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Rīgas Stradiņa universitāte

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KIDNEY TRANSPLANTATION FROM A DONOR AFTER CARDIOCIRCULATORY DEATH

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine
Speciality – Transplantology

Riga, 2016

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The Doctoral Thesis can be found in the RSU Library and the RSU website: www.rsu.lv

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The Abbreviations and Acronyms Used

ALT – Alanine aminotransferase (U/L)

AST – Aspartate aminotransferase (U/L)

ATG – anti-T-lymphocytic globulin

CIT – cold ischemia time

CPR – cardiopulmonary resuscitation

DBD – donor after brain death

DCD – donor after cardiocirculatory death

DGF – delayed graft function

ER – early rejection

HD – haemodialysis

HLA – Human Leucocyte Antigen

LTC – Latvian Transplant Center

NS – statistically not significant

PD – peritoneal dialysis

PF – primary graft function

PRA – panel reactive antibodies

R-DBD – recipient, who received a kidney from a donor after diagnosed brain death

R-DCD – recipient, who received a kidney from a donor after cardiocirculatory death

S-bradykinin – serum bradykinin (pg/ml)

S-creatinine – serum creatinine (mmol/l)

S-FoxP3 – forkhead box P3 (pg/ml)

U-NGAL – urinary neutrophil gelatinase- associated lipocalin (mg/ml)

Used Definitions

Back table procedure is a procedure for preparing kidney transplant just before the transplantation surgery is started.

Delayed graft function (DGF) is defined in this Doctoral Thesis according to the definition approved by the Latvian Association of Transplantologists as the need for dialysis during the first seven days after transplantation (2, *131–154*). In other cases, graft function is considered as a primary graft function (PF).

Donor organ allocation means matching of a compatible donor-recipient pair.

Early rejection (ER) means rejection that developed during the first month after kidney transplant**.**

Early post-transplant period is the first year after kidney transplantation.

Hypoperfusion time – the time from the beginning of CPR to perfusion of the organs with cold preservative solution.

Pre-transplant puncture biopsy is biopsy of a donor kidney in the donor's body before a process of conservation is started. After histological examination of the tissue, proportion of glomerular sclerosis and interstitial sclerosis expressed as a percentage are determined.

TABLE OF CONTENTS

1. INTRODUCTION

Treatment of patients using organ transplantation including kidney transplantation is currently being carried out incompletely that is connected with the shortage of donor organs.

Better kidney transplantation results can be achieved through transplants from living donors (8, *1259–1266*). However, by using living donors, provision of sufficient number of organs is not possible. In Europe in 2014, 21.7% of all kidney transplantations were performed from living donors (7, *3–31*). That is why deceased donors remain the basic source of donor organs. There are approximately 20,000 kidney transplantations made annually in Europe, out of which 75–80% are obtained from deceased donors. In the 70-ies of the previous century, a concept of brain death was defined and methods were developed for removal of organs from donors after diagnosed brain death (DBD) under the conditions when artificial lung ventilation is provided and blood circulation is preserved (3, *337–340*). Young donors without significant intercurrent medical conditions with diagnosed brain death caused by traumatic cerebral lesions are considered optimal donors for fully functional organs being removed. Extension of indications for organ transplantation, an increasing number of transplantation centers and transplantation activity result in increasing shortage of donor organs despite a wide use of donors with diagnosed brain death. In addition, data were obtained on negative influence of long-lasting state of brain death to function of several organs and organ systems (10, *227–287*).

Therefore, a number of donor organs increased in early 2000-s, which have been obtained from donors after irreversible asystole, under conditions of cardiocirculatory and circulatory death. Initial definition of "*Non-heart beating donor"* was changed to "*Donation after Cardiac/Circulatory Death*", because this definition complies with ethical principles, including the concept of death.

The resulting amount of donor kidneys from donors after cardiocirculatory death (DCD) continues to grow. The largest number of DCD use in European countries in 2004 was 6.81 donor per 1 million inhabitants in the Netherlands. By 2014, DCD rate in the Netherlands increased up to 7.9 donors per 1 million inhabitants. A number of DCD increased almost 8 times in the United Kingdom and Belgium between 2005 and 2014. The increase was also observed in other countries that used DCD (6, *23–38;* 7, *3–31*). In Latvia, there are 6 DCD at an average annually per 1 million inhabitants.

This source of donor organs is widely used for liver transplantation (9, *474–485*). Moreover, reports on successful transplantation of lungs and even heart from donors after cardiocirculatory death appear every year (4, *2585–2591;* 5, *2031–2036*). In its turn, that contributed to the need to address other problems of ethical and medical nature. There is some debate about classification of controlled and uncontrolled types of heartbeat cessation. There are no data on optimal and generally accepted duration of non-touch period that must elapse between the cardiocirculatory death and beginning of organ explantation. Assessment algorithms for several aspects are changing inter alia duration of agonal period, maximum duration of warm and cold ischemia time, etc.

Considering the necessity to improve the results of kidney transplantation from donors after cardiocirculatory death and to extend the use of such donors, there is need to determine the role of clinical and biochemical and morphological criteria for determining functionality of transplants from donors after cardiocirculatory death.

THE GOAL, OBJECTIVES AND HYPOTHESES OF THE THESIS

The Goal of the Research

The goal of the research is to improve the results of kidney transplants obtained from donors after cardiocirculatory death.

Tasks of the Research

- Comparative analysis of functionality of kidney transplants obtained from donors after cardiocirculatory death and from donors after diagnosed brain death.
- Identification of significance of using biomarkers of acute renal failure, renal function, immunologic reactivity, inflammation and hemodynamic state of the donor for assessment of functionality of kidney transplant.
- Exploration of the role of pre-transplantation biopsy for forecasting the outcome of kidney transplantation.
- Creating a set of criteria for forecasting the functional state of kidney transplant in the first year after transplantation.

Hypotheses of the Research

- Kidney transplantation using organs obtained from donors after cardiocirculatory death can provide good transplant outcome.
- Introduction of additional clinical and laboratory investigations of the donors enables prognosing of post-transplant results that way improve donor organ allocation and transplantation outcomes.

NOVELTY OF THE THESIS

Comprehensive research of functional restoration of kidney transplants obtained from donors after cardiocirculatory death in Latvia was conducted. The collected data suggest that functional status of kidney transplants in early post-transplant period does not depend on a type of deceased donor (donors after cardiocirculatory death or donors after diagnosed brain death). It resulted in slightly worse graft function during the first year after transplantation using kidneys from DCD.

Comprehensive assessment of functionality of kidney transplants was conducted by supplementing it with determination of biomarkers in the body of the donor after cardiocirculatory death. It was established that supplementing examination of donors after cardiocirculatory death with determination of serum bradykinin level enabled more precise prediction of the development of delayed graft function.

Transplantation of kidneys from DCD with mild degree of glomerular or interstitial sclerosis does not impact early post-transplant period.

A new method for comprehensive assessment of kidney transplants obtained from donors after cardiocirculatory death was developed.

APPROBATION OF RESEARCH RESULTS

Publications of study results (original papers)

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2. MATERIAL AND METHODS

2.1 Population under Research

The research included 60 donors: 36 donors (60.0%) complied with the definition of DCD and 24 donors (40.0%) complied with the definition of DBD.

Kidney transplantations from 36 DCD were performed in 71 recipient. One kidney was not accepted for transplantation due to poor perfusion quality of preservative solution. Out of 71 recipient, 59 recipients were included in the research according to the recipient selection criteria (R-DCD).

Kidney transplantations from 24 DBD were performed in 48 recipients, out of whose 42 recipients were included in the research according to the recipient selection criteria (R-DBD).

2.2 Examination of Donors and Recipients Included in the Research

Examination of the donors included in the research was carried out on the basis of algorithm accepted by the Latvian Transplantation Center (1, *39–46*), by supplementing it with sampling of blood and urine for determination of biomarkers. Obtaining biological material takes place just before beginning of organ explantation procedure.

The following parameters were defined and further analysis was carried out for all the donors: age (years), duration of hospitalization (days), serum creatinine (mmol/l), cystatin C level in serum (mg/l), serum FoxP3 (*forkhead box P3*) level (pg/ml), serum bradykinin level (pg/ml), urinary neutrophil gelatinase- associated lipocalin (NGAL) level (mg/ml), WBC count (thousand/L), RBC count (million/L), haemoglobin concentration (g/L), haematocrit (%), platelet count (thousand/L), serum ALT (U/L), serum AST

(U/L), glomerular sclerosis of transplant (%), interstitial sclerosis of transplant (%), and hypoperfusion time (min).

The following parameters were defined and further analysis was carried out for all the recipients: age (years), cold ischemia time (CIT) (hours), duration of transplantation surgery (minutes), weight of graft (grams), the recipient's body weight (kilograms), recipient/graft weight ratio (g/g), gender, the first or repeated transplantation, the right or the left kidney transplant, type of dialysis before the transplantation, medications applied for induction immunosuppression (*Basiliximab* or anti-T-lymphocytic globulin (ATG)).

Graft function was evaluated according to the recipient's serum creatinine on day 7, 14, and 30 after transplantation, and at 2, 3, 6, 9, and 12 months after transplantation. At all relevant times, glomerular filtration rate (GFR) was calculated using the Cockcroft and Gault formula.

Transplantation outcomes were evaluated according to the following criteria:

- Delayed or primary graft function,
- Histologically proven acute rejection in the first month after transplantation,
- GFR at month 12 after transplantation is higher or lower than 60 ml/min.

Survival analysis of the graft and recipient was carried out.

U-NGAL level was determined in the last urine sample of a donor by using the ARCHITECT appliance (*Abbott Laboratories*). U-NGAL analysis is chemiluminescent microparticle immunoassay analysis (CMIA) for quantitative determination of neutrophil gelatinase-associated lipocalin in human urine. Reference range of the analysis is between 0 and 130 ng/ml.

Bradykinin level in serum was counted in serum samples of a donor by using bradykinin detection *Abcam® in vitro* by means of ELISA (*Enzyme-*

Linked Immunosorbent Assay) method. Detection range is from 11.7 pg/ml to 30,000 pg/ml.

Cystatin C level in serum is determined in serum samples of a donor by using the N Latex Cystatin C in vitro for quantification by means of BN II Nephelometer (Siemens). Reference range is from 0.62 to 1.11 mg/L.

FoxP3 level in serum is determined in serum samples of a donor by using *Quantitative Sandwich ELISA* detection method. Detection range is from 31.2 pg/ml to 1,000 pg/ml.

Serum creatinine level was determined by using blood Chemistry Analyzer *Ilab ARIES (Instrumentation Laboratory)*. Reference range in women is from 0.06 to 0.11 mmol/l, whereas reference range in men is from 0.08 to 0.12 mmol/l.

Glomerular filtration rate (GFR) was calculated according to the Cockcroft and Gault equation:

$$GFR = \frac{(140 - age) * body\ weight\ (kg)}{Serum\ creatinine\ (\mu mol/L) * 0.81}$$

The result obtained for women is multiplied by 0.85.

To express serum creatinine level in units of μmol/L, the result expressed in mmol/L is multiplied by 1,000.

Clinical blood panel (WBC count, RBC count, haemoglobin concentration (Hgb); Haematocrit (Hct), platelet count (PLT)) was carried out by using *Beckman Coulter* Instrument – HMx (5 diff.).

Pre-transplant kidney biopsy was performed during explantation of donor organs before preservation process was started by using a disposable automatic biopsy needle *Angiotech Tru-Core^{TM} 18 ga*. The obtained histological material after fixation in 4% formalin solution was coloured by using haematoxylin-eosin or Masson (trichrome) colouring methods, and was

examined by means of light microscopy method. For further analysis, volume of glomerular sclerosis and interstitial sclerosis expressed as a percentage was used. Puncture biopsy was recognised as informative if five or more glomeruli were detected in the obtained material.

Acute rejection is diagnosed by graft puncture biopsy with subsequent histological examination of the material obtained.

Weighting of donor kidney is done at the end of donor kidney preparation (*back table*), by using electronic scales *Comfort LBS-6019* with weighing precision of ± 1 gram. Before weighing procedure of each donor kidney, control weighing is carried out with weight control of 100 grams.

Weighting of recipients was carried out by using electronic scales *CAS DB-II* with precision of ± 50 grams. Scales are calibrated by laboratory Metrolab Ltd.

The following analysis included weight of donor kidney (grams), body weight of recipient (kilograms), and ratio of the body weight of recipient and the weight of donor kidney (expressed in grams).

After transplantation, serum creatinine level of the recipient at day 7, 14, and 30 after transplantation, and at month 2, 3, 6, 9, and 12 after transplantation was determined. At all relevant times, GFR was calculated.

2.3 Organ Explantation, Preservation, and Transplantation Technique

Access to organ preservation and explantation is ensured by midline laparotomy incision and subsequent *in situ* organ perfusion with *HTK* (Custodiol®) solution. For perfusion of preservative solution, right-side iliac artery access is used, for drainage of venous system – the lower vena cava access. Organ explantation occurs by exciding the right and then the left kidney with feeding blood vessels and other anatomical structures.

Allocation of donor kidney is carried out based on ABO compatibility and results of lymphocytotoxic cross-match. Allocation also takes place following the principle of the *old-to-old*, when a donor organ of elder person is transplanted to an elderly recipient. Size of donor kidney and weight of a recipient are taken into account. HLA compatibility was not followed.

Cross-match and determination of panel reactive antibodies (PRA) were conducted at the Histocompatibility Laboratory of the National Blood Donor Centre. Cross-match was performed by means of *flow cytometry*. PRA titres are determined by HLA antibody screening by using 20 randomized donor panel.

Kidney transplantation is performed in the left or right hypogastrium in the retroperitoneal space. Transplant blood flow is provided by using the iliac vascular access of a recipient. Urinary drainage from the donor kidney is ensured by establishment of neoureterocystostomy.

Induction immunosuppression therapy is started just before or during the kidney transplantation. Medication containing interleukin-2 receptor anta gonist – Basiliximab (Simulect, Novartis) 20 mg i/v on the day of surgery and the fourth postoperative day is used or medication containing polyclonal antibodies against T-lymphocytes (ATG, Fresenius Biotech) 1.5–3 mg/kg i/v for the first 3–5 postoperative days.

Maintenance immunosuppressive therapy includes a combination of calcineurin inhibitors (Cyclosporine or Tacrolimus), mycophenolate (Mycophenolate mofetil or mycophenolic acid) and corticosteroids (Prednisone or methylprednisolone) combination.

In the event of acute rejection, treatment is initiated with 500 mg methylprednisolone i/v 3–5 days in turn. In the event of steroid-resistant acute rejection, treatment is continued with anti-T lymphocyte globulin 1.5–3 mg/kg a day i/v for 10–14 days.

2.4 Statistical Data Processing

For development of a database for obtained results, software Microsoft Excel 2013 was applied, and a program IBM SPSS Statistics Version 22 was used for statistical analysis thereof. All indexes are indicated as a mean ± standard deviation (st. dev.) or a numeric value and as a percentage.

Independent Samples T-Test was applied for analysis of parametric data. Non-parametric data analysis involved Pearson Chi-square test.

Odds Ratio (OR) was defined that is probability of one event occurrence in one group against probability of the same event occurrence in the second group. Confidence Interval (CI) is quantified as 95%.

Graft and recipient survival was analysed by means of Kaplan-Meier surveillance test. Comparison of two groups included Log Rank (Mantel-Cox) criterion.

In order to predict probability of transplantation outcome, binary logistic regression was applied.

Statistical significance of results was defined at $p < 0.05$.

3. RESULTS

3.1 Comparison of transplantation results between donors after cardiocirculatory death and donors after brain death

Incidence of delayed graft function (DGF) does not vary between R-DCD and R-DBD groups (16.9% and 14.3% respectively, p = 0.718) (Figure 3.1).

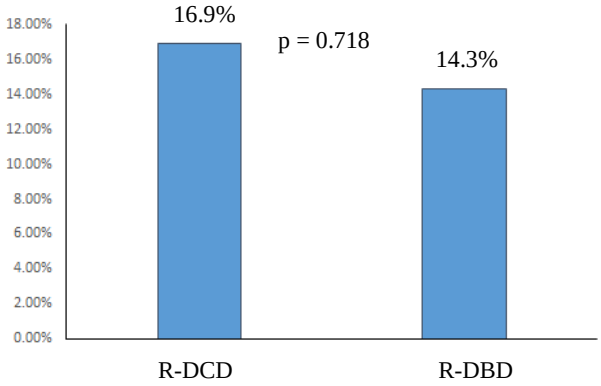

Fig. 3.1 **Delayed graft function**

Incidence of early rejection (ER) was also not statistically significantly different between R-DCD and R-DBD groups (18.6% and 23.8%, respectively, p = 0.528), Figure 3.2.

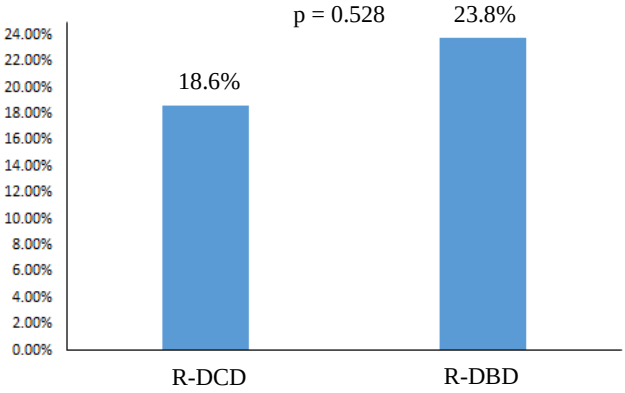

Fig. 3.2 **Early rejection**

When comparing GFR during the first year after transplantation, statistically significantly lower GFR was observed in R-DCD group in month 1, 9, and 12 after transplantation than the one in R-DBD group, where $p < 0.05$ in all cases. A trend for lower GFR level in R-DCD group was observed on day 14, and in month 2 and 3 after transplantation (Figure 3.3).

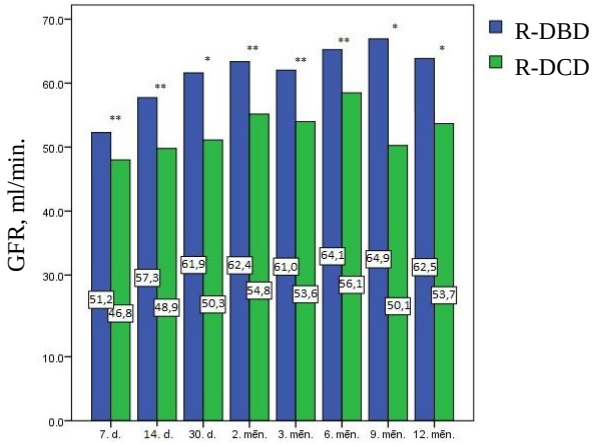

Fig. 3.3 **GFR comparison between R-DCD and R-DBD**
* $p < 0.05$; ** $p \geq 0.05$

One-year death censored graft survival in R-DCD group was 94.9%, which is not statistically significantly different from a one-year graft survival rate in R-DBD group, 95.2% (Log Rank (Mantel-Cox) 0.009, p = 0.923) (Figure 3.4).

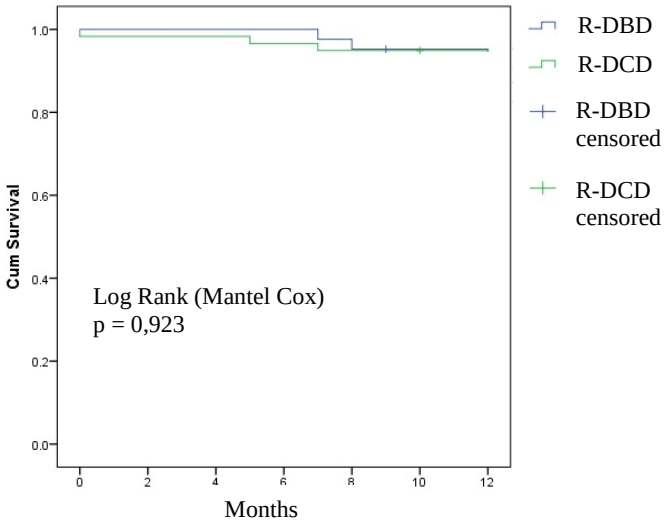

Fig. 3.4 **One-year death censored graft survival**

One-year recipient survival rate also does not differ statistically significantly between R-DCD and R-DBD groups (98.3% and 97.6% respectively, Log Rank (Mantel Cox) 0.063, p = 0.802) (Figure 3.5).

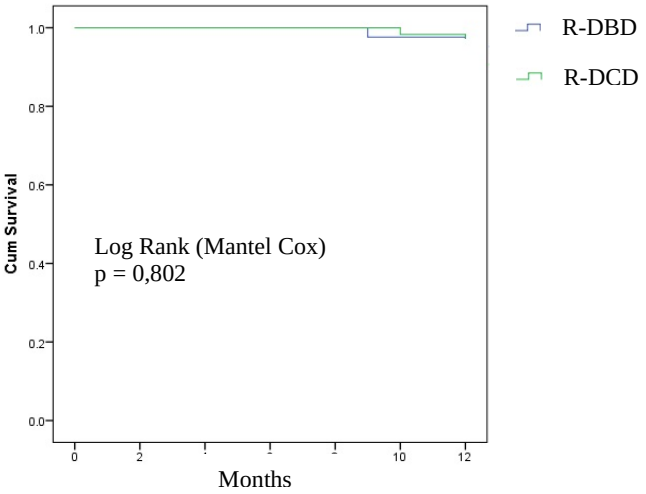

Fig. 3.5 **One-year recipient survival rate**

Summary:

1. Having analysed the outcomes of transplantation from DCD and DBD, it was found that graft survival, recipient survival, and DGF and ER incidence did not differ between the two groups.

2. It resulted in slightly worse graft function during the first year after transplantation in R-DCD group.

3.2 Delayed Graft Function and Factors Affecting It, when using Donors after Cardiocirculatory Death

Having analysed development of delayed graft function in recipients, it was concluded that recipients, who experienced delayed graft function (DGF), received a kidney from donors with a statistically significantly lower cystatin C level in serum (Figure 3.6), the highest bradykinin level in serum (Figure 3.7), and the highest ALT level in serum (Figure 3.8), statistically significantly lower

WBC count (Figure 3.9), and lower platelet count (Figure 3.10) when compared to the recipients who experienced primary graft function (PF).

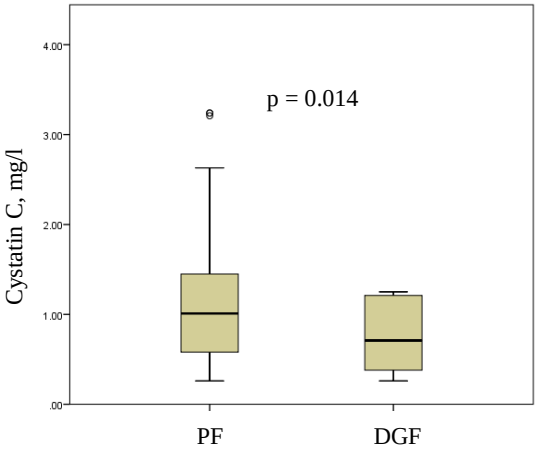

Fig. 3.6 **Cystatin C level in serum of the donor in PF and DGF groups**
(1.2 ± 0.8 mg/l vs. 0.76 ± 0.4 mg/l, p = 0.014)

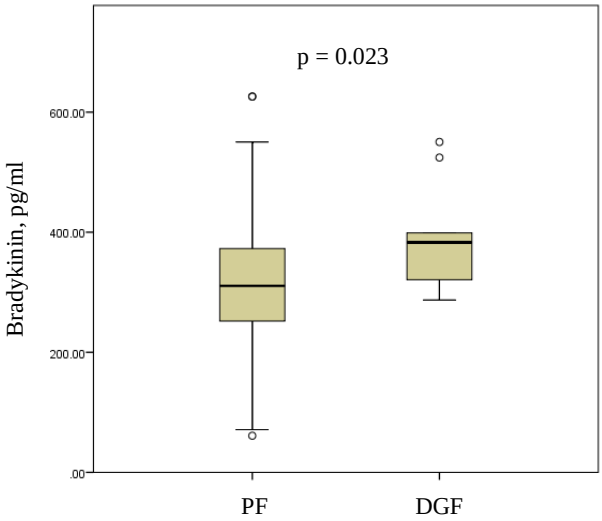

Fig. 3.7 **Bradykinin level in serum of the donor in PF and DGF groups**
(308.75 ± 138.5 pg/ml vs. 391.31 ± 86.1 pg/ml, p = 0.023)

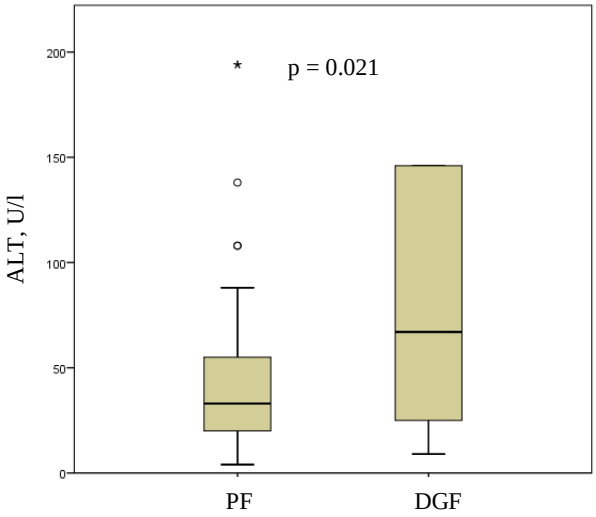

Fig. 3.8 **ALT level in serum of the donor in PF and DGF groups**
(60.9 ± 85.1 U/l vs. 147.6 ± 178.0 U/l, p = 0.021)

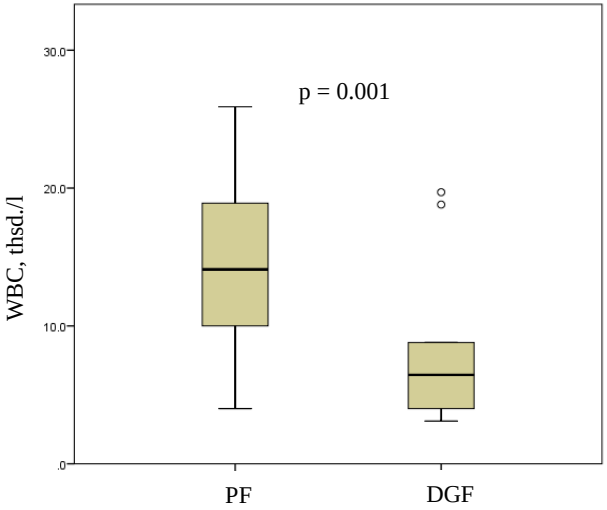

Fig. 3.9 **WBC count of the donor in PF and DGF groups**
(14.5 ± 5.8 thsd./l vs. 8.4 ± 6.1 thsd./l, p = 0.013)

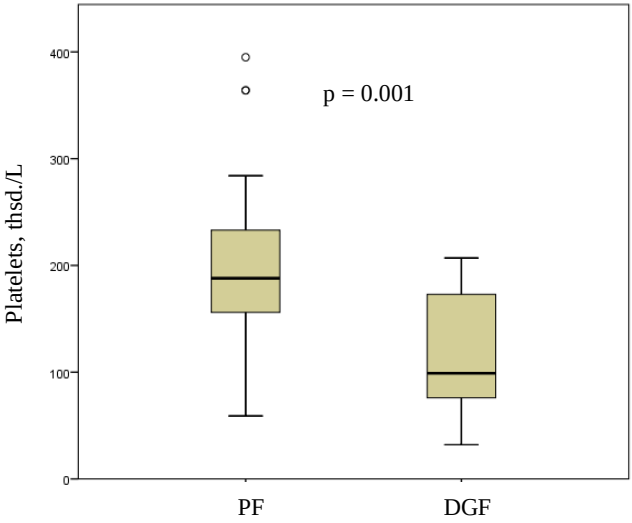

Fig. 3.10 **Platelet count of the donor in PF and DGF groups**
(199.1 ± 71.7 thsd./l vs. 112.7 ± 59.9 thsd./l, p = 0.001)

A trend towards lower serum creatinine and lower serum FoxP3 levels in donors was observed in cases, associated with the development of the delayed graft function. Other donor factors did not differ statistically significantly between the groups of recipients with delayed or primary graft function ($p > 0.05$ in all cases).

Having analysed the factors of recipients, it was stated that the DGF group had longer CIT (Figure 3.11) and higher recipient/graft weight ratio (Figure 3.12).

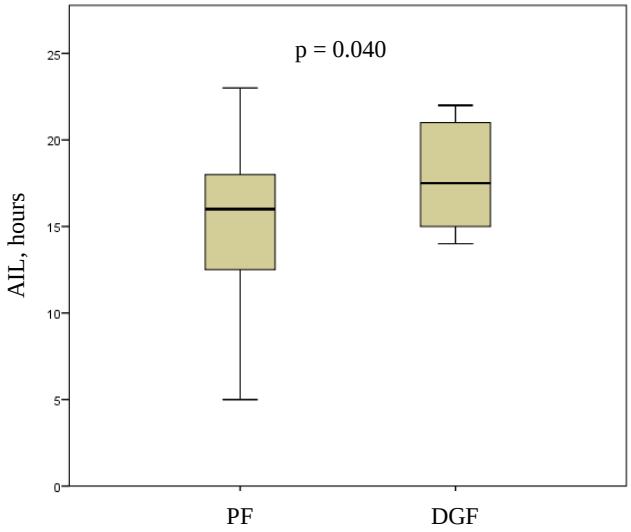

Fig. 3.11 **CIT in PF and DGF groups**
(15.2 ± 4.2 hours vs. 17.8 ± 3.1 hours, p = 0.040)

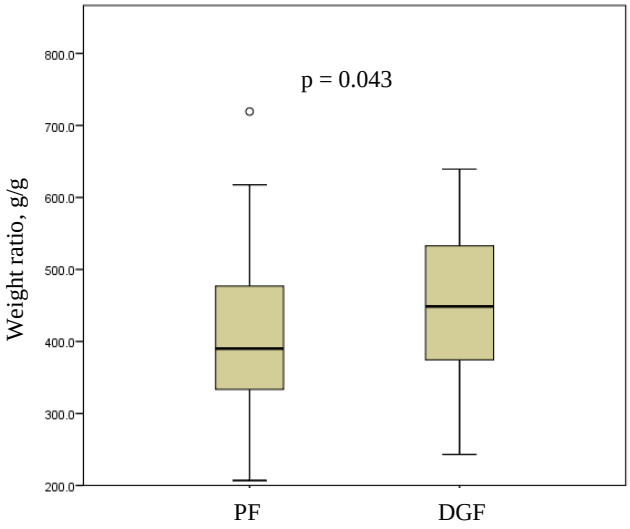

Fig. 3.12 **Recipient/graft weight ratio in PF and DGF groups**
(402.8 ± 111.6 g/g vs. 504.5 ± 145.9 g/g, p = 0.043)

Other factors of recipients did not differ statistically significantly in the event of delayed or primary graft function ($p > 0.05$ in all cases).

While carrying out logistic regression analysis in order to predict probability of delayed graft function, an equation with values given in Table 3.1 was obtained.

Table 3.1

Results of Logistic Regression Analysis for Prediction of Delayed Graft Function

Factor	Values ± st. error	p	OR (95 % CI)
S-Bradykinin	0,008 ± 0,004	0,071	1,090 (0,993–1,196)
ALT	0,009 ± 0,004	0,024	1,009 (1,001–1,017)
CIT	0,360 ± 0,157	0,022	1,434 (1,053–1,951)
Recipient/graft weight ratio	0,006 ± 0,003	0,069	1,006 (1,000–1,012)
Constant	−13,629 ± 4,50	0,002	

The index characterising quality of an established logistic analysis model, Nagelkerke $R^2 = 44.5\%$. The developed equation enables us classifying 88.1% of patients correctly.

The model also included other donor and recipient specific factors, but these values did not affect simulation results statistically significantly (p = NS in all cases).

According to the model developed, probability of delayed graft function can be calculated by the following formula:

$$P_{DGF} = \frac{e^{-13.63+0.008*S-Brady+0.009*ALT+0.36*CIT+0.006*RGW}}{1+e^{-13.63+0.008*S-Brady+0.009*ALT+0.36*CIT+0.006*RGW}}$$

e = 2.71;
S-Brady – serum bradykinin (pg/ml);
RGW – recipient/graft weight ratio (g/g).

When conducting Pearson Chi-square test, it was found that bradykinin level in serum of a donor above 300 pg / ml, ALT level above 75 U/l, CIT over

21 hour were statistically significantly associated with delayed graft function (Table 3.2).

Table 3.2

Pearson Chi-Square Test for Probability of Delayed Graft Function

Factor	p	OR (95% CI)
Serum bradykinin ≥ 300 pg/ml	0,023	8,640 (1,016–73,476)
Serum ALT ≥ 75 U/l	0,050	3,900 (0,942–16,151)
CIT ≥ 21 stundas	0,052	4,821 (0,885–26,276)

In patients with delayed graft function, GFR was statistically significantly lower in the first month after transplantation, whereas no differences in GFR were stated later on (Figure 3.13).

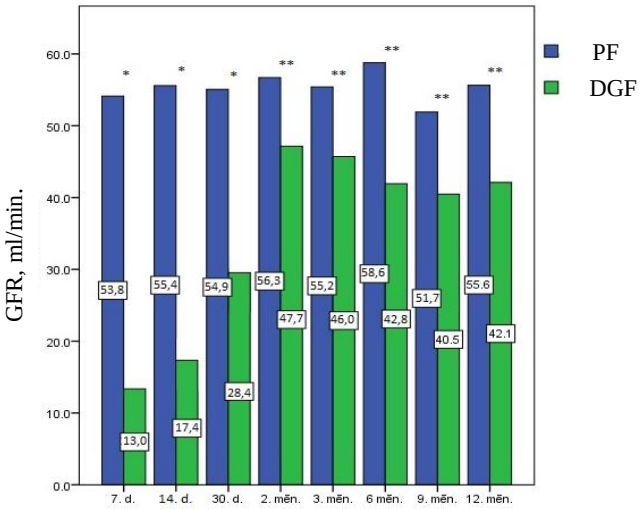

Fig. 3.13 **The first post-transplant year GFR in the event of primary and delayed graft function**

* $p < 0.05$; ** $p \geq 0.05$

Summary:

1. Increased bradykinin level in serum of a donor above 300 pg/ml, ALT level in serum of a donor above 75 U/l, and CIT longer than 21 hour are risk factors for delayed graft function.

2. Development of delayed graft function worsened the graft function during the first postoperative month statistically significantly, whereas no statistical significance was observed later on.

3. Results of pre-transplantation puncture biopsy showed no association with delayed or primary graft function.

4. A model for predicting delayed graft function with the following characteristics of the donor and the recipient was drafted: bradykinin level in serum of a donor, ALT level in serum of a donor, CIT, recipient/graft weight ratio.

3.3 Early Rejection and the Factors Affecting It, when using Donors after Cardiocirculatory Death

When comparative analysis of the donor specific factors for recipients with early rejection (ER) and recipients without early rejection (without ER) was carried out, statistically significant differences in serum creatinine levels (Figure 3.14), WBC count (Figure 3.15), and platelet count (Figure 3.16) were stated.

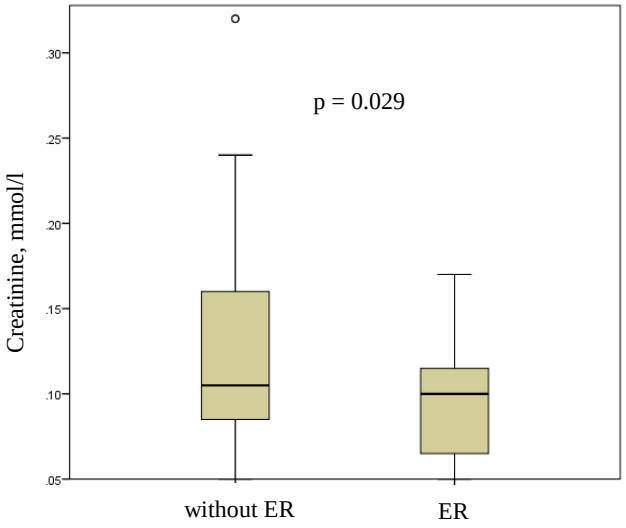

Fig. 3.14 **Donor S-Crea level in groups without ER and with ER**
(0.13 ± 0.08 mmol/l vs. 0.09 ± 0.04 mmol/l, p = 0.029)

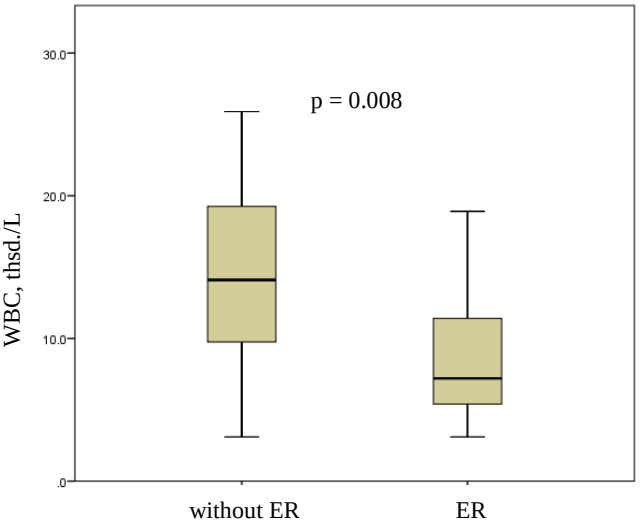

Fig. 3.15 **Donor WBC count in groups without ER and with ER**
(14.5 ± 6.0 thsd./L vs. 8.9 ± 5.4 thsd./L, p = 0.008)

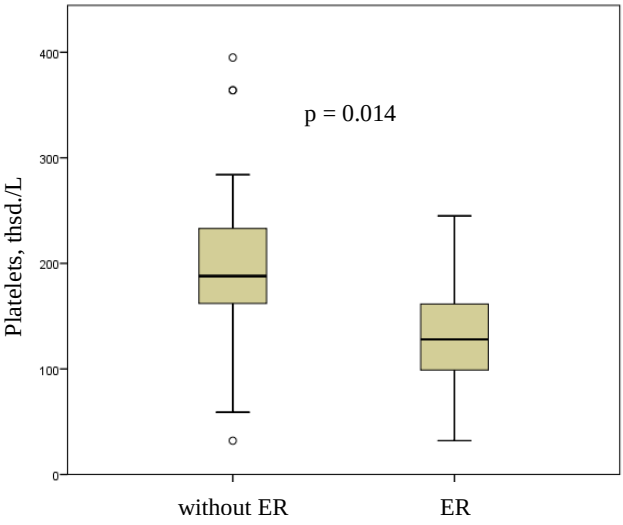

Fig. 3.16 **Donor platelet count in groups without ER and with ER**
(195.8 ± 74.7 thsd./L vs. 134.8 ± 64.3 thsd./L, p = 0.014)

Other donor specific factors did not differ statistically significantly in groups with ER and without ER ($p > 0.05$ in all cases).

When recipient specific factors were analysed, it concluded that there was statistically significantly longer CIT (Figure 3.17) and higher recipient/graft weight ratio (Figure 3.18) in the ER group.

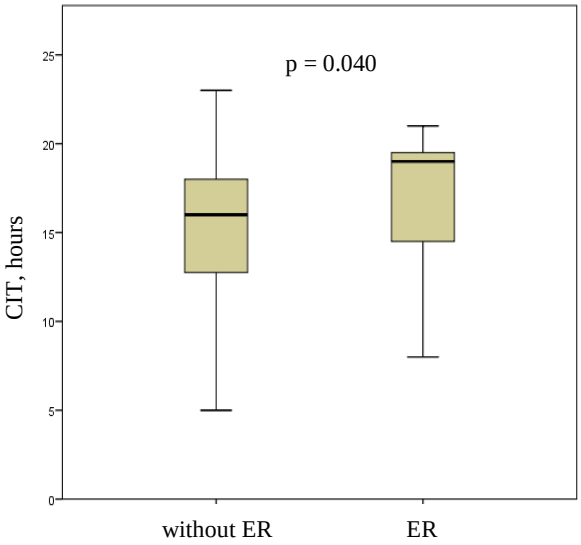

Fig. 3.17 **CIT in groups without ER and with ER**
(15.4 ± 4.1 hours vs. 17.0 ± 4.0 hours, p = 0.040)

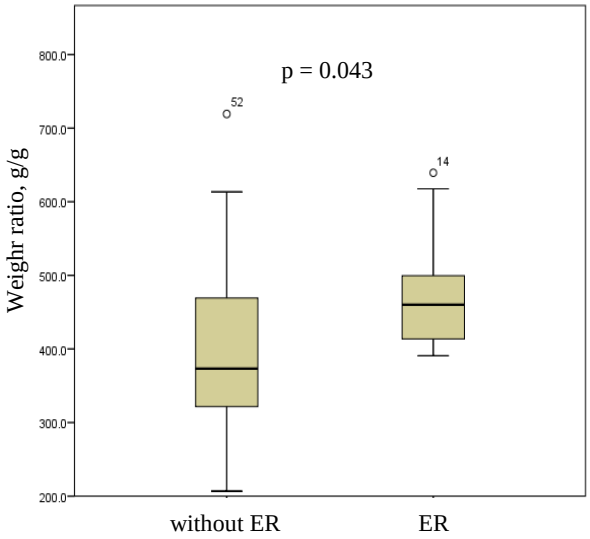

Fig. 3.18. **Recipient/graft weight ratio in groups without ER and with ER**
(407.2 ± 153.9 g/g vs. 476.2 ± 84.5 g/g, p = 0.043)

When Pearson Chi-square test was made, it was found that early rejection more often developed in the event of repeated transplantation (χ^2 = 4.32, p = 0.038) (Figure 3.19).

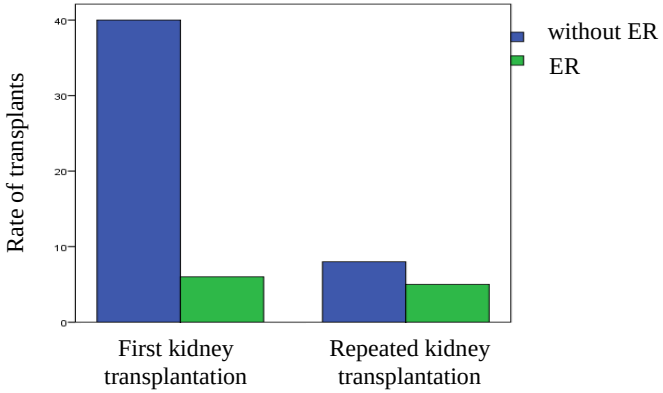

Fig. 3.19 **ER in the event of the first and repeated transplantation**

Pearson Chi-square test also showed a trend towards more frequent development of early rejection in recipients with delayed graft function (χ^2 = 3.62, p = 0.057) (Figure 3.20).

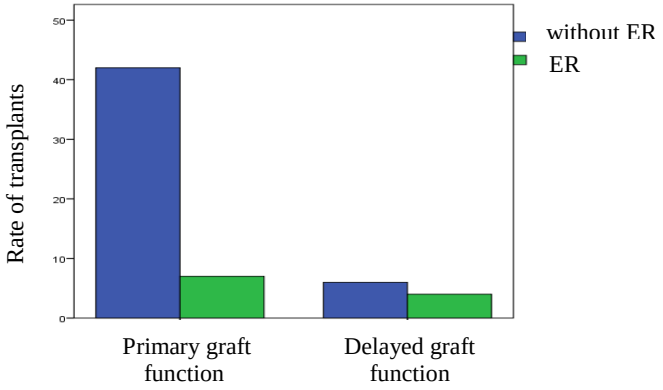

Fig. 3.20 **ER in the event of delayed and primary graft function**

Other recipient specific factors did not differ statistically significantly in groups with early rejection and without it (p> 0.05 in all cases).

Having made logistic regression analysis to predict early graft rejection, an equation was obtained with the values given in Table 3.3.

Table 3.3

Results of Logistic Regression Analysis when Predicting Early Rejection

Factor	Values ± st. error	p	OR (95% CI)
Repeated transplantation	2,496 ± 1,068	0,019	12,137 (1,498–98.355)
Delayed graft function	3,281 ± 1,295	0,011	26,590 (2,101–336,460)
Age of recipients	−0,094 ± 0,036	0,010	0,910 (0,848–0,978)
Age of donors	0,098 ± 0,058	0,095	1,102 (0,983–1,236)
Constant	−3,701 ± 2,609	0,156	

The index characterising quality of an established logistic analysis model, Nagelkerke R^2 = 41.9%. The developed equation enables us classifying 83.1% of patients correctly.

The model also included other donor and recipient specific factors, but these values did not affect simulation results statistically significantly (p= NS in all cases).

According to the model developed, probability of delayed graft function can be calculated by the following formula:

$$P_{AR} = \frac{e^{-3.7+2.496*\text{Rep.Tr.}+3.281*DGF+(-0.094*RA)+0.098*DA}}{1+e^{-3.7+2.496*\text{Rep.Tr.}+3.28*DGF+(-0.09*RA)+0.098*\text{DA}}}$$

e = 2.71;
Rep. Tr. – repeated transplantation;
DGF – delayed graft function;
RA – age of recipient;
DA – age of donor.

Recipient age under 40 years was statistically significantly associated with development of early acute rejection (OR = 7.58; 95% CI = 1.82 – 31.57, p = 0.003).

GFR in recipients with early rejection was statistically significantly lower in the first week after transplantation, whereas GFR differences did not reach statistical significance later on (Figure 3.21).

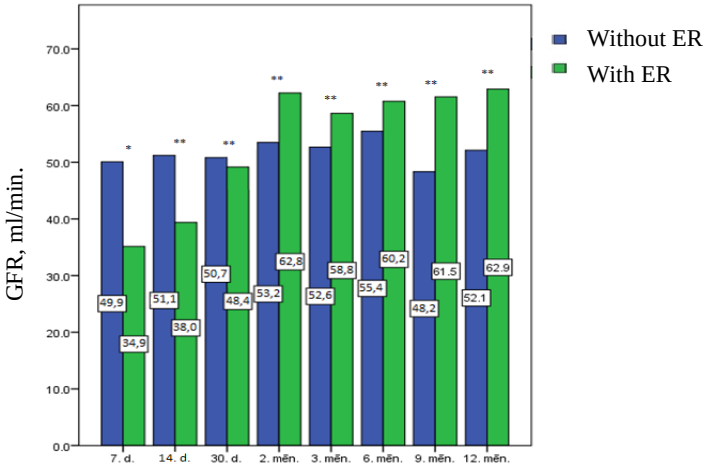

Fig. 3.21 **GFR in recipient groups without ER and with ER**
* $p < 0.05$; ** $p \geq 0.05$

Summary:

1. Delayed graft function, repeated kidney transplantation, age of recipient under 40 years are risk factors for development of early rejection.

2. Development of early rejection had no effect on graft functionality in the early post-transplant period.

3. Donor U-NGAL (biomarker for acute renal failure), Cystatin C in serum (biomarker for functional condition of kidney), serum FoxP3 (biomarker for immunological reactivity of donor) and serum bradykinin (biomarker for

inflammation and haemodynamic status), and results of pre-transplantation biopsy were not predictive for development of early graft rejection.

4. A model for predicting early rejection with the following characteristics of the donor and the recipient was drafted: repeated or the first kidney transplant, delayed or primary graft function, age of the recipient and age of the donor.

3.4 Graft Function at the 12th Month after Surgery and the Factors Affecting It, when using Donors after Cardiocirculatory Death

Having analysed graft function at the 12th month after surgery, it was stated that the recipients, whose GFR was less than 60 ml/min., received transplants from older donors (Figure 3.22) and from donors with longer duration of hospitalization (Figure 3.23).

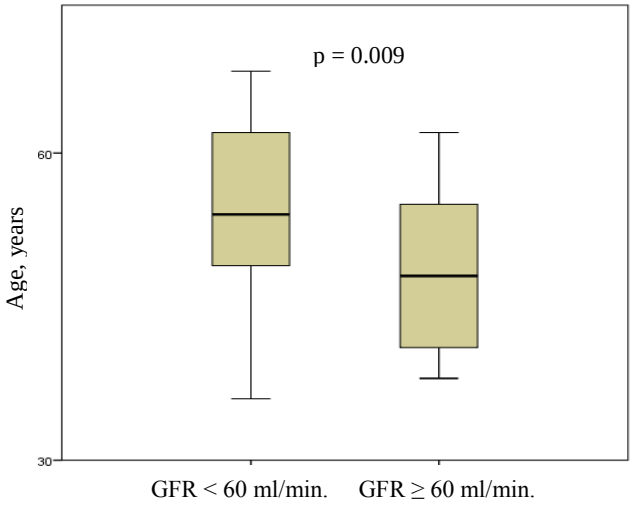

Fig. 3.22 **Age of donors in recipient groups with GFR ≥ or < 60 ml/min.** (54.5 ± 8.4 years vs. 48.4 ± 7.6 years, p = 0.009)

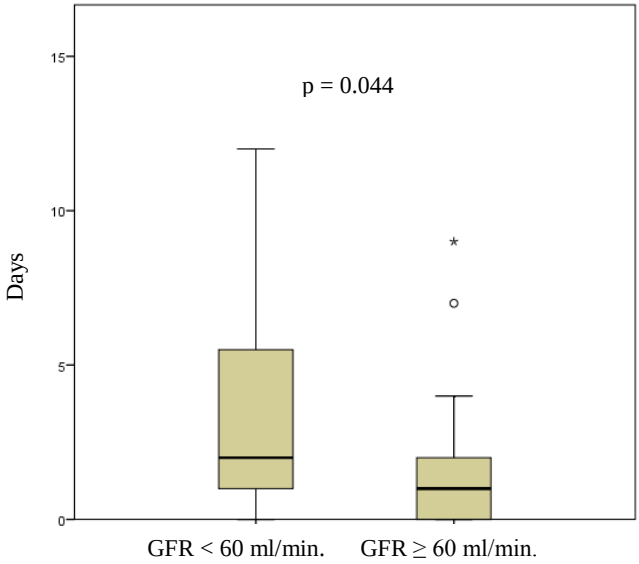

Fig. 3.23. **Duration of donor hospitalisation in recipient groups with GFR ≥ or < 60 ml/min.**
(4.4 ± 5.3 days vs. 1.8 ± 2.4 days, p = 0.044)

Other donor specific factors did not differ statistically significantly between the groups of recipients with GFR ≥ or <60 ml/min. ($p > 0.05$ in all cases).

The recipients with GFR < 60 ml/min. were statistically significantly older (Figure 3.24), and their body weights were lower (Figure 3.25) if compared with the recipients with GFR ≥ 60 ml/min. at the 12th month after surgery.

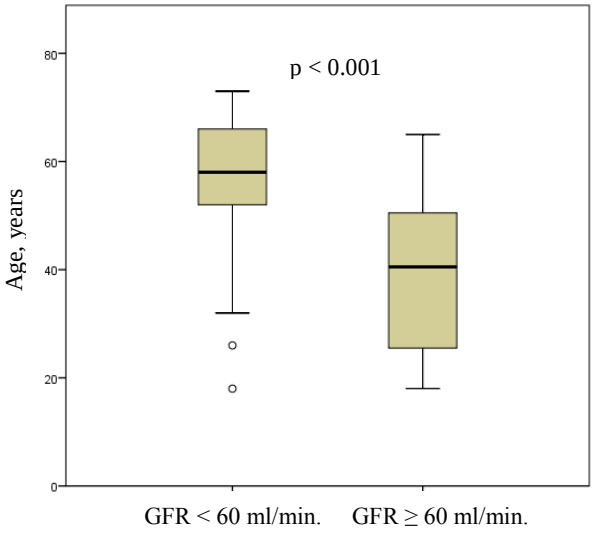

Fig. 3.24 **Age of recipients in groups with GFR ≥ 60 ml/min and GFR < 60 ml/min.**
(55.9 ± 13.5 years vs. 39.7 ± 15.2 years, p < 0.001)

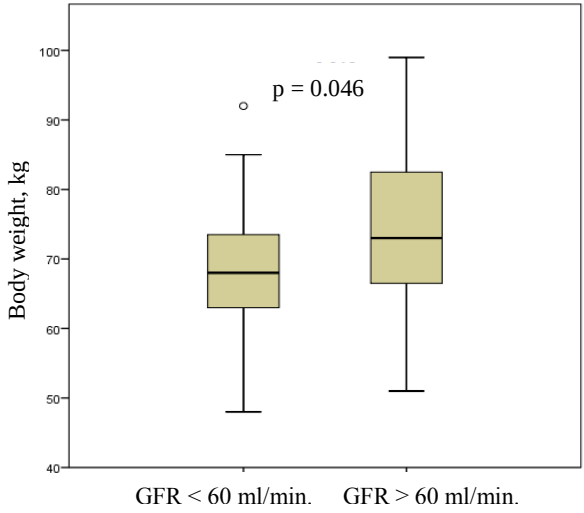

Fig. 3.25 **Body weight of recipients in groups with GFR ≥ 60 ml/min and GFR < 60 ml/min.**
(68.8 ± 8.7 kg vs. 75.2 ± 12.1 kg, p = 0.046)

Other recipient specific factors showed no statistical difference between the groups of recipients with GFR ≥ or < 60 ml/min. ($p > 0.05$ in all cases).

While carrying out logistic regression analysis in order to predict probability that GFR would be above 60 ml/min. a year after transplantation, an equation with values given in Table 3.4 was obtained.

Table 3.4

Results of Logistic Regression Analysis when Predicting GFR ≥ 60 ml/min. at the 12th month after transplantation

Factor	Values ± st. error	p	OR (95% CI)
U-NGAL	−0,004 ± 0,002	0,075	0,996 (0,991–1,000)
Age of recipient	−0,128 ± 0,038	0,001	0,880 (0,816–0,948)
Delayed graft function	−2,503 ± 1,532	0,100	0,082 (0,004–1,647)
Body weight of recipients	0,169 ± 0,061	0,005	1,184 (1,052–1,334)
Constant	−5,890 ± 3,222	0,068	0,003

The index characterising quality of an established logistic analysis model, Nagelkerke $R^2 = 59.9\%$. The developed equation enables us classifying 81.8% of patients correctly.

The model also included other donor and recipient specific factors, but these values did not affect simulation results statistically significantly (p = NS in all cases).

According to the model developed, probability that GFR would be above 60 ml/min. at the 12th month after transplantation can be calculated by the following formula:

$$P_{\text{GFR} \geq 60 \text{ ml/min}} = \frac{e^{-5.89+(-0.004*NGAL)+(-0.13*RA)+(-2.5*DGF)+0.169*RW}}{1+e^{-5.89+(-0.004*NGAL)+(-0.13*\text{RA})+(-2.5*DGF)+0.169*\text{RW}}}$$

e = 2.71;
NGAL – urinary neutrophil gelatinase- associated lipocalin (mg/ml);
RA – age of recipients;
DGF – delayed graft function;
RW – body weight of recipients;

Age of recipient under 40 years was statistically significantly associated with better graft function (GFR $\geq$ 60 ml/min) at the 12th month after transplantation (OR = 6.00; 95% CI = 1.651 – 21.801, p = 0.004).

Summary:

1. Age of recipient over 40 years of age are risk factor for worse graft function at the 12th month after transplantation.

2. Donor U-NGAL level is one of the factors affecting graft function.

3. Results of pre-transplantation biopsy showed no association with graft function at the 12th month after transplantation.

4. A model for predicting good graft function with the following characteristics of the donor and the recipient was drafted: donor U-NGAL level, age of the recipient, delayed or primary graft function, and body weight of the recipient.

4. CONCLUSIONS

1. Kidney transplantation using donors after cardiocirculatory death is not associated with unacceptably higher frequency of development of post-transplant complications and worse graft and recipient survival.

2. Use of donors after cardiocirculatory death is linked to reduced renal graft function in the first year after transplantation.

3. Delayed graft function does not significantly affect functionality of the graft in the first year after transplantation.

4. Determination of biomarker for inflammation and haemodynamic status of donors (serum bradykinin) enables to predict the risk of delayed graft function.

5. Determination of biomarker for acute kidney injury of donors (urinary NGAL) is predictive of preservation of functionality of kidney grafts during the first year after transplantation.

6. Mild interstitial and glomerular sclerosis detected by means of pre-transplantation puncture biopsy of the donor kidney do not correlate with recovery of graft function in the early post-transplant period.

7. Risk-measurement models drafted in the doctoral thesis enable predicting of the outcome of kidney transplantation, that way improving donor organ allocation and transplantation outcomes.

SCIENTIFIC AND PRACTICAL SIGNIFICANCE OF THE THESIS

Kidney transplantation from donors after cardiocirculatory death is a relatively safe and must be developed. In each case appropriate and professional intensive care and resuscitation measures must be administered and recovered kidneys must be transplanted as soon as possible within 20 hours after explantation.

Following the set of criteria defined, risk-measurement models for predicting delayed graft function, acute rejection, and functional wholesomeness of the graft are developed that enables more secure allocation of donor organs and can facilitate the follow of the patient during post-operative period.

Determination of biomarkers (serum bradykinin, U-NGAL) when using donors after cardiocirculatory death, is useful and makes improving of organ allocation process possible. Identification of other biomarkers used in transplantology is a promising research direction.

Mild interstitial and glomerular sclerosis detected by means of pre-transplantation kidney puncture biopsy does not significantly affect the outcomes of transplantation in the first year after transplantation, where organs from donors after cardiocirculatory death are used.

Determination of recipient/graft weight ratio plays an important role in the process of organ allocation, when kidneys from donors after cardiocirculatory death are used.

Research of both biomarkers and other factors being predictive of outcomes of transplantation is promising direction for development of entire field of transplantology.

PRACTICAL RECOMMENDATIONS

In order to predict the outcomes of kidney transplantation, it is recommended to supplement examination of a donor after cardiocirculatory death with determination of the following biomarkers:

- Serum bradykinin;
- U-NGAL;
- Recipient/graft weight ratio.

Avoiding kidney transplants from donors after cardiocirculatory death with serum bradykinin level above 300 pg/ml, ALT level above 75 U/L and cold ischemia time exceeding 21 hour for the following recipients:

- Recipients younger than 40 years;
- Recipients with increased risk of acute rejection;
- Recipients with dialysis access problems.

Use of the risk calculation formulas offered for predicting delayed graft function, acute rejection, and functional wholesomeness of the graft in organ allocation.

Mild glomerular and interstitial sclerosis detected by means of pre-transplantation puncture biopsy does not define the one-year outcomes of transplantation and is not a contraindication for kidney transplantation.

When expanding selection criteria for donors after cardiocirculatory death, special attention should be paid to the risk factors defined in this Doctoral Thesis.

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