Functional Activity of the Complement System in Deceased Donors in Relation to Kidney Allograft Outcome

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ABSTRACT

Complement activation is considered one of the mediators of renal ischemia-reperfusion injury. Elevated levels of C5b-9, C3a, and C5a are detected in sera of deceased kidney donors. The goal of the study was to characterize the functional activity of complement pathways in donor sera and to assess their influence on transplant outcome.

Materials and methods. Sixty-four deceased kidney donors (age 45 ± 16 years; 28 female, 36 male) and 27 healthy controls (age 42 ± 12 years; 14 female, 13 male) were enrolled in the study. The results of transplantation for the respective 122 kidney recipients were included in the analysis. The functional activities of classical (CP), lectin (LP), and alternative (AP) pathways were measured using Wielisa-kit (reference normal level = 100%). In most cases, decreased functional activity reflects the activation status of the pathway.

Results. The median (interquartile range) functional activities of the pathways in donor sera were CP 118 (89–150)%, LP 80 (20–127)%, and AP 74 (50–89)%, and did not differ from the control values CP 110 (102–115)%, LP 81 (26–106)%, AP 76 (61–88)%. The frequency of pathway activation observed in controls was CP 0%, LP 11%, and AP 0%. Deceased donors did not differ in activation of classical (11%) and lectin (13%) pathways, but presented a higher rate of alternative pathway activation (19%, $P = .03$). No significant influence of any pathway functional activity or its activation was proved to influence the transplant outcome.

Conclusion. Complement activation via alternative pathway was observed in diseased donor sera. No predictive potential of donor complement functional activity on the transplant outcome could be proved.

KIDNEY transplantation is currently considered the most effective method of renal replacement therapy positively influencing patient life quality and duration. The best outcomes are achieved with kidneys harvested from living donors [1]. Nevertheless, the vast majority of organs come from deceased donors. Donor morbidities and brain death cause hemodynamic changes, metabolic and hormonal dysregulation, and systemic immunological activation. Among the many pathophysiological mechanisms directly influencing transplanted kidney function, the complement system plays a crucial role. The complement cascade is the main defense mechanism of the innate immune system. It consists of soluble and membrane-bound proteins and proteolytic enzymes that are sequentially activated via 3 different pathways: the classical (CP), lectin

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(LP), and alternative pathway (AP). Activation of complement system leads to elimination of pathogens, immune complexes, and apoptotic cells from circulation and tissues [2]. Systemic and local complement activation induced in donors by morbidities and brain death has been suggested to contribute to loss of allograft function after transplantation [3–5]. Increased levels of C5b-9/membrane attack complex and anaphylatoxins C3a and C5a are observed in deceased donor sera [5,6]. Complement is also an important mediator of renal ischemia-reperfusion injury. A contribution of the complement system to ischemia-reperfusion tissue injury has been demonstrated in various animal models [7–9].

Although the impact of some aspects of peritransplant complement activation on allograft outcome has been described in literature, there are no data linking a degree of the complement functional activity and posttransplant events in recipients.

The aim of the study was to assess how donor CP, LP, and AP functional activity are linked to kidney transplant outcome.

PATIENTS AND METHODS

The study was approved by the Bioethical Committee of Wroclaw Medical University and performed in accordance with the World Medical Association Declaration of Helsinki.

The study involved prospectively included diseased kidney donors. The recipients, transplanted in the Department of General, Vascular and Transplant Surgery and followed in the Department of Nephrology and Transplantation Medicine, Wroclaw Medical University (Wroclaw, Poland), were included in the study after their informed consent.

The study included 64 deceased kidney donors (aged 11–76 years, mean 45±16 years; 28 female, 36 male). Kidneys were received by 122 recipients (aged 19–73 years, mean 49±13 years; 53 female, 69 male) followed for up to 12 months. The control group included 27 healthy volunteers with no kidney disease (aged 24–65 years, mean 42±12years; 14 female, 13 male). Donor serum samples were obtained immediately before organ harvesting and stored at −80°C until analysis.

Complement Functional Activity

Measurement of the 3 functional complement pathways was performed using a commercially available ELISA test (Wielisa-kit COMP 300) according to the manufacturer's instruction. The functional complement activity for each pathway was expressed as a percentage of the activity of the positive control serum. Reference normal level was 100%. In most cases, decreased functional activity reflects the activation status of the pathway. According to the manufacturer's protocol, drop <69% for CP, <30% for AP, and 0% for LP was assumed to reflect an increased activation.

Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet in Renal Disease study formula.

Statistical Analysis

All statistical analyses were performed using Statistica 12.0 (StatSoft, Krakow, Poland). The normality was tested with the Shapiro-Wilk test. The numerical data were presented as mean ± SD for normally distributed variables and median and interquartile range for the remaining data. As the functional complement activity was not normally distributed, nonparametric Mann-Whitney U test and Spearman rank correlation were used for data analysis. P value $< .05$ was considered statistically significant.

Table 1. Donor and Recipient Characteristics, Median (IQR)

Donors	
Donor sex, female/male	28/36
Donor age, y	45 (34–56)
BMI, kg/m^2	24.7 (22.3–26.7)
Donor cause of death	56 cerebrovascular accident, 8 others
Donor last creatinine, mg/dL	1.2 (0.9–1.6)
Donor last serum urea, mg/dL	4.6 (27.9–60.5)
C-reactive protein mg/L	180 (88–273)
Hourly diuresis, (average for the last 6 hours) mL	150 (120–220)
Daily diuresis (last 24 hours), mL	4465 (2957–5460)
Sudden cardiac arrest	12 (18%)
Days in intensive care unit	3.9 (2.2–7.7)
Cold ischemia time, h	25.5 (22–28.5)
Warm ischemia time >0, min	32 ± 10.9
Warm ischemia time = 0, min	77
Recipients	
Recipient sex (female/male)	53/69
Recipient age, y	49 ± 13.1
Dialysis (HD/PD)	88/21
Time of dialysis before transplantation, mo (n = 98)	24 (14–42)
Primary kidney disease:	
Diabetic nephropathy	5
Chronic glomerulonephritis	58
Hypertensive nephropathy	13
Polycystic renal disease	16
Other/unknown	30
Delayed graft function	20
Acute rejection (first year posttransplant)	11
Max PRA >20%	17
Last PRA >20%	7
HLA-A mismatch (0/1/2)	13/63/46
HLA-B mismatch (0/1/2)	14/53/55
HLA-DR mismatch (0/1/2)	41/60/21

Abbreviations: BMI, body mass index; HD, hemodialysis; IQR, interquartile range; PD, peritoneal dialysis; PRA, panel reactive antibodies.

RESULTS

Donor and recipient demographic and clinical characteristics are presented in Table 1.

Functional Complement Activity in Study Groups

Functional complement activity of classical, lectin, and alternative pathways in donor sera was CP 118% (89%-150%), LP 80% (20%-127%), and AP 74% (50%-89%). The median of donor functional activity levels did not significantly differ from those observed in the healthy control group: CP 110% (102%-115%), LP 81% (26%-106%), and AP 76% (61%-88%). Eleven percent of donors presented activated CP, 13% presented activate LP, and