ABSTRACT

Introduction: The complement system is a part of the innate immune system. Its major functions are recognition and elimination of pathogens, but they are also involved in removal of immune complexes and apoptotic cells from the circulation and tissues. There are three known pathways of complement activation: the classical (CP), lectin (LP) and alternative pathway (AP). In the final stage of complement activation C5b-9 membrane attack complex (MAC) is formed leading to cell membrane damage and cell lysis. Among the many pathophysiological mechanisms directly influencing transplanted kidney function, the complement system plays a crucial role. Complement cascade activation caused by death of donor's brain, ischemia reperfusion injury and early renal allograft rejection is also considered amongst the main factors leading to transplanted organ injury.

Aim of study: The goal of the study was to characterize the functional activity of complement pathways in donors after brain death and recipients sera, and to assess their influence on transplant outcome.

Material and Methods: The first study group included 64 deceased kidney donors. Donor serum samples were obtained immediately before organ harvesting. The second study group enrolled 42 kidney recipients. Blood samples from kidney allograft recipients were collected prior to the initiation of immunosuppression, just before the transplantation, up to 1 hour after transplantation proceduresurgery and 1, 3, and 7 days after transplantation. Healthy volunteers were also included in the study as a control group.

Measurement of the three functional complement pathway was performed using a commercially available ELISA test (Wielisa®-kit COMP 300, Sweden) according to the manufacturer's instruction.

Results: The median (IQR) functional activities of the pathways in donors 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%) and, 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 CP (11%) and LP (13%), but presented higher rate of AP activation (19%, p=0.027). No significant influence of any pathway functional activity or its activation was proved to influence the transplant outcome.

We observed that the baseline functional activity of the alternative pathway is higher in chronic kidney disease patients compared to healthy controls (101% (78%-117%) vs 73% (62%-88%), p=0,001). In addition, the AP functional activity was influenced by pre-transplant dialysis type. From the three pathways, AP functional activity was significantly higher in patients treated with peritoneal dialysis then in patients treated with hemodialysis (135% (129% – 143%) vs 93% (75% – 107%), p<0.001). Kidney transplantation induced decrease in AP functional activity that was most pronounced 1 hour after reperfusion (101% (78%-117%) vs 99% (71%-107%), p=0.044). When the peri-surgical parameters were analyzed, functional complement activity was influenced by warm ischemia. In particular, recipients operated with graft surface cooling presented significantly higher level of AP functional complement activity 1 hour and 24 hours after reperfusion, than patients who were operated with using standard procedures. Functional complement activity of AP measured 3 days after transplantation was significantly lower (72% (62%-88%) vs 88% (71%-108%), p=0.04) in recipients experiencing a delayed graft function compared to those with immediate graft function. Moreover, post-transplant glomerular filtration rate was associated with AP functional activity 3 days after KTx (3^{rd} day eGFR r=0.36, p=0.019; 7^{th} day eGFR r=0.39, p=0.010; 3^{rd} month eGFR r=0.40, p=0.042). CP and LP functional activity were not related to graft function.

Conclusions:

1. Increased complement activation via alternative pathway was observed in deceased donor sera.
2. No predictive potential of donor complement functional activity on the transplant outcome could be proven.
3. Functional complement activity of alternative pathway measured in donors sera after brain death correlated with CRP.
4. Changes in the functional activity of complement system in kidney transplant recipients indicate involvement of the alternative pathway in early phase after kidney transplantation.
5. Renal transplant recipients who developed delayed graft function had significantly lower level of AP functional complement activity 3 days after transplantation, moreover, post-

transplant glomerular filtration rate was associated with AP functional activity 3 days after transplantation.

6. In chronic kidney disease patients the level of AP functional activity was higher compared to healthy controls, moreover dialysis modality has influence on complement activation. In peritoneal dialysis treated patients, AP functional activity was significantly higher than in patients treated with hemodialysis.
7. Elimination of the second warm ischemia results in decreased activation of the alternative complement pathway.