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Our first experiences in applying an original method for removal of ABO-isoagglutinins in ABO-incompatible kidney recipients

Naša prva iskustva u primeni originalne metode za snižavanje titra ABO izoaglutinina kod ABO inkompatibilnih transplantacija bubrega

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Abstract

Background/Aim. Due to improved methods for removal of ABO isoagglutinins and novel immunosuppressive protocols, short and long term outcome in blood group incompatible is similar to blood group compatible kidney transplantation. The aim of this study was to determine the efficacy of our original method for removal of ABO isoagglutinins from the blood in ABO-incompatible kidney allograft recipients. **Method.** Between 2006 and 2008 twelve patients were transplanted from ABO incompatible living donors. Titers of ABO isoagglutinins were 4–128 (IgG). Immunosuppressive therapy started 14 days before kidney transplantation with rituximab, followed by a triple therapy (prednisone + tacrolimus + mycophenolate mofetil) and the first plasma exchange (PE) procedure, in which one plasma volume was substituted with albumin and saline on day 7 before transplantation. For selective extracorporeal immunoadsorption, the removed plasma was mixed with donor blood type filtered red blood cells, centrifuged and the supernatant separated and preserved. In the next PE procedure, the removed plasma was replaced with immunoadsorbed plasma, and so on. Titers of ABO agglutinins, renal allograft function and survival were followed-up. **Results.** The pre-transplant treatment consisting of 1–5 PE procedures and immunosuppressive therapy resulted in target ABO agglutinins titers below 4. During a 10–24 month follow-up three patients had an early acute rejection, one patient acute rejection and hemolytic anemia, two patients surgical complications and one of them lost his graft. In the post-transplant period, the titers of ABO antibodies remained below 4. All the patients had stable kidney allograft function with mean serum creatinine ±SD of 129 ± 45 μmol/l at the end of the study. **Conclusion.** Our method for removal of ABO antibodies was effective in a limited series of patients and short-term follow-up.

Key words:
kidney transplantation; abo blood-group system; blood group incompatibility; plasmapheresis; antibodies; graft rejection; graft survival.

Apstrakt

Uvod/Cilj. Zahvaljujući poboljšanju metoda za snižavanje titra ABO izoaglutinina i novim imunosupresivnim protokolima, kratkoročno i dugoročno preživljavanje transplantiranih bubrega je slično kod bolesnika kojima je transplantacija urađena preko krvno grupne barijere u odnosu na krvno grupno podudarne. Cilj ovog ispitivanja bio je određivanje efikasnosti naše originalne metode u smanjivanju titra ABO izoaglutinina u krvi bolesnika kojima je bubreg transplantiran preko krvno grupne barijere. **Metod.** Od 2006. do 2008. urađeno je dvanaest transplantacija bubrega od krvno grupno nepodudarnih živih davalaca. Titar ABO izoaglutinina bio je 4–128 (IgG). Sa imunosupresivnom terapijom počinjalo se 14 dana pre transplantacije rituksimabom, a nastavljalo se sedam dana pre transplantacije trojnom terapijom (prednizon+takrolimus+mikofenolat mofetil) i plazmeferezom u kojoj je izdvojena plazma supstituisana albuminima i fiziološkim rastvorom. Tokom selektivne ekstrakorporalne imunoadsorpcije (SEKIA) izdvojena plazma mešana je sa eritrocitima davaočeve krvne grupe, centrifugovana, a supernatant je odvajan i konzerviran. Tokom sledeće plazmafereze, izdvojena plazma supstituisana je imunoadsorbovanom plazmom. Broj procedura određivan je postizanjem ciljnog titra izoaglutinina < 4 (IgG). Tokom praćenja registrovani su titar izoaglutinina, funkcija i preživljavanje grafta. **Rezultati.** U pretransplantacionoj pripremi urađeno je 1–5 plazma izmena i sa imunosupresivnom terapijom postignut je titar izoaglutinina < 4. Tokom praćenja od 10 do 24 meseca tri bolesnika su imala epizodu ranog akutnog odbacivanja, jedan akutno odbacivanje i hemolitičku anemiju, dva hirurške komplikacije, od kojih je jedan izgubio graft. U posttransplantacionom periodu titrovi izoaglutinina ostali su < 4. Na kraju praćenja svi bolesnici imali su stabilnu funkciju alografta sa serumskim kreatininom 129 ± 45 μmol/l. **Zaključak.** Na malom broju bolesnika sa bubregom transplantiranim preko krvno grupne barijere i uz nedovoljno dugo praćenje naš protokol za smanjenje titra ABO antitela pokazao se efikasnim.

Ključne reči:
transplantacija bubrega; abo sistem; krvne grupe, nepodudaranje; plazmafereza; antitela; graft, odbacivanje; graft, preživljavanje.

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Introduction

In the past, preparations for kidney transplantation across blood groups were demanding, and often included extensive surgery [1]. Over the last 10 years, methods for removal of ABO isoagglutinins (plasma exchange, immunoabsorption) have improved considerably, and new advances in immunosuppressive therapy have been made [2–6]. Contrary to early studies with poor results for kidneys transplantated across blood groups, recent reports showed short and long term outcome in kidney transplantation across blood groups to be similar to those with the same blood type [7, 8].

Due to legislation and organizational problems at the national level concerning kidney transplant programs from deceased donors, only a limited number (about 7%) of kidneys are transplanted from cadavers in our institution. In order to increase the number of transplanted patients, we started with preemptive kidney transplantations from unrelated, but emotionally related donors and from incompatible living donors (with positive cross match and across the blood group barrier). We developed an original method for removal of ABO isoagglutinins from the recipient's blood, which includes two procedures: plasma exchange (PE) and selective extracorporeal immunoadsorption (SECIA) of removed plasma with filtered donor blood group erythrocytes, as "a closed circuit" [9]. The immunosuppressive protocol was adapted from a Swedish preposition for pre- and post-transplant management in kidney transplantation across blood groups [10, 11]. The aim of the present study was to investigate the efficacy of our therapy protocol in a kidney transplant program across blood groups.

Methods

In the period from April 1, 2006 to April 30, 2008 twelve ABO-incompatible transplantations, in 6 males and 6 females (mean age 40.17 ± 7.64 years) were performed at the Military Medical Academy, Belgrade. The etiology of end-stage renal disease was diabetic nephropathy in one patient and unknown in eleven patients. Donors were living, 11 related, one unrelated (emotionally related spouse) (mean age 61.92 ± 6.27 years), and with at least 50% Human Leukocyte Antigens (HLA) matching. Baseline titers of recipients' ABO isoagglutinins were 4 to 128 (IgG). The age and blood type of donors and recipients, HLA matching and cross match are shown in Table 1. Seven recipients with blood type O had a B type donor, three O type recipients had an A type donor, one A type recipient had a B type donor and one B type recipient had an A type donor. The lymphocytotoxic pre-transplant cross match was negative in all patients, although one patient had a historically positive cross match, successfully treated with PE, double immunosuppressive therapy (mycophenolate mofetil and tacrolimus) and rituximab.

The study was approved by the Ethical Committee of the Medical Military Academy and a written informed consent was obtained from each donor and recipient.

The day of transplantation was designated as day 0. Pre-transplant preparation started on day – 14 with rituximab (375 mg/m^2), followed from day – 7 with triple immunosuppressive therapy: prednisone [0.5 mg/kg body weight (BW)], mycophenolate mofetil in the standard dose of 500 mg bid, and tacrolimus adjusted to a target C0 concentration of 15–20 ng/ml. Plasma exchange and SECIA were also performed on day -7. The first four patients received anti-CD 25 antibodies (daclizumab) twice, as induction therapy on day -1 (2 mg/kg BW) and on day +13 (1 mg/kg BW).

The first large volume PE was performed on day -7 with removal of 3.8 ± 0.5 l of plasma and replacement with 20% human albumin in saline. Plasma was exchanged on a BCT-Spectra instrument (Gambro, Sweden). The removed plasma underwent SECIA with filtered erythrocytes of the donor blood type, using the original method of Balint et al. [9] as described in detail elsewhere. After immunoadsorption, the supernatant was centrifuged, separated and preserved. In the second PE procedure, the immunoadsorbed plasma was used as the replacement fluid. The removed plasma in the second procedure underwent SECIA, and so on. The number of PE and SECIA procedures depended on the titer of ABO isoagglutinins, with the target titer less than 4 (IgG).

The immunosuppressive protocol in the post-transplant period consisted of prednisone (tapered by standard proto-

Table 1

Basic patients data

Patient (n)	Donors' age (years)	Recipients' age (years)	Donors' blood type	Recipients' blood type	HLA* matching	Cross match
1	58	36	B+	O+	2/4	–
2	58	34	B+	O+	2/4	hystoric +
3	64	59	B+	O+	2/9	–
4	64	42	A1+	O+	2/4	–
5	55	29	B+	O-	2/4	–
6	65	44	B+	O+	3/9	–
7	73	40	B+	A2+	2/4	–
8	58	41	B+	O+	2/4	–
9	64	36	B+	O+	2/4	–
10	50	34	A2+	B+	2/4	–
11	67	46	A1-	O+	2/4	–
12	67	41	A1+	O+	2/4	–

*HLA – human leukocyte antigens

col), tacrolimus (dose adjusted to C0 blood concentration) and mycophenolate mofetil in a standard daily dose of 2 g for the first 1-3 months and then 1 g daily divided into two doses. In the follow up period the titers of ABO isoagglutinins (IgG), allograft function (through serum creatinine) and survival, episodes of rejections and infections were monitored.

Titers of ABO isoagglutinins were measured using a standard procedure described in detail in the Technical Manual of the American Association of Blood Banks, (Mark E Brecher, 15th ed., 1999). Blood groups and Rh factor were tested with blood grouping reagents ABO-Rh / Reverse grouping cassette (Ortho-Clinical Diagnostics, USA). The concentration of tacrolimus before the morning dose was measured with the Abbot IMx Tacrolimus II Assay, based on Micro particle Enzyme Immunoassay (MEIA). Serum creatinine concentrations were determined colorimetrically by the Bonsnes and Taussky method on a Dade Behring instrument.

According to transplantation protocol of our institution diagnosis of early acute rejection (in the first 15 postransplant days) was based on clinical presentation, laboratory findings and response to steroid pulse therapy. In the cases resistant to steroid therapy kidney allograft biopsy was performed. The cut-off level for diagnosis of reactivated cytomegalovirus (CMV) was 400 copies/ml [Polymerase Chain Reaction (PCR) Amplicor, Hoffman La Roche]. Bacterial infections were diagnosed according to positive cultures, hematological and biochemical parameters and clinical presentation.

The efficacy of the PE + SECIA procedure is shown as individual IgG isoagglutinin titers before and after the procedure, and as a mean percentage of the antibody reduction per procedure. The reduction of ABO isoagglutinins titers per procedure was analyzed with the Wilcoxon matched pairs test using a standard statistical program (Stat for Windows, R.4.5, Stat Inc, SAD 1993). Differences were considered statistically significant at $p < 0.05$.

Results

The main criteria for the number of PE procedures with immunoadsorbed plasma in the pre-transplant period was the titer of ABO isoagglutinins (IgG). Most of our patients had 1–3 procedures. Three patients with hemolytic ABO antibodies had 5 pre-transplant modified PE procedures. For these patients in first two PE procedures, instead of immunoadsorbed auto-plasma, immunoadsorbed allo-plasma or AB blood type plasma was used. None of the patients exhibited side effects after rituximab, PE with immunoadsorbed plasma and the triple immunosuppressive protocol. The first PE+SECIA procedure was very effective in reducing anti-donor BG antibodies (antibodies to white cell antigen remnants on red cells), but all patients showed the rebound phenomenon after the first procedure. After 3–5 procedures there were no rebounds, and the titers of ABO isoagglutinins (IgG) were within the reference range (below 4). The percentage reduction of ABO isoagglutinins (IgG) for PE with immunoadsorbed plasma was: 65.9 ± 12.61% after the first, 47.72 ± 26.11 after the second, 60 ± 12.8 after the third, 75% after the fourth, 81.25 ± 8.83% after the fifth and 50% after the sixth procedure (Table 2).

Selective extracorporeal immunoadsorption was very efficient in reducing ABO isoagglutinins: 91.4 ± 17.3% after the first, 88.75 ± 17.13% after the second, 88.75 ± 17.13% after the third, 78.13 ± 21.35 after the fourth, and 100% after the fifth, procedure (Table 3).

In the post-transplant period 1–3 preemptive PE procedures with immunoabsorbed plasma were performed in spite of the very low isoagglutinin titers only in the first three patients. Neither of these procedures was performed in the remaining eight patients. In all the patients the titer stayed very low during the whole follow up period, so we did not have to administer additional immunosuppressive therapy or PE with immunoadsorbed plasma procedures. The titers of ABO isoagglutinins in the post-transplant months are presented in Table 4.

Table 2

The titers of ABO isoagglutinins (IgG) at the start and in the end of the plasma exchange (PE) with human albumines (PE 0) and immunoadsorbed plasma (PE 1–5)

Patient (n)	PE 0		PE 1		PE 2		PE 3		PE 4		PE 5	
	start	end	start	end	start	end	start	end	start	end	start	end
1	32	8	16	4	8	4	8	2				
2	16	8	8	4	8	2						
3	128	32	32	8	16	8	32	8	16	4	2	1
4	64	32	32	16	16	8	16	4				
5	4	2	4	2								
6	32	8	16	16	16	4						
7	32	8	2	1	2	1						
8	8	2	4	2	4	2						
9	16	4	8	4	16	8	8	4	8	1		
10	32	8	8	2	8	2	32	4	8	4		
11	32	16	4	4	8	2						
12	64	16	16	8	8	4						
% of reduction	-65.91		-47.72		-60.0		-75.0		-81.25		-50.0 ± 0	
± SD	± 12.61		± 26.11		± 12.9		± 0.0		± 8.8		± 0.0	
p	0.003		0.007		0.005		0.068		–		–	

Table 3

The titers of ABO isoagglutinins (IgG) at the beginning and in the end of selective extaacorporeal immunoadsorption (SECIA)

Patient	1. SECIA		2. SECIA		3. SECIA		4. SECIA		5. SECIA	
	start	end	start	end	start	end	start	end	start	end
1	16	1	8	0	4	0	4	0	1	0
2	8	0	4	0	4	0	1	0		
3	64	H*	16	4	16	4	8	1		
4	32	H*	8	H*	16	2	16	4		
5	2	1	2	1	1	0				
6	8	1	16	0	8	0				
7	4	0	2	0	2	0				
8	4	0	2	0	2	0				
9	8	4	4	2	8	2	8	0		
10	16	H*	4	1	4	1	4	2		
11	8	0	8	0	4	0				
12	16	0	8	1	2	1				
% of reduction	-91.4		-88.75		-88.75		-78.125		-100	
±SD	± 17.33		± 17.13		± 17.12		± 21.34		± 0	
p	0.003		0.005		0.005		0.067			

*H – value than normal ones higer

Table 4

ABO isoagglutinin titers (IgG) in months after transplantation

	Post- transplant months																	
Patient	1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24
1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
2	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	
3	2	1	1	1	1	1	2	1	2	1	1	1	0	1	0	0	0	
4	2	4	4	4	4	2	2	1	1	0	0	0	0	0				
5	1	1	0	0	0	0	0	0	0	0	0	0	0	0				
6	1	1	1	1	1	1	1	1	1	0	0	0	0	0				
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
8	0	0	0	0	1	0	1	0	1	1	1	1	1	1				
10	4	4	4	4	1	1	2	2	4	4	4	2	1					
11	1	0	1	0	1	1	2	1	0	0	0							
12	2	0	0	2	1	0	0	1	0	0								

The first four patients received anti-CD25 antibodies and two of them experienced clinical and laboratory signs of acute steroid sensitive rejection. At the time of acute rejection tacrolimus C0 was somewhat below 15 ng/ml in both patients. Anti-CD25 antibodies were not used in the next eight patients. Their C0 concentration of tacrolimus before transplantation reached the target value of 15–20 ng/ml. Two of these eight patients had early episodes of acute rejection, and both were successfully treated with 5 × 500 mg methylprednisolone pulses. One to three months after the transplantation, the dose of mycophenolate mofetil was decreased by 50%, without further rejection episodes.

Two of our patients had serious surgical complications. The first had life threatening bleeding in the abdominal wall requiring massive transfusions of filtered red blood cells and donor-type fresh frozen plasma. He also had urinoma and repeated episodes of acute pyelonephritis, which resolved with successful surgical treatment. The second patient had repeated surgical treatments for bleeding from the operative field complicated with recurrent sepsis with resistant *Pseudomonas aeruginosa* and the syndrome of multi-organ dysfunction (MODS). He lost kidney graft function within 6 days. These two patients had no abnormalities in coagulation or increased titers of alloantibodies or isoagglutinins.

All the patients had at least one symptomatic or asymptomatic urinary tract infection during the follow up. Four patients with reactivated cytomegalovirus infection were successfully treated with intravenous gancyclovir. One patient had primary cytomegalovirus infection, and received intravenous gancyclovir for 28 days, and then oral acyclovir 400 mg daily for 6 months.

With the exception of the single patient (sepsis and MODS), who lost kidney function early after transplantation, all the remaining patients had stable renal function (estimated by serum creatinine) during the follow up. Mean creatinine level in these patients was 129 ± 45 μmol/l. Only one patient (with urinoma and multiple surgical treatments) had a creatinine concentration slightly above 200 μmol/l, which remained stable during the follow up. Individual serum creatinine levels in the examined patients throughout the post-transplant period are presented in Figure 1.

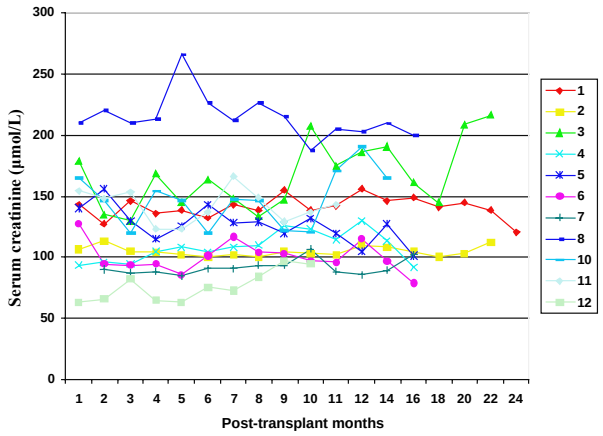

Fig. 1 – Serum creatinine in post-transplant period

Discussion

Unlike the majority of transplant professionals who use PE for removal of anti-donor BG antibodies in the pre- and post-transplant period, Tyden et al. [10, 11] proposed selective, antigen-specific immunoadsorption as a safe and effective procedure. This method had several advantages over the standard PE procedures including minimal loss of coagulation factors, proteins and electrolytes and to be relatively ease of application. The remaining problem is a prediction of the right number of columns for immunoadsorption per patient, because the number of columns depends on the achieved isoagglutinins titer. The number reported varied from 7 to 26 columns per patient. In addition, considerable time is necessary to acquire the columns, which may not be acceptable in emergency situations. Our centre proposed a combination of PE and consecutive SECIA of ABO isoagglutinins with filtered erythrocytes of the donor blood type. The proposed procedure was tested and shown to be safe and effective in preserving plasma composition and reducing isoagglutinin titers [9]. After a pilot study, it was used in twelve kidney transplant recipients who were the subject of this study. The great advantage of this procedure compared to antigen specific immunoadsorption is the low price, combined with good safety and efficacy. Patients who had ABO agglutinin antibodies needed 2–3 procedures prior to transplantation, while those with hemolytic antibodies required 3–5 procedures pre-transplant. In the post-transplant period, extra procedures were not necessary, but, due to lack of experience, this was done pre-emptively in the first three patients. As part of the pre-transplant preparation, all our patients were given only one dose of rituximab adjusted to the body surface area, and none of them needed it in the post-transplant period. In the postoperative period the patients received only a maintenance immunosuppressive protocol, with the exception of four patients with signs of acute rejection who were treated with methylprednisolone pulses.

The kidney donors had blood types A1, A2, and B and the recipients O, A2 and B. Tyden et al. [10, 11] also proposed an immunosuppressive protocol without splenectomy for the same blood group combinations and reported encouraging results in graft function, an acceptable number of acute rejection episodes and antibody mediated rejections with good response to standard therapy. In comparison to the immunosuppressive protocol proposed by Tyden's group, in our protocol intravenous immunoglobulin was excluded and, after the first four transplantations, induction therapy with monoclonal anti-CD 25 antibodies was also excluded. The use of intravenous immunoglobulin was proposed in order to scavenge HLA alloantibodies and minimize the chance of antibody-mediated rejection. However, as intravenous immunoglobulin contains small amounts of alloantibodies, they can promote an antibody-mediated rejection (own unpublished data). In the follow-up period we noticed acceptable rates of acute steroid-sensitive rejections (30%). Other authors also found similar rates of acute rejections [10, 11].

Considering that most kidney donors were marginal with a mean age of 61.92 ± 6.27 years, renal allograft function was satisfactory, with mean serum creatinine of 129 ± 45 μmol/L, which remained stable during the follow-up. Allograft function and complications after kidney transplantation across blood groups appeared with similar frequency and clinical features as in the ABO compatible kidney transplantations performed in our institution [12, 13].

Limitations of the study: This is a case-series study presenting preliminary experience in a single centre with a specific, modified protocol applied in the care of these complex patients. The study does not compare the results with those for patients with matching ABO donors or with different treatment protocols. However, our results are encouraging and warrant further prospective trials that would address these issues. The diagnosis of acute rejection was made according to clinical and laboratory findings and the response to steroid pulse therapy but was not confirmed by kidney biopsy. Although this is a limitation of the study, the post-transplant course and response to steroid pulse therapy in the patients with the clinical presentation of acute rejection indicated that acute rejection was not a significant problem in the presented incompatible living kidney donor transplantations.

Conclusion

Our original method for removal of anti-donor BG abs in patients receiving kidney transplant from ABO-incompatible donors appears to be safe and effective. The overall survival, complication rates and post-transplant kidney function are comparable to the historical data from ABO-compatible patients.

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R E F E R E N C E S

1. *Gloor JM, Lager DJ, Fidler ME, Grande JP, Moore SB, Winters JL*,et al.A Comparison of splenectomy versus intensive post-transplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation. Transplantation. 2005; 80(11): 1572–7.
2. *Segev DL, Simpkins CE, Warren DS, King KE, Shirey RS, Maley WR*, et al. ABO incompatible high-titer renal transplantation without splenectomy or anti-CD20 treatment. Am J Transplant 2005; 5(10): 2570–5.
3. *Stegall MD, Dean PG, Gloor JM*. ABO-incompatible kidney transplantation. Transplantation 2004; 78(5): 635–40.
4. *Tanabe K, Tokumoto T, Ishikawa N, Shimizu T, Okuda H, Ito S*, et al. ABO-incompatible living donor kidney transplantation under tacrolimus immunosuppression. Transplant Proc 2000; 32(7): 1711–3.
5. *Sonnenday CJ, Warren DS, Cooper M, Samaniego M, Haas M, King KE*, et al. Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. Am J Transplant 2004; 4(8): 1315–22.
6. *Gloor JM, Lager DJ, Moore SB, Pineda AA, Fidler ME, Larson TS*, et al. ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. Transplantation 2003; 75(7): 971–7.
7. *Takahashi K, Saito K, Takahara S, Okuyama A, Tanabe K, Toma H*, et al. Japanese ABO-Incompatible Kidney Transplantation Committee. Excellent long-term outcome of ABO-incompatible living donor kidney transplantation in Japan. Am J Transplant 2004; 4(7): 1089–96.
8. *Cohney SJ, Walker RG, Haeusler MN, Francis DM, Hogan CJ*. Blood group incompatibility in kidney transplantation: definitely time to re-examine! Med J Aust 2007; 187(5): 306–8.
9. *Balint B, Pavlovic M, Jevtic M, Hrvacevic R, Ignjatovic L, Ostojic G*, et al. Simple "closed-circuit" group-specific immunoadsorption system for ABO-incompatible kidney transplants. Transfus Apher Sci 2007; 36(3): 225–33.
10. *Tydén G, Kumlien G, Genberg H, Sandberg J, Sedigh A, Lundgren T*, et al. The Stockholm experience with ABO-incompatible kidney transplantations without splenectomy. Xenotransplantation 2006; 13(2): 105–7.
11. *Tydén G, Kumlien G, Genberg H, Sandberg J, Lundgren T, Fehrman I*. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. Am J Transplant 2005; 5(1): 145–8.
12. *Ignjatović L, Vavić N, Paunić Z, Rabrenović V, Kovačević Z, Hrvačević R*. Renal allograft function in the patients with double immunosuppressive protocol. XVIII Danube Symposium, September 26–30, 2006; Novi Sad, Serbia: Abstract book, p. 144.
13. *Rabrenović V, Kovačević Z, Jovanović D, Ignjatović Lj*. Complications in the kidney transplant patients previously treated by peritoneal dialysis – 10 years experience in a single center. BANTAO J 2007; 5: 93–5.

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