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The impact of C4d deposits in peritubular capillars on living related donor kidney transplantation outcome

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In the Department of Kidney Transplantation of Petrovsky National Research Centre of Surgery, the investigations of kidney biopsy specimens for C4d started in 2009. The study was performed in 119 patients with impaired function of kidneys allografted from living related donors. The staining for C4d was negative in 85 cases, and positive in 34 cases. The groups were compared on the following parameters: the patient mortality, and allograft loss. Besides, morphologic findings in the biopsy were compared by nature between the groups. There were 6 deaths among 85 patients in Group 1, and 16 deaths among 34 patients in Group 2. Comparative analysis of results, using Fischer's criterion, demonstrated a statistically significant difference in mortality between the groups (χ 2 = 4.86, p = 0.0275). As for graft losses, and the nature of assessed morphology findings, they were nearly similar in both groups. Therefore, according to our data, the presence of C4d protein was associated with increased mortality. The differences between the groups in all other parameters were not statistically significant.

Keywords: transplantation, kidney, living related donors, immunosuppression.

Introduction

The acute humoral rejection in patients with impaired function of allografted kidney is generally diagnosed on the basis of the following three criteria: 1) kidney allograft biopsy (showing polymorphocellular infiltration in peritubular capillaries [PTCs]); 2) diffuse circular deposition of monoclonal C4d in peritubular capillaries; 3) serologic evidence of donor-specific antibodies [1]. The presence of PTC C4d depositions is a risk factor. The impact of various factors on the kidney live related donor transplant outcomes have been studied in the Department for Kidney Transplantation of our Center for the recent 15 years [2-6].

The diagnosis of kidney acute humeral rejection is difficult when either two of the above criteria are absent, or two of these signs are not present simultaneously; or the extent of PTC C4d staining is at least 50% to be considered as diffuse [7]. All these symptoms may indicate an antibody aggression against the grafted kidney and therefore adversely affect the outcomes of kidney transplantation from a living related donor, and from a cadaveric donor. There are contradictory opinions on this issue in the world literature [8-11]. In connection with the above, we decided to review our own experience and present our point of view on this issue.

Material and Methods

Renal allograft biopsy testing for C4d has been made in *Petrovsky National Research Centre of Surgery* since 2009. Not all, but only those

patients were tested who demonstrated a deterioration of allografted kidney parameters over time, and the test was aimed at identifying the cause of this phenomenon. The study was conducted from 2009 to April 2015 and included 119 patients aged from 1 to 63 years (21.05±12.50), among whom 60 were adults and 59 were children. There were 65 male and 54 female patients. The causes of end-stage chronic renal failure are presented in the Table.

Table. Causes of end-stage chronic renal failure

Diagnosis	Number of patients	Per cent
Chronic glomerulonephritis	29	24.27
Vesicoureteral reflux	12	10.29
Hemolytic-uremic syndrome	9	7.35
Renal hypoplasia	13	11.02
Alport syndrome	3	2.94
Kidney dysplasia	5	4.41
Multicystic kidney disease	2	2.21
Obstructive uropathy	7	5.52
Fanconi's nephronophthisis	3	2.21
Nephrotic syndrome	5	4.04
Focal segmental glomerulosclerosis	3	2.57
Diabetic nephropathy	2	2.21
Other	26	20.96
Total	119	100.00

Biopsies were negative for C4d in 85 patients (the 1st group), and positive in 34 patients (the 2nd group). Matching of HLA A, B, Dr histocompatibility antigens made 2.55±1.00 in the 1st group, and 2.91±1.24 in the 2nd group. Although the compatibility was slightly higher in the 2nd group than in the 1st one, however, the difference did not reach the statistical significance, as analyzed by using Student's t-test, ($t=1.96$; $p>0.05$). So, the groups were comparable by the degree of matching of histocompatibility antigens.

The results of the studied renal biopsy morphology were classified into four categories (patterns): 1) *normal*; 2) *borderline abnormalities* (BA); 3) *acute rejection* (AR1-2); 4) *chronic allograft nephropathy* (CAN). Renal biopsy morphology findings are graphically summarized in Fig. 1.

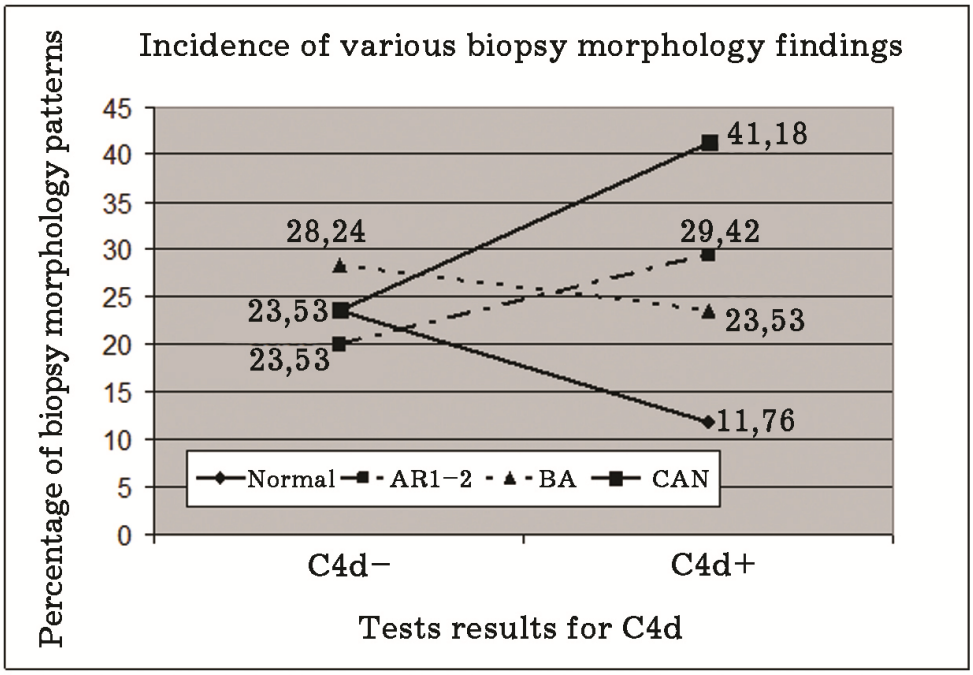

Fig. 1. Incidence of various biopsy morphology findings.

As demonstrated by the curve in Fig. 1, the percentage of biopsies with normal results in the 1st group (C4d-) was almost twice higher than in the 2nd group (C4d+). The same trend was observed with the biopsies that yielded "BA" results. As for the biopsies with "AR1-2" and "CAN" results, the trend was quite opposite: their numbers were twice lower in the 1st group (C4d-) compared to those in the 2nd group (C4d+). Therefore, the obtained data suggest a negative impact of C4d depositions on biopsy morphology. We also compared the severity of abnormal biopsy morphology parameters. The results are shown in Fig. 2.

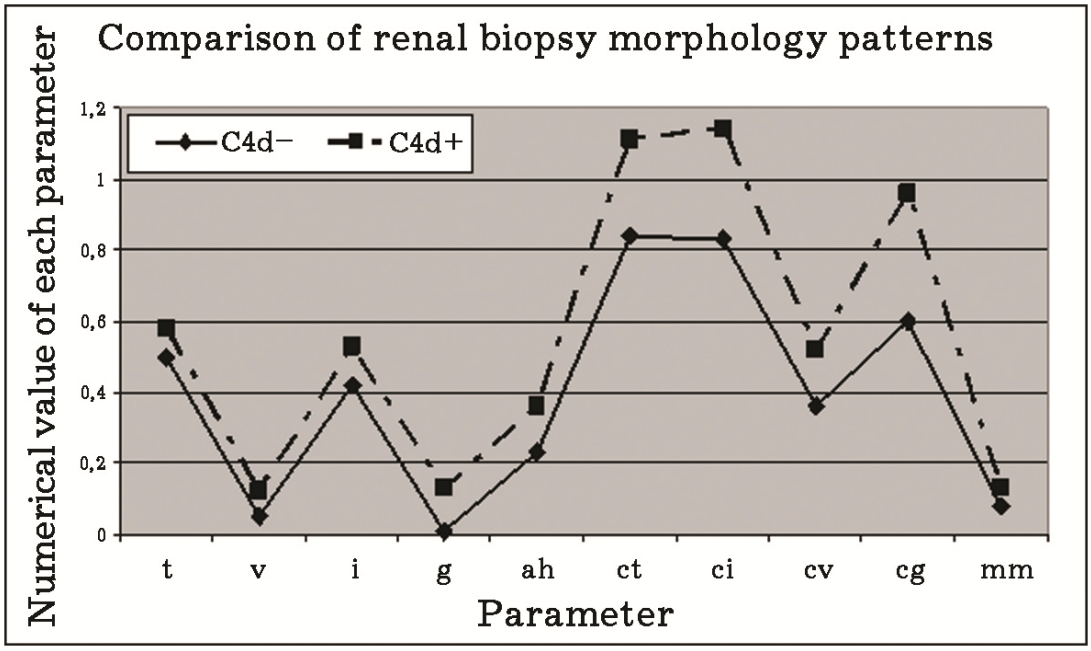

Fig. 2. Comparison of renal biopsy morphology patterns

The graph presented in Fig. 2 demonstrates that the incidences of various biopsy morphology findings in the two groups are almost similar, but the numerical value of each parameter in the 2nd group is somewhat

higher than in the 1st group. Comparative analysis using Student's t-test demonstrated the numerical difference between the groups not being statistically significant ($t=0.671$; $p>0.05$).

To answer the question of whether the related donor age affects the probability of C4d depositions in renal allograft, we calculated the mean age of donors in both groups. The mean donor age was 40.92 ± 9.62 years in the 1st group, and 45.68 ± 10.75 in the 2nd group. The difference between the groups was not statistically significant ($t=0.33$; $p>0.05$), as shown by mathematical processing using the Student's t-test. Therefore, the related donor age does not affect the probability of C4d depositions.

The review of outcomes demonstrated 6 deaths of 85 patients in the 1st group (7.06%), and 8 deaths out of 34 patients in the 2nd group (23.53%). Mathematical analysis using Fisher's exact test found the difference being statistically significant ($\chi^2=4.86$; $t=0.0275$). Therefore, C4d depositions in the renal allograft have a negative impact on the recipient mortality rates. As for the graft loss, there were 7 lost grafts among 85 recipients in the 1st group, and 1 lost graft among 34 recipients in the 2nd group. Mathematical analysis using Fisher's exact test showed that difference was not statistically significant ($\chi^2=0.41$; $p=0.5243$). So, it seems that C4d depositions pose no impact on the incidence of graft loss.

Similar results were obtained by using the mathematical analysis with the Kaplan-Meier calculation of cumulative survival and Willcox cumulative risk analysis. The results are summarized in Fig. 3 and 4.

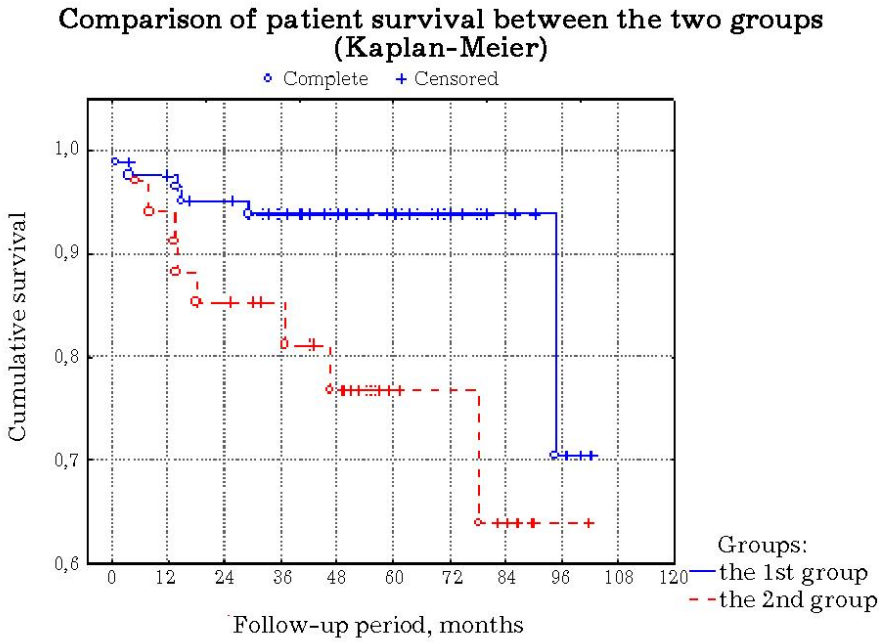

Fig. 3. Comparison of patient survival between the two groups (Kaplan-Meier)

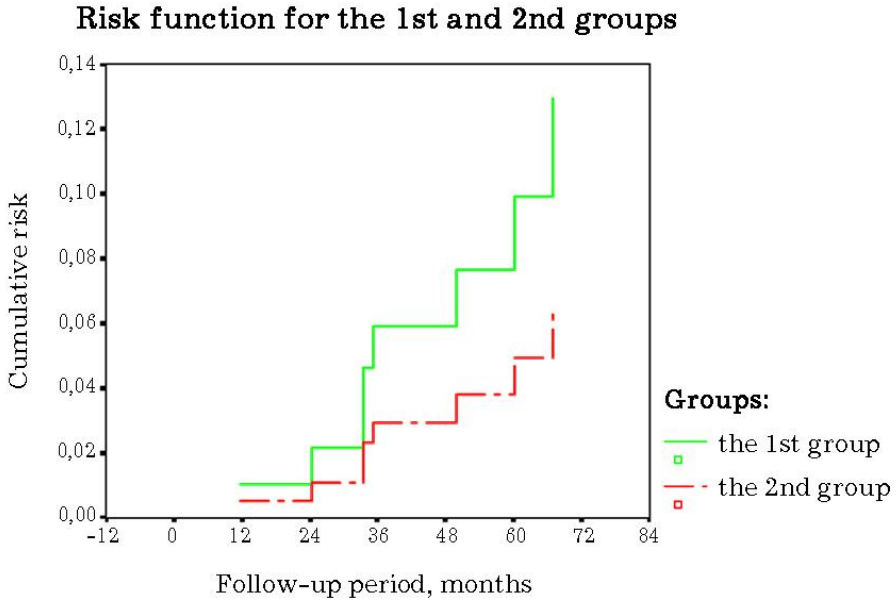

Fig. 4. The cumulative risk of allograft loss for the 1st and 2nd groups

The graph in Fig. 3 clearly shows that the recipient cumulative survival in the 1st group (C4d-) was significantly higher than in the 2nd group (C4d+). Statistical analysis using log-rank test showed the statistical significance of the difference (t=2.46840; p=0.01357). A similar trend was observed in the calculation of the cumulative risk (Fig. 4) using Cox model statistics.

Mathematical analysis revealed the statistically significant difference between the groups (p=0.020). So, C4d depositions pose a negative impact on recipient survival and have virtually no effect on the incidence of graft loss.

Discussion

Immune inflammatory response in the renal allograft may lead to the development of an acute rejection in some cases. The histological evaluation of the renal allograft biopsy specimens obtained in the phase of an emerging antibody-mediated acute rejection demonstrated the following morphological findings: the presence of neutrophilic capillaritis, glomerulitis, fibrinoid necrosis of arterioles or glomeruli. In our study, we wished to find out whether the presence of C4d depositions affected the nature of morphological changes in renal allograft biopsy, and had an impact on kidney transplantation outcomes. Our study has shown that C4d depositions in PTCs have a negative impact on the morphological pattern of renal allograft biopsy. Although a quantitative difference in the kidney allograft morphology findings between the 1st and 2nd groups was not statistically significant, however, the fact remains: the number of histologically evaluated biopsies with "Normal" and "BA" results in the 1st

group was significantly higher than in the 2nd group. As for the biopsies with "AR1-2" and "CAN" results, their number was higher in the 2nd group.

The impression is that all biopsy specimens should be tested for C4d even those obtained from allografted kidneys demonstrating a satisfactory function. This tactics would help to identify the patients with a high risk of rejection and to take timely and appropriate measures aimed at the prevention of this complication by using a slightly intensified immunosuppression protocol.

Conclusion

Our study has proved that C4d depositions in renal allograft pose a negative impact on the recipient survival and do not affect the rate of renal allograft loss.

References

1. Calvin R.B. Renal transplant pathology. In: Jenette J.C., et al. *Heptinstalls pathology of the kidney.* 5th ed. Philadelphia: Lippincott-Raven, 1988. 1409–1540.

2. Kaabak M.M., Sandrikov V.A., Ragimov A.A., et al. Analiz vyzhivaniya pochechnogo allotransplantata po dannym registra Rossiyskogo dializnogo obshchestva i vozmozhnye puti uluchsheniya otdalennykh rezul'tatov [Survival analysis of renal allograft according to the register of the Russian society of dialysis and possible ways to improve the long-term results]. *Vestnik transplantologii i iskusstvennykh organov.* 2006; 4: 31–36. (In Russian).

3. Goryaynov V.A., Kaabak M.M., Molchanova E.A. Plazmaferez dlya lecheniya reperfuzionnoy travmy pri peresadke pochki. Vliyanie na blizhayshie i otdalennyy rezul'tat [Plasmapheresis in the treatment of reperfusion injury in kidney transplantation. The influence on the immediate and long-term outcome]. *Vestnik Rossiyskoy akademii meditsinskikh nauk.* 2002; 5: 43–45. (In Russian).

4. Kaabak M.M., Goryaynov V.A., Zokoev A.K., et al. Desyatiletniy opyt primeneniya rannego plazmafereza posle peresadki pochki [Ten-year experience with plasmapheresis early after kidney transplantation]. *Vestnik transplantologii i iskusstvennykh organov.* 2009; 11 (1): 28–33. (In Russian).

5. Goryaynov V.A., Kaabak M.M., Babenko N.N., et al. Allotransplantatsiya rodstvennykh pochek u detey [Related kidney allografts in children]. *Khirurgiya*. 2008; 6: 58–62. (In Russian).

6. Kaabak M.M., Babenko N.N., Samsonov D.V., et al. Alemtuzumab induction in pediatric kidney transplantation. *Pediatric Transplantation.* 2013; 17 (2): 168–178.

7. Kayler L.K., Kis L., Sharma V., et al. Acute renal allograft rejection: diagnostic significance of focal peritubular capillary C4d. *Transplantation.* 2008; 85 (6): 813–820.

8. Racusen L.C., Solek K., Colwin R., et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int.* 1999; 55 (2): 713–723.

9. Trpkov K., Campbell P., Pazderka F., et al. Pathologic features of renal allograft rejection associated with donor-specific antibody. Analysis

using the Banff grading schema. *Transplantation.* 1996; 61 (11): 1586–1592.

10. Baldwin W.M., Halloran P.F. Clinical syndromes associated with antibody in allograft. In: Racusen L., et al., eds. *Kidney transplant rejection.* 3rd ed. New York: Marcel Dekker, 1998. 127–147.

11. Halloran P.F., Wadgymar A., Ritchie S., et al. The significance of the anti-class 1 antibody response. *Transplantation.* 1990; 49 (1): 85–91.