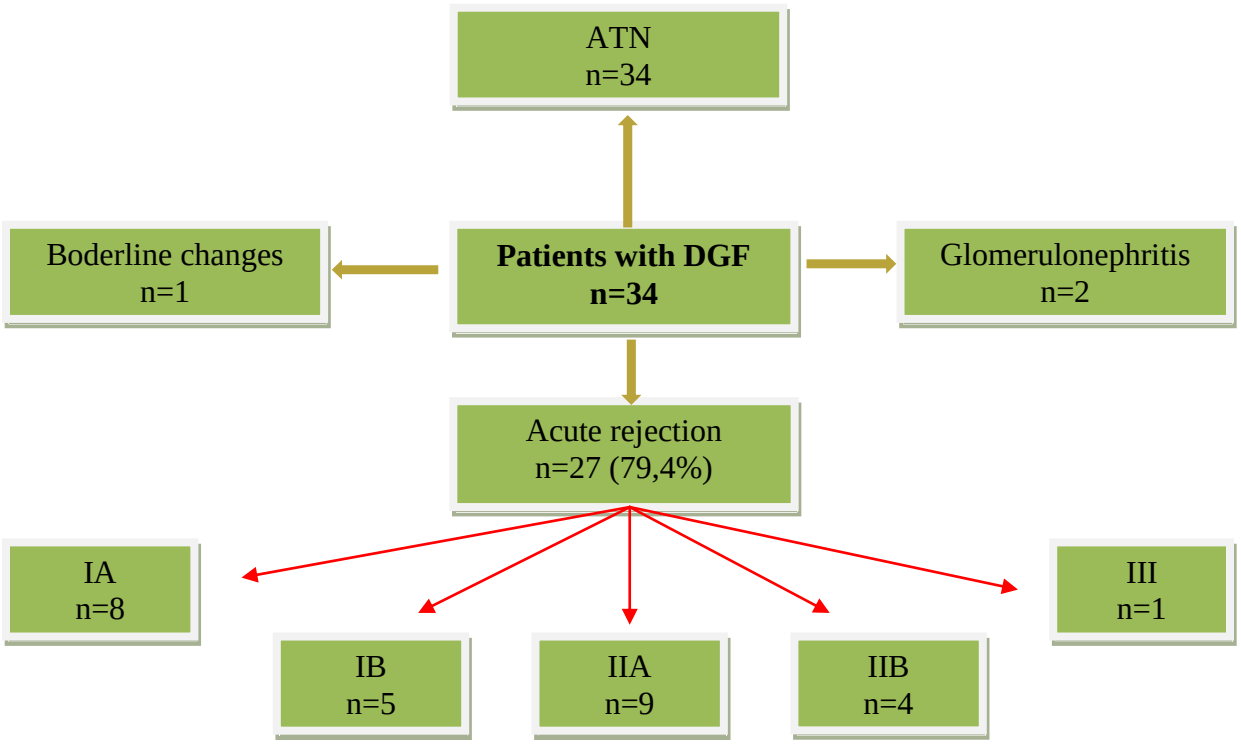


Figure 10. Core biopsy results in patients with delayed renal graft function

These rejections in patients with delayed graft function may be called “hidden”, because they do not manifest usual clinical symptoms and may be diagnosed only with biopsy.

In 21 patients “hidden” rejection was discovered already after the first biopsy, but in 6 patients with DGF rejection was discovered with second protocol biopsy, although the first biopsy gave no signs of rejection.

Comparing the group of patients with primary (PF) renal graft function (n=215) and the group with delayed graft functions and “hidden” rejection discovered by biopsy (n=27) the results were as follows:

- the age of donors and recipients and the cold ischemia time were statistically credibly higher in patients with delayed function (Table 6)

Table 6. Comparison of renal graft function and clinical details in patients with primary (group 1) and delayed (group 2) renal graft function.

	Group 1 (n=215) Patients with PF	Group 2 (n=27) Patients with DGF	Fisher's Exact (p)
Recipient age (years)	43,9±14,5	54,1±8,7	<0,05
Cold ischemia time (hours)	16,0±4,7	18,3±4,4	<0,05
Donor age (years)	42,2±13,6	50,2±12,5	<0,05
Sr creatinine after 3 years (mmol/l)	0,157±0,061	0.140±0,039	NS
GFR after 3 years (ml/min)	53,6±15,7	55,1±13,2	NS

- after three years both serum creatinine levels and GFR were similar in both groups. Graft function was even slightly better in patients with delayed function and discovered rejections than in patients with primary graft function, but they were not statistically significant (figure 11).

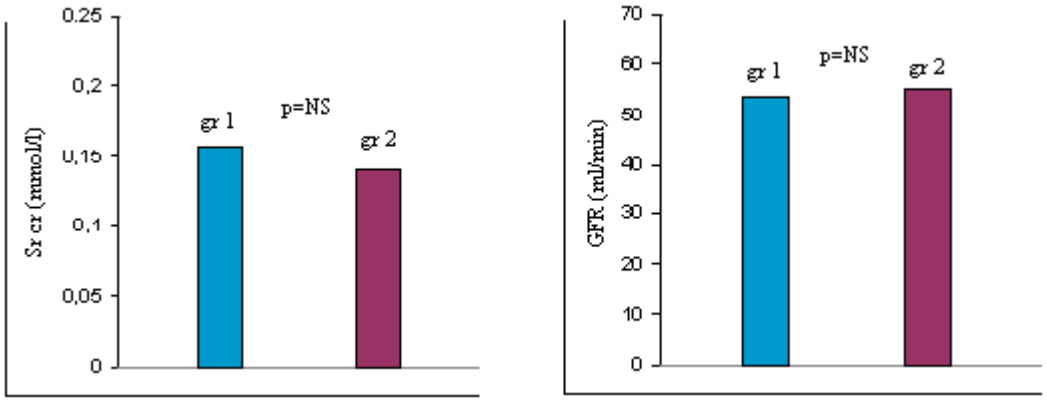


Figure 11. Level of Sr cr and GFR 36 months after transplantation in patients with primary (group 1) and delayed (group 2) renal graft function.

- The analysis of comparison results showed that the incidence of acute rejection in the late postoperative period was similar in both groups (figure 12).

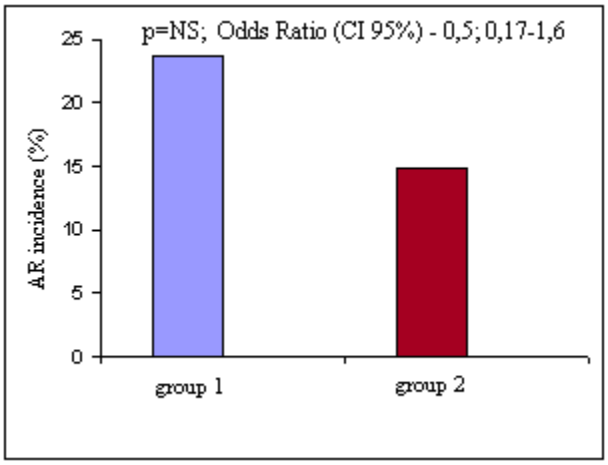


Figure 12. Incidence of AR in patients with primary (group 1) and delayed (group 2) renal graft function.

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- three years renal graft survival had 86% of patients with primary function and 82% of patients with delayed function and “hidden” rejections (figure 13).

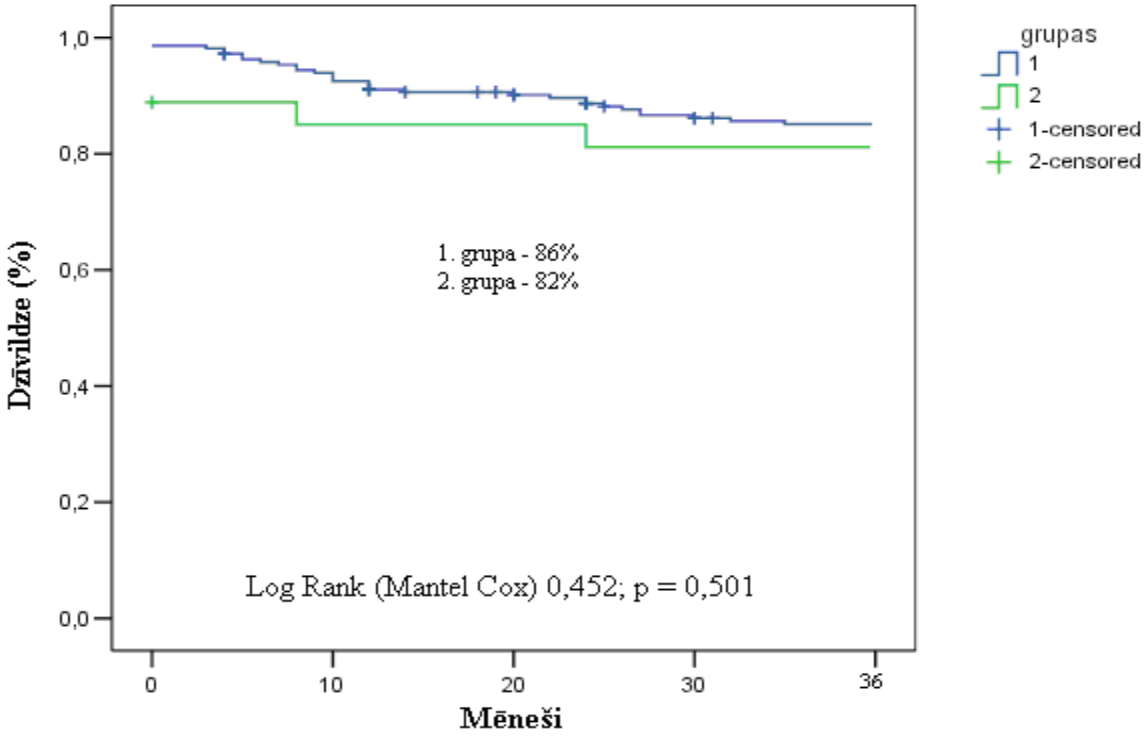

Figure 13. Three years graft survival in patients with primary (group 1) and delayed (group 2) renal graft function.

Summary:

1. Protocol biopsies in 27 recipients with DGF (79.4%) showed “hidden” rejection.

2. In six patients rejection was discovered during the second biopsy, although no rejection signs were stated in the first biopsy specimen.

3. Renal function after three years was similar in patients with delayed graft function and diagnosed and treated “hidden” rejections and in patients with primary graft function.

4. The risk of acute rejection and 3 years graft survival was similar in patients with DGF and treated “hidden” rejection and in patients with primary graft function.

5.2.2. Protocol biopsy in case of primary renal graft function

Histological investigation results were as follows (figure 14):

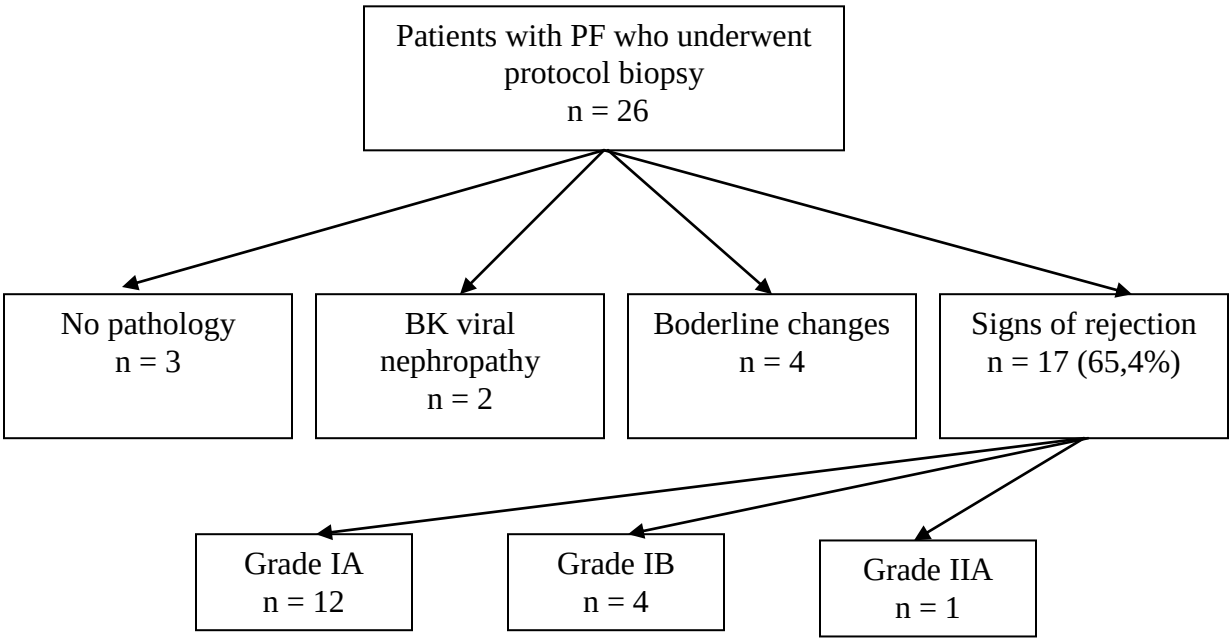

Figure 14. Results of protocol biopsy in patients with primary kidney graft function.

Subclinical rejection (SR) was treated with steroids in 9 patients – Solu-Medrol for 3 days 500 mg per day. In eight patients subclinical rejection was not treated.

The group comparison results were as follows (Table 7).

- the age of donors and recipients and the cold ischemia time was similar in all groups (p=NS);

Table 7. Comparison of renal graft function and clinical details in patients without histological signs of SR (group A), with treated SR (group B), with no treatment of SR (group C) and control group patients (group D).

	Group A (n=9)	Group B (n=9)	Group C (n=8)	Group D (n=54)
Recipients age (years)	52,0±11,5	46,0±15,1	49,0±7,5	46,1±16,0
Cold ischemia time (hours)	17,9±1,7	16,7±2,1	16,3±2,8	16,7±5,0
Donors age (years)	42,1±12,9	51,1±13,5	48,6±8,4	44,9±16,2
Sr cr 1 month after transplantation (mmol/l)	0.119±0,022	0,118±0,027	0.125±0,035	0,106±0,021
Sr cr 24 months after transplantation (mmol/l)	0,139±0,022	0,139±0,022	0.145±0,057	0,130±0,052
GFR 1 month after transplantation (ml/min)	69,8±17,5	70,1±22,5	59,8±11,6	73,1±15,2
GFR 24 month after transplantation (ml/min)	57,6±16,1	57,1±14,9	53,5±14,3	61,6±16,5
Incidence of clinical acute rejection during follow up (%)	33,3% (3)	22,2% (2)	25% (2)	16,7% (9)

- serum creatinine levels and GFR both in one month and in 24 months after transplantation do not statistically differ in all groups, including the group in which subclinical rejection was not treated (figure 15, 16);
- the incidence of clinical acute rejections was also similar in all groups (figure 16);
- 2 years renal graft survival did not statistically differ in all groups (figure 17).

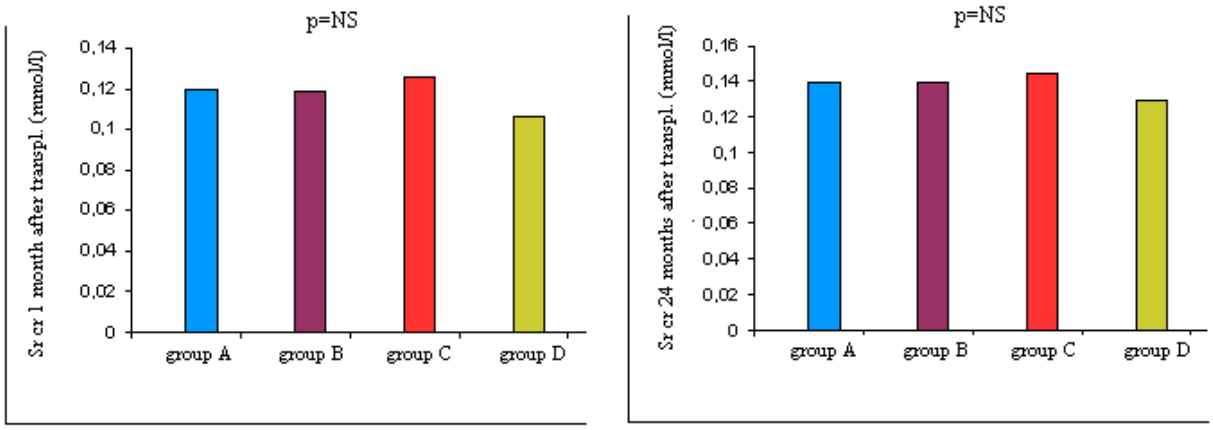

Figure 15. Sr cr levels 1 month and 24 months after transplantation in patients without histological signs of SR (group A), with treated SR (group B), with no treatment of SR (group C) and control group patients (group D).

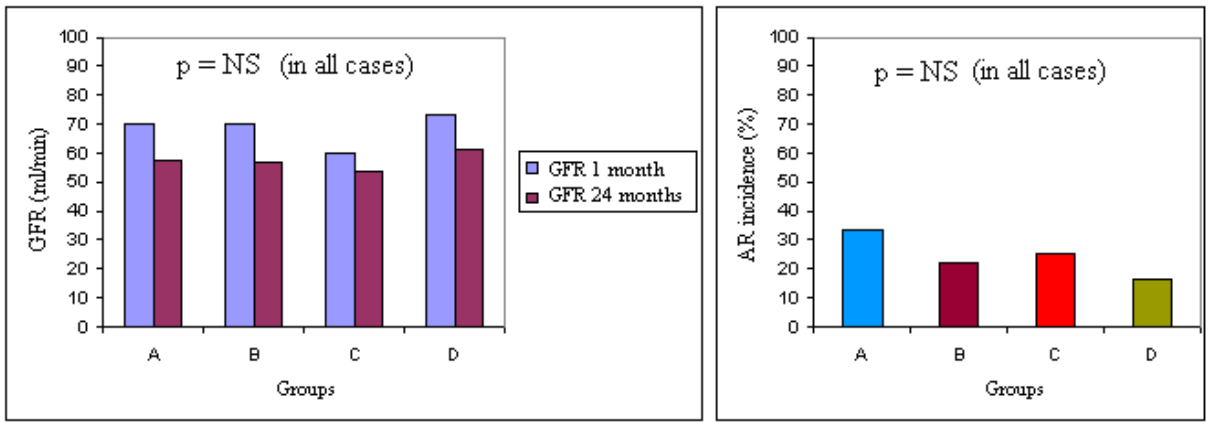

Figure 16. GFR 1 month and 24 months after transplantation and incidence of clinical AR in patients without histological signs of SR (group A), with treated SR (group B), with no treatment of SR (group C) and control group patients (group D).

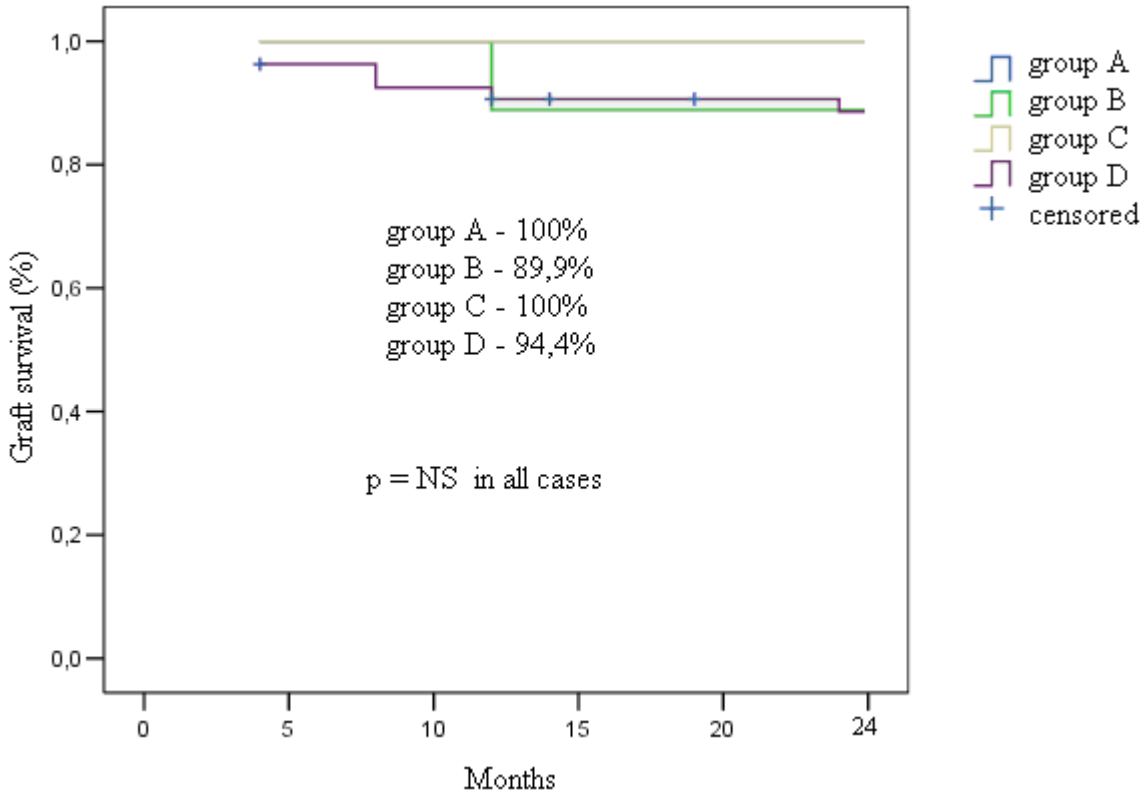

Figure 17. 2 years graft survival in patients without histological signs of SR (group A), with treated SR (group B), with no treatment of SR (group C) and control group patients (group D).

Summary.

1. With protocol biopsies different degrees of subclinical rejection were discovered in 65.4% of research patients with primary and stable renal graft function.
2. Graft function, graft survival and the incidence of clinical AR did not statistically differ in patients with subclinical rejection and in patients without them during two years, even if SR was not treated.

5.3. Analysis of acute biopsy results.

Histological investigation results were as follows (figure 18):

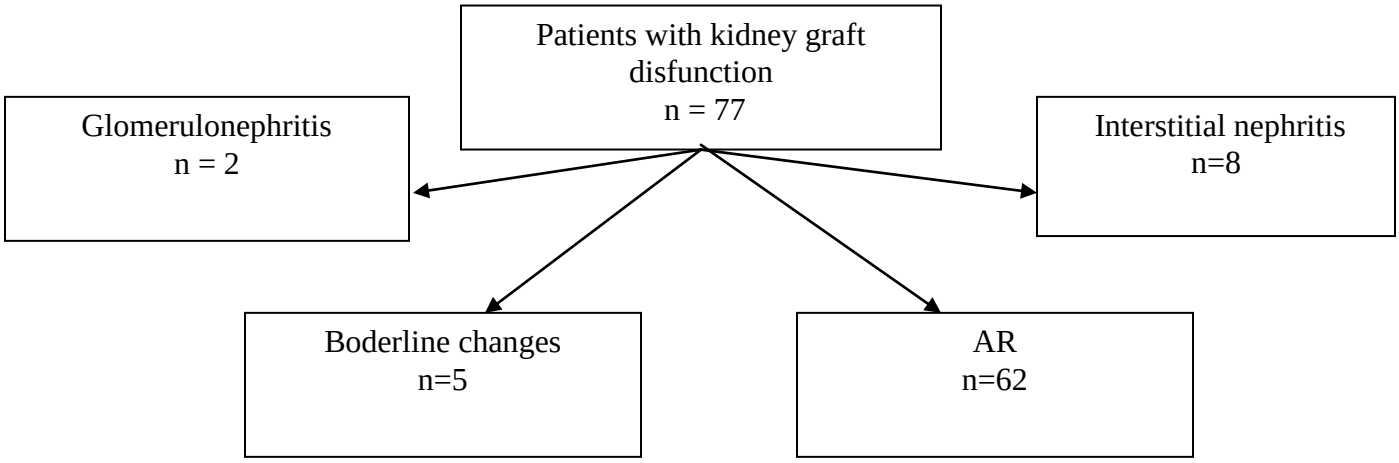

Figure 18. Core biopsy findings in patients with acute renal graft dysfunction early post transplantation

Acute rejection was defined as presence of histological changes from grade IA and higher (Table 8).

Table 8. Grades of acute rejection in patients with acute renal graft dysfunction early post transplantation

Grade of AR	Number of patients
IA	19
IB	13
IIA	21
IIB	4
III	4
Antibody-mediated rejection	4

In two patients with delayed renal graft function antibody-mediated rejection was discovered retrospectively, when C4d deposits were found in peritubular capillaries during the morphological examination of the specimen.

Analyzing the influence of early acute rejection on the late graft function (Table 9) it was stated that after three years serum creatinine levels were slightly higher, but GFR was slightly lower in patients with acute rejection in the early postoperative period, but the difference was not statistically significant (figure 19).

Table 9. Comparison of renal graft functions 3 years after operation in patients with incidence of AR early post transplantation (group 1) and without it (group 2).

	1. grupa (n=62)	2. grupa (n=158)	Fisher's Exact (p)
Sr cr 3 years after transplantation (mmol/l)	0,165±0,058	0.155±0,062	NS
GFR 3 years after transplantation (ml/min)	51,6±16,0	54,2±15,6	NS

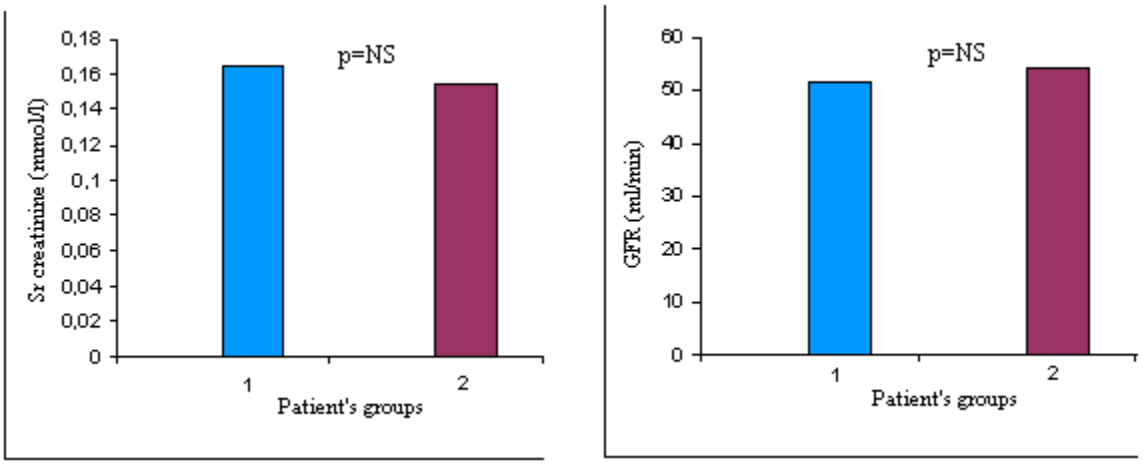

Figure 19. . Level of Sr cr and GFR 36 months after transplantation in patients with incidence of AR early post transplantation (group 1) and without it (group 2).

Comparing the incidence of acute rejection in the late postoperative period, it was stated that it is more likely in patients who already had AR in the early post-transplant period (figure 20).

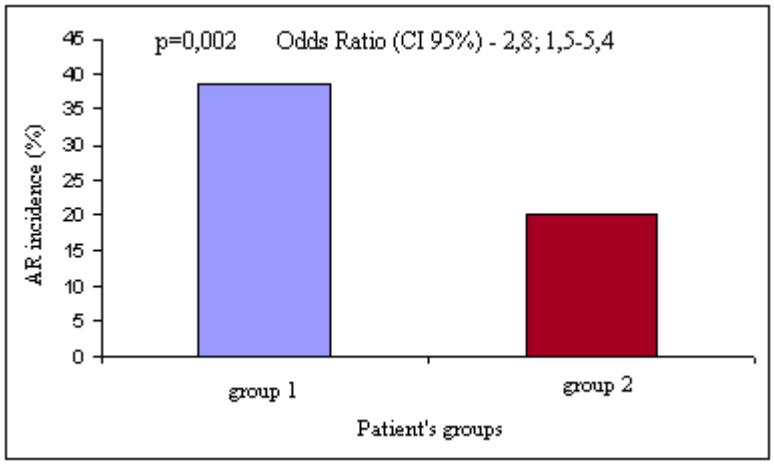

Figure 20. The incidence of AR late after transplantation in patients with incidence of AR early post transplantation (group 1) and without it (group 2).

Graft survival also was shorter in patients who had AR in the early post-transplant period (figure 21).

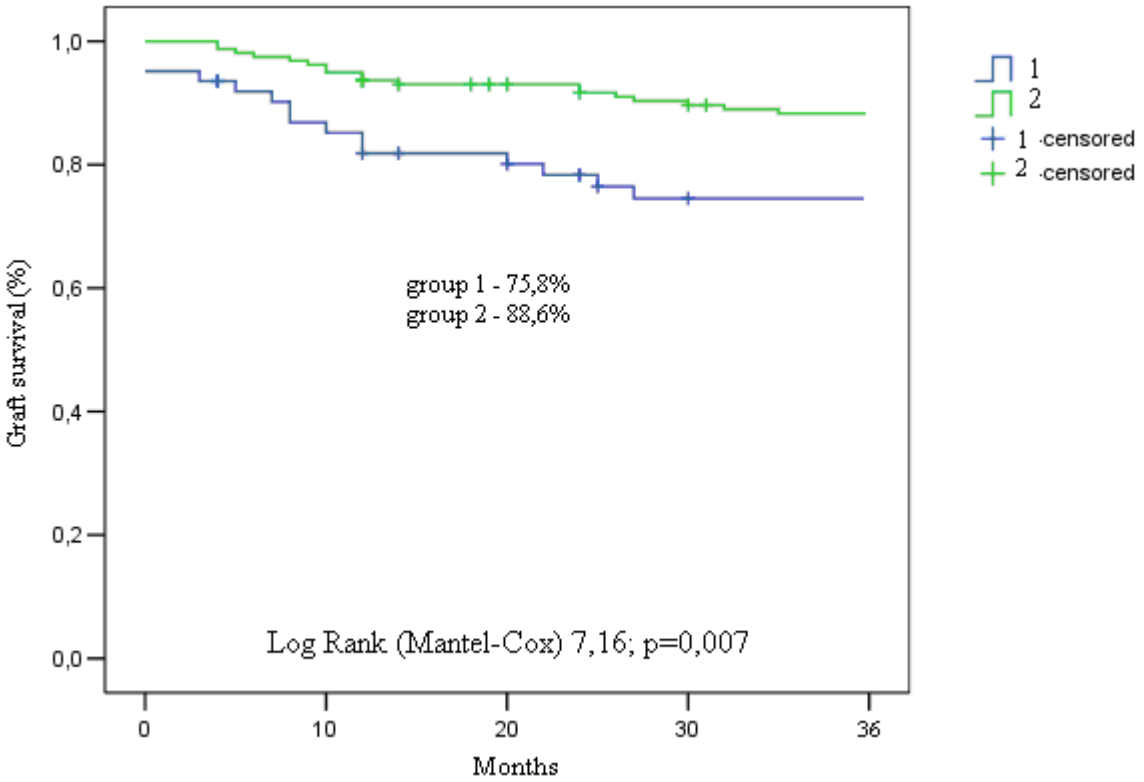

Figure 21. Three years graft survival in patients with incidence of AR early post transplantation (group 1) and without it (group 2).

Comparing graft function after three years in patients with different degrees of acute rejections (Table 10), it was stated that it is similar in both groups (figure 22), but the recipients with more severe degree of acute rejection tend to demonstrate more frequent development of the rejection in the late post-transplant period (figure 23).

Table 10. Renal graft function 36 months after transplantation in patients with different grades of early AR.

	Group 1 (n=30) AR grade I	Group 2 (n=32) AR grade II, III and antibody-mediated rejections.	Fisher's Exact (p)
Sr cr 3 years after transplantation (mmol/l)	0,166±0,062	0,164±0,054	NS
GFR 3 years after transplantation (ml/min)	52,4±16,9	53,3±17,3	NS

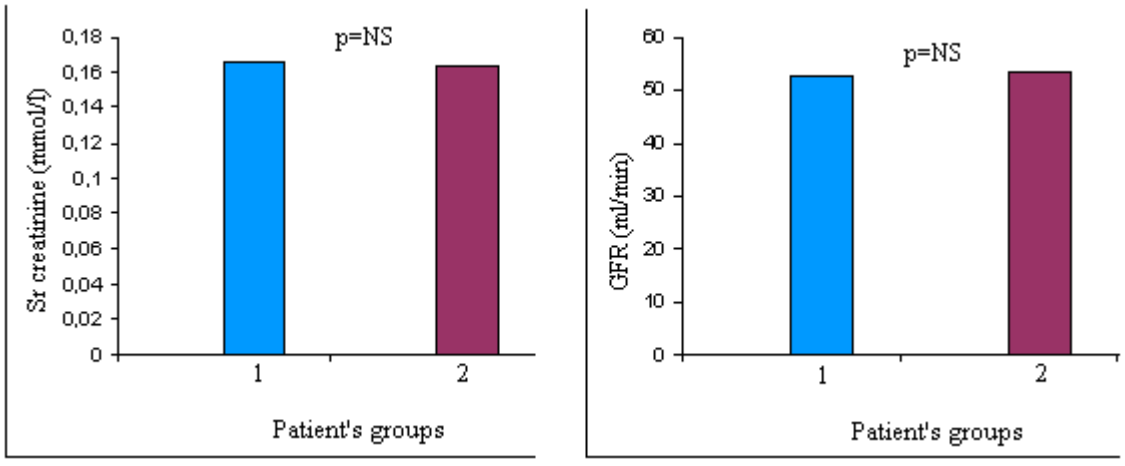

Figure 22. Level of Sr creatinine and GFR 36 months after transplantation in patients with different grades of early AR.

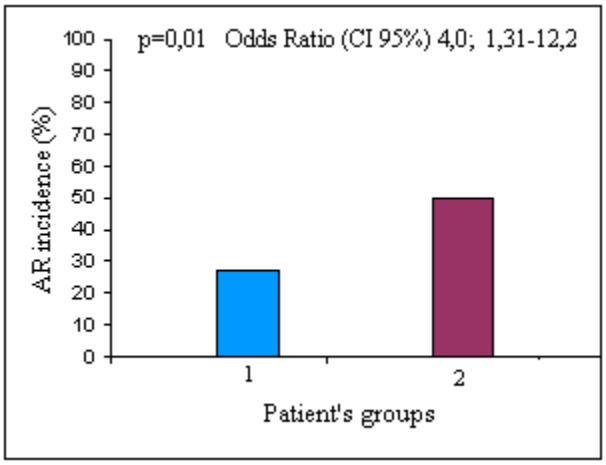

Figure 23. The incidence of late AR in patients with different grades of early AR

Graft survival was shorter in patients with more severe degree of acute rejection (figure 24).

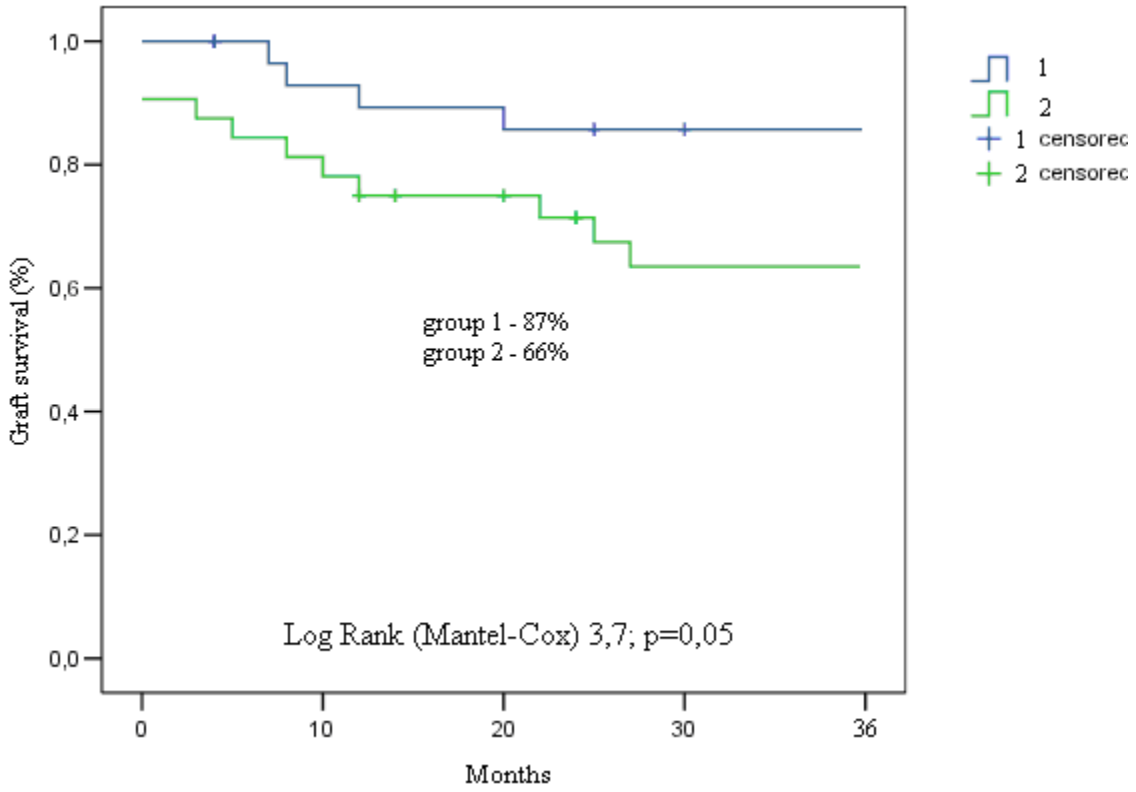

Figure 24. Three years graft survival in patients with different grades of early AR.

Summary.

1. Core biopsy in case of renal graft dysfunction (acute biopsy) helps to establish the cause of dysfunction.
2. The cause of dysfunction in sixty two (80.5%) of 77 patients with sudden deterioration in renal graft function was acute rejection.
3. Acute clinical rejection in the early postoperative period increases the risk of rejection also in the late postoperative period, as well as reduces graft survival.
4. Patients with more severe degree of rejection tend to have shorter renal graft survival and higher AR risk in the late period.

6. CONCLUSIONS

1. Core biopsy is safe and informative method of diagnostics of pathological conditions of renal graft with a minimum risk of complications.
2. Time-zero biopsy specimen provides a possibility to forecast renal graft function in the early and late postoperative period and to select an optimal type of therapy.
3. Patients with delayed graft function showed "hidden" rejection in 79.4% of cases. An early (using protocol biopsy) diagnosis and treatment of "hidden" rejection may reduce the influence of delayed function on the outcome of transplantation.
4. Performing protocol biopsy to patients with primary renal graft function subclinical rejection was diagnosed in 65.4% of cases. Two years' observation results certify that even untreated, subclinical rejection does not affect further graft function.
5. Performing acute biopsy in the early postoperative period, it was stated that the cause of dysfunction in patients with sudden deterioration in renal graft function in the majority of cases (80.5% of cases) was acute rejection.
6. Acute clinical rejection in the early postoperative period increases the risk of rejection in the late postoperative period. The higher is the degree of rejection, the higher is the risk.

7. PRACTICAL RECOMMENDATIONS.

- Taking into account safety and informative value of core biopsy, the use of this manipulation in the estimation of the renal graft condition shall be extended.
- Information acquired from time-zero biopsies shall be used to select the tactics and the treatment schedule for the post-transplant period.
- Starting on the 5^{th}-7^{th} day after the surgery all patients with delayed renal graft function shall have protocol biopsy which shall be repeated every 6-7 days until the function restores. If "hidden" rejection is discovered, biopsy has to be repeated after treatment.
- Patients with primary graft function, without clinical signs of dysfunction and without previous pathologies in the donor kidney require no protocol biopsy, because, according to our data, subclinical rejections discovered in a such way do not influence graft function.
- When treating clinical acute rejections, biopsy has to be repeated in all patients after the treatment course to estimate the effectiveness of the treatment.
- A core biopsy algorithm was developed to optimize the diagnostics of pathological conditions of renal grafts in the early postoperative period (figure 25).

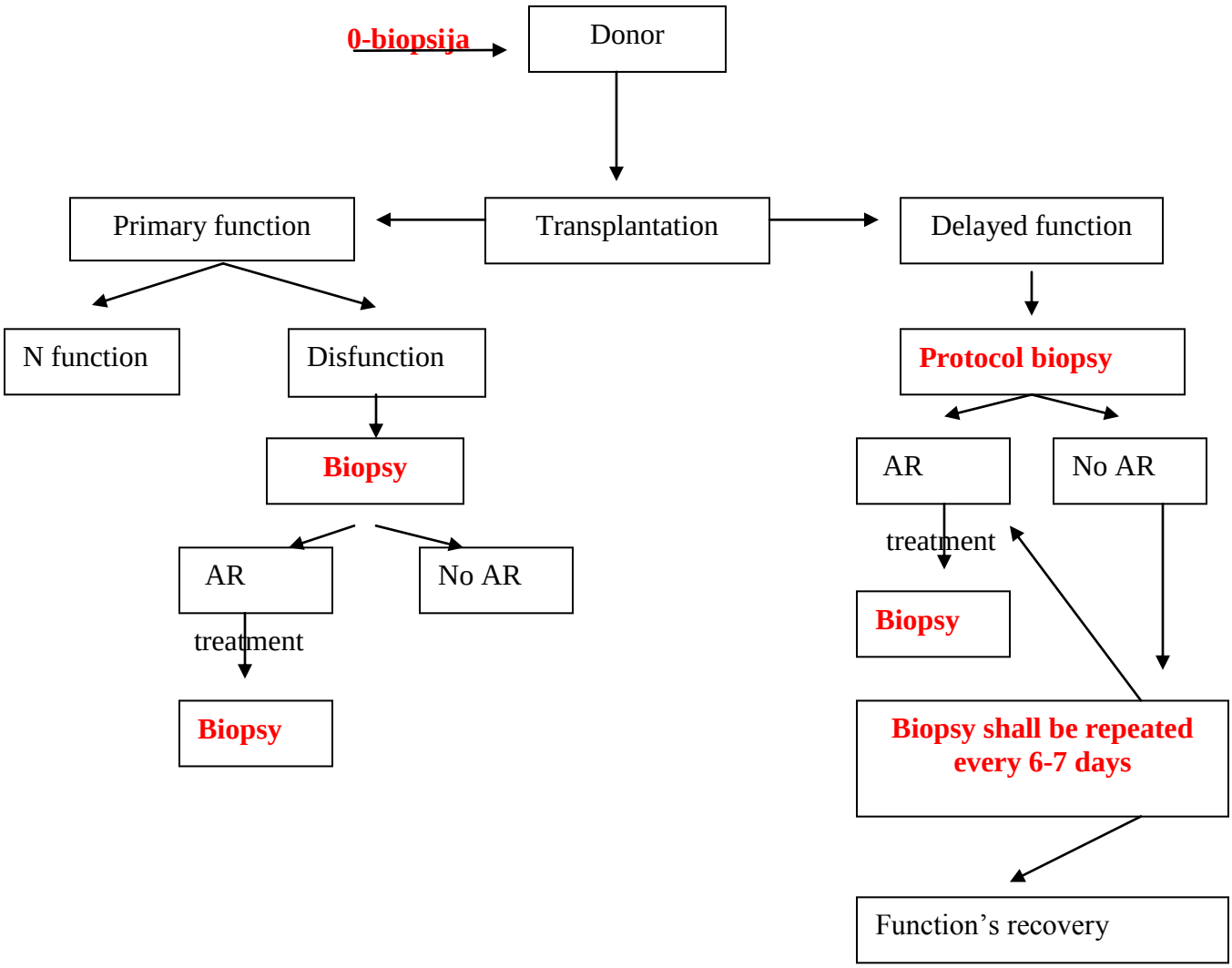

Figure 25. The algorithm of renal graft core biopsy in the early postoperative period

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11. ABSTRACTS

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- Suhorukovs V, Jushinskis J. Impact of subclinical rejection on kidney graft function during the first year after transplantation. Abstracts of the Scandinavian Transplantation Society XXV Congress, 2010, O-19, Abstracts book: 43.