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Organ transplantation in Bulgaria

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Abstract The transplantation program in Bulgaria started in 1968 with renal transplantations to a child and adult woman. In 1986 the first heart transplantation was performed. To date a total of 10 heart transplants have been performed, including one combined heart/lung. A liver transplantation program was launched in 2005 with a total number of 16 transplantations—7 from living donors and 9 from deceased donors. The highest transplantation activity is registered in the field of renal transplantation. During the period 1980–2006, 462 Bulgarian recipients of kidney were transplanted in Bulgaria. The ratio between transplantations from deceased and living related donors is approximately 1:0.9. Annual transplantation activity varies among the years from 1 to 12 renal transplantations p.m.p./per year. The 1- (80.7% vs. 63.1%), 5- (57.86% vs. 39.0%) and 10-year (42.65% vs. 23.62%) graft survival rates are higher for recipients of living donor kidneys compared to those of deceased donor. In 1983 a National kidney waiting list was established. Currently the number of the registered patients eligible for renal transplantation is 885. The proportion of sensitized patients in the waiting list is 20.45% and 4.34% of them are hyperimmunized. Recently HLAMatchmaker program has been implemented not only for sensitized patients but also for those with rare alleles and haplotypes. Post-transplant immunological monitoring showed a strong association between alloantibody presence and delayed graft function (Chi-square = 10.73, $P < 0.001$), acute rejection (Chi-square = 14.504, $P < 0.001$), chronic rejection (Chi-square = 12.84, $P < 0.001$) and graft loss (Chi-square = 20.283, $P < 0.001$). Based on the experience in our transplant center a strategy for improvement of long-term renal graft survival was developed and implemented.

Keywords Anti-HLA antibodies · Histocompatibility · HLAMatchmaker · Organ transplantation · Rejection · Selection approaches

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Transplantation of organs is a life-enhancing and technologically advanced form of therapy in medical practice today. The first successful renal transplant was performed in 1954. Advances in histocompatibility testing and immunosuppressive therapy made it a clinical reality in the 1960's.

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child and adult woman. In 1986 the first heart transplantation was performed. To date a total of 10 heart transplants have been performed, including one combined heart/lung. A liver transplantation program was launched in 2005 with a total number of 16 transplantations—7 from living donor and 9 from deceased donor. In 2000 the National Centre ''Bultransplant'' was established, transformed in Executive Agency for Transplantation in 2004, aimed at providing coordination and control of transplantation process in Bulgaria.

Kidney transplant activity

The highest transplantation activity is registered in the field of renal transplantation (RT). During the period 1980–2006, 462 Bulgarian recipients of kidney were transplanted in Bulgaria. The ratio between transplantations from deceased and living related donors is approximately 1:0.9. The transplantations performed from 1980 to 2006 are illustrated on Fig. 1. The variations between years are due to the number of kidneys available per year. Outline waves of higher activity could be noted in 1998–2000, 2003 and 2006. After 1994 the introduction of the program for living related donors has resulted in a significant increase of the number of transplants. On the other hand in 1999 due to the highest number of kidneys from deceased donors ($n = 54$) the transplantations from living related donors decreased ($n = 16$). The problem in organ donation become more serious after 2004 due to changing the transplant law into the presume consent. The number of used kidneys per million population (p.m.p.)/per year for the whole reported period amounted to 1. Substantial increase was present only in 1999 when 70 RT were carried out or 9.5 p.m.p.—the highest results among the Balkan countries.

Kidney waiting list

In 1983 a National Kidney waiting list was established. During the period 1983–2006, 3020 potential recipients have been immunologically tested for kidney transplantation. Most patients await a first transplant and only 2.17% a repeat transplant. Currently the number of the registered patients eligible for RT is 885 and still highly exceeds the number of transplantations performed.

Analysis of HLA haplotype distribution in the kidney waiting list showed significant heterogeneity due to the prevalence of rare haplotypes. The percentage of rare haplotypes was higher (79.1% out from the whole haplotype number) in the patients group due to the accumulation of recipients with rare for the population HLA haplotypes, who have lower probability for HLA matched transplantation. Frequently such patients are excluded from the selection processes and this is resulting in their long time stay in the waiting list.

The probability such patients to receive a kidney transplant with a greater compatibility

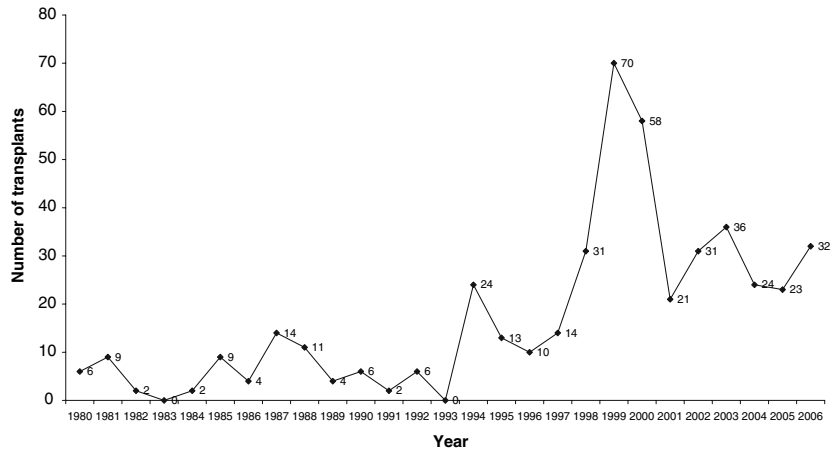

Fig. 1 Transplant activity per year

based on the major HLA allele groups is highly decreased in case the conventional selection criteria are applied. This leads to find alternative strategies for organ allocation that permits greater access to better-matched organs for patients with rare HLA specificities such as HLAMatchmaker computer program (Duquesnoy 2002a, b). The application of this algorithm as a selection criterion increases the probability of finding a matched donor on amino acid triplet level in patients with rare HLA antigens compared to the healthy population. For example, for a patient with HLA-A*01, *02; B*51, *78; DRB1*01, *1001 genotype carrying the rare for the Bulgarian population B*78 allele, the likelihood to find a fully compatible donor using the conventional methods is 0.607%, while applying the HLA-Matchmaker analysis it rises to 1.037%. This is due to the identification of additional antigen specificities with zero triplet mismatch for this recipient—A*29, *36, *69; B*35, *53, some of which (B*35) with high frequency in our population. By this selection approach the phenotypes of the potential donors could be a combination between the patient HLA antigens and other fully matched at structural level antigens. Practically by applying the HLAMatchmaker algorithm it is possible to achieve a decrease of the waiting time of patients with rare HLA specificities. On the other hand significant heterogeneity and accumulation of rare haplotypes emphasize the importance of international organ exchange programs. Comparisons with other populations by phylogenetic analysis based on HLA-A, -B, -DRB1 allele frequencies showed that healthy individuals and recipients for kidney transplantation from the Bulgarian population are more closely related to Romanians, Greeks, Turkish, Italians than to other European populations (Naumova and Ivanova 2006).

Sensibilization on the waiting list

The regular monitoring of the anti-HLA antibodies shows that the greater portion of patients in the waiting list are non-sensitized (Fig. 2). The highly sensitized (> 85% PRA) recipients in the register are few which is most likely due to the application of erythropoetin instead of regular hemotransfusions for the treatment of the secondary anemic syndrome. Relatively low is the percentage of the sensitized recipients (5–85% PRA) as well. One hundred and thirty eight sera (25.2% sensitized recipients) were found to be positive in the alloantibody screening in 2005. The analysis of HLA Class I and Class II antibody reactivity shows a predominant sensitization simultaneously to Class I and Class II (12.4%) and to Class I antigens only (9.2%). The portion (3.6%) of recipients with alloimmune response restricted for Class II specificities is small. A similar distribution of sensitization was established in other registers (Hennessy et al. 2000). The simultaneous presence of antibodies against both HLA Class I and Class II antigens is accepted as a signal for higher level of alloreactivity that should be taken into consideration in transplantation of such recipients (Süsal and Opelz 2004).

A subject of more complex pretransplant immune testing are the ''hyperimmunized'' patients for whom the possibility for a negative crossmatch is very small. In regard to this and the new trends in finding the most appropriate approach of donor/recipient selection we have studied the opportunity of the HLAMatchmaker program application in sensitized recipients. Based on the antibody specificity, the presence of common epitopes from crossreacting HLA antigen groups and the amino acid polymorphisms in the HLA molecules ''acceptable'' mismatched donor antigens could be identified. The HLA-Matchmaker algorithm considers each HLA antigen as a string of polymorphic triplet sequences in antibody-accessible positions of HLA molecules (Duquesnoy 2002a). Through intralocus and interlocus comparisons the program assesses histocompatibility by determining which triplets on mismatched HLA molecules are different or shared between donor and recipient. Antigens with no or few triplet differences are structurally similar to recipient antigens, it is not expected to induce a humoral immune response and could be considered as compatible. For example in a sensitized patient (68% PRA) with HLA genotype A*02, *32; B*35; DRB1*13 (DRB*3) the analysis shows that the antibodies are not directed

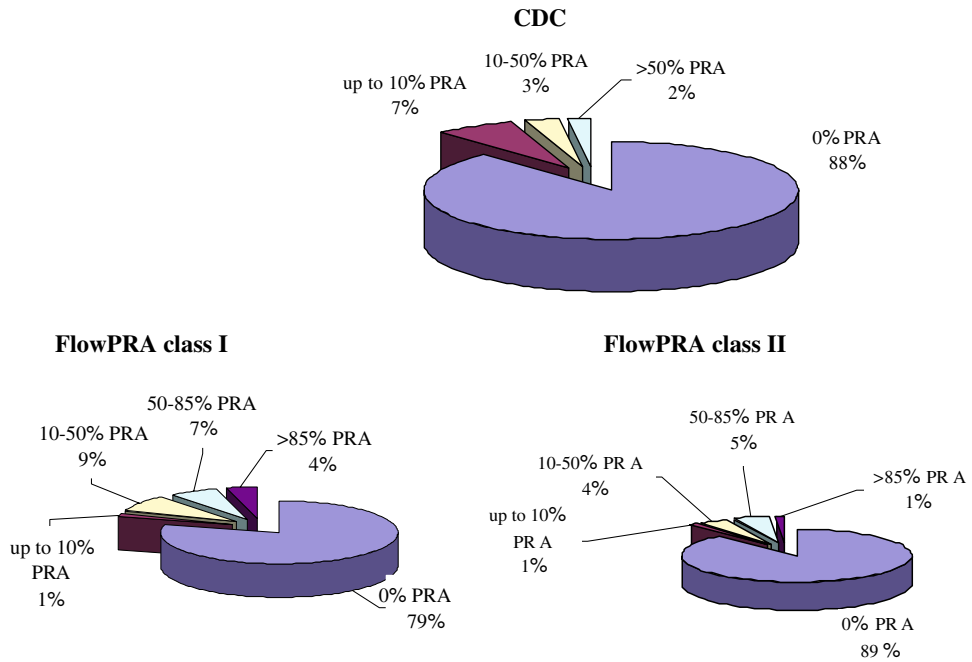

Fig. 2 CDC—complement dependent cytotoxicity; FlowPRA—panel reactive antibodies detected by flow cytometry bead-based techniques; PRA—panel reactive antibodies; RT—renal transplantation

against HLA-A2, 34, 66, 68, 69; B18, 35, 38, 45, 52, 62, 65 antigens which are identified as acceptable specificities. The application of HLAMatchmaker algorithm extremely increases the probability of finding a fully matched donor (4.87%) compared to conventional selection criteria (0.121%) and to acceptable antigen mismatches as well (2.598%). Based on the HLA genotype of this patient and the negative reactions resulting from the antibody specification, the program can detect additional specificities: A25, 74; B39, 49, 50, 51, 53, 59, 64, 70, 71, 72, 75, 77, 78 as antigens with zero triplet mismatch. In this way the number of acceptable antigens is increasing as well as the probability of finding a compatible donor for the hypersensitized patients for whom a selection of highly degree HLA matched transplant is necessary.

Factor influencing kidney graft survival rate

Survival of the graft and the recipient after RT is a function of multiple factors. The effect of donor source on graft survival is shown in Fig. 3. The 1- (80.7% vs. 63.1%), 5- (57.86% vs. 39.0%) and 10-year (42.65% vs. 23.62%) graft survival rates differed for the two donor groups (living related and deceased donors). As could be expected the graft survival is higher for recipients of living donor kidneys compared to those of deceased donor. Additional analysis is necessary to explain the slump of graft survival during the first year after RT. One of the causes could be a relatively high incidence of delayed graft function (DGF) observed in our patients transplanted from deceased donor. The effect of DGF on graft survival is illustrated in Fig. 4. Our data showed nearly 96% graft survival rates for recipients with normal graft function compared to 54% for the recipients with DGF ($P = 0.0006$). In addition, 51.8% of the patients with delayed graft function developed anti-HLA antibodies and significant, positive correlation was observed between allo-antibody presence after transplantation and DGF association (Chi-square = 7.659, $P < 0.01$). Further, recipients with post-transplant HLA-reactive antibodies were more likely to have acute allo-graft rejection as compared to those with no

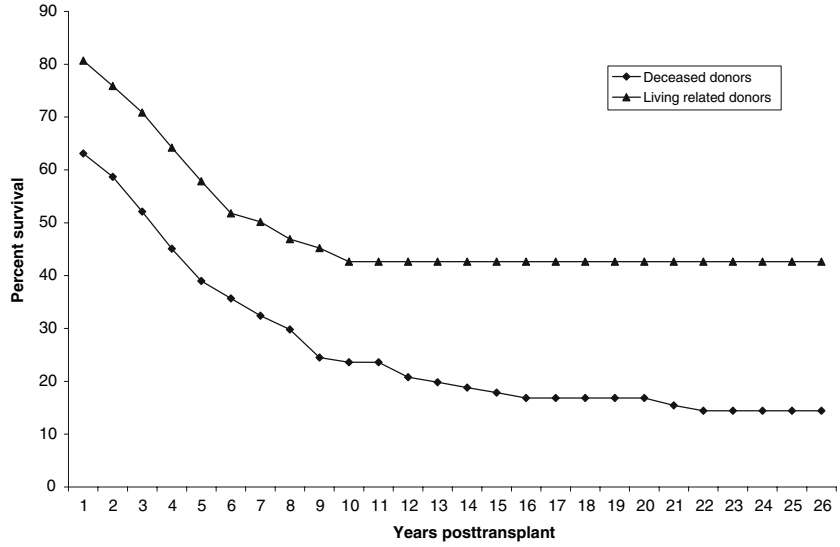

Fig. 3 Graft survival rate (1980–2006) according to donor source

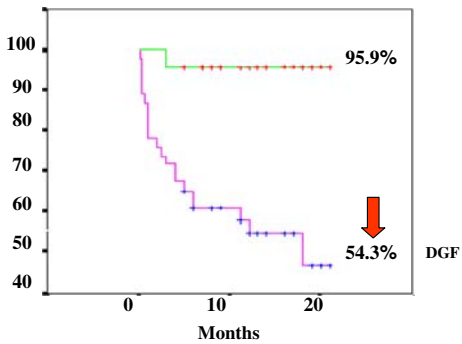

Fig. 4 The survival rate was estimated using the Kaplan–Meier method. The statistical significance was assessed by log rank test. $P < 0.05$ was accepted as statistically significant

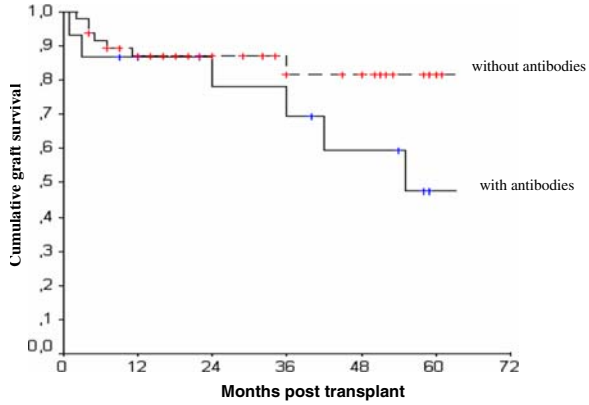

Fig. 5 The survival rate was calculated using the Kaplan–Meier method. The statistical significance was assessed by log rank test

detectable alloantibodies (37.5% vs.3.6%, Chi-square = 14.504, $P < 0.001$). A similar association was also observed with chronic rejection (43.75% vs.7.1%, Chi-square = 12.84, $P < 0.001$). Among patients who developed alloantibodies, 56.25% lost their graft due to immunological causes, compared to only 7.1% in the antibody-negative group (Chi-square = 20.283, $P < 0.001$). In addition, a trend was observed ($P > 0.05$) in which antibody-positive recipients had a lower graft survival rate compared to those without detectable humoral alloimmunization (Fig. 5). This trend was more apparent after the third year post transplant.

Based on this data we have implemented in our transplant center a strategy for improvement of long-term renal graft survival:

- Determination of HLA compatibility at structural level using HLAMatchmaker algorithm for patients with rare HLA alleles and sensitized patients.
- Application of HLAMatchmaker algorithm to predict crossmatch results for sensitized patients.
- Post-transplant monitoring (production of anti-HLA antibodies) as a marker for: recipient alloreactivity and development of

acute and chronic allograft rejection; prediction of long-term allograft survival; assessment of the effect of immunosuppressive therapy on HLA antibody production.

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