Immunobiology 224 (2019) 485–489

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Importance of HLA typing, PRA and DSA tests for successful parathyroid allotransplantation

Emrah Yucesan[a,*], Beyza Goncu[b], Burcu Ozdemir[b], Oguz Idiz[c], Yeliz Emine Ersoy[d], Erhan Aysan[d,1]

[a] Bezmialem Vakif University, Institute of Life Sciences and Biotechnology, Istanbul, Turkey
[b] Bezmialem Vakif University, Experimental Research Center, Istanbul, Turkey
[c] Istanbul Teaching and Research Hospital, General Surgery Clinic, Istanbul, Turkey
[d] Bezmialem Vakif University, Faculty of Medicine, Department of General Surgery, Istanbul, Turkey

ARTICLE INFO

Keywords:
Parathyroid allotransplantation
HLA typing
PRA
DSA
CXM
CDC

ABSTRACT

Parathyroid allotransplantation is increasingly practiced for patients who have permanent hypoparathyroidsm. Parathyroid allotransplantation success is varied, and no defined criteria about immunologic monitoring for pre-/post-transplantation follow-up. This study sought to evaluate the possible role of immunological tests. Four unrelated recipients and one living donor who have chronic kidney disease were evaluated for HLA-typing, PRA, CXM tests to conduct parathyroid allotransplantation. Parathyroid glands were obtained and resected from the donor, then cells were isolated and cryopreserved. Upon histologic examination, cells were cultivated and injected into muscle of four recipients. Recipient's were followed for parathormone and calcium levels for four years. PRA screening were monitored and *de novo* DSA was evaluated as well. In two of the recipients, allografts continued to be functional more than four years. In one recipient, allograft remained functional for two years and another recipient lost function after one year. Two out four were negative for *de novo* DSA and three out of four of the recipients remained negative for PRA. Neither HLA-matching nor *de novo* DSA positivity and PRA screenings seems significant for successfull parathyroid allotransplantation. This study has considerable potential for immunological monitoring of parathyroid allotransplantation.

1. Introduction

Recent advances in transplantation, immunologic monitoring before and after transplantation and including development of new immunosuppression regimens have led to long-term improvements in survival of the transplanted graft. Nonetheless allograft survival is the most critical challenge and overcoming immune response in transplantation starts with post-operative management (Jung et al., 2017). Therefore clinical, immunolological and genetic heterogeniety between donor and recipient will require assessment of risk to prevent rejection (Cravedi and Heeger, 2012).

There are several types of rejection and they can be divided by their mechanisms. Major rejection types are associated with being hyperacute, acute or chronic. In hyperacute rejection the anti-donor antibodies existing in the recipient before transplantation act rapidly and this leads to necrosis of the graft (Moreau et al., 2013). Moreover acute rejection directly acts on the graft and lasts for several months after transplantation. Chronic rejection also is a major cause of rejection. Mainly two types of immune response generates the processes: humoral and cellular rejection which can be mentioned also as antibody-mediated rejection (AMR), T cell-mediated rejection (TCMR) and B cell-mediated rejection (BCMR) while acting alone or in combination (Moreau et al., 2013; Garces et al., 2017). Ideal immunosuppression regimens are determined according to the transplanted graft and increased risk of acute rejection favors allograft whereas their efficacy still remains a promising strategy (Townamchai et al., 2013; Marin et al., 2018). Another immune response is microcirculation inflammation and is directly associated with reduced capillary perfusion, adhesion of leukocytes and activation of many mediators to induce inflammation cascades which are responsible for poor graft survival after transplantation with combination of other responses especially *de novo* donor spesific antibodies (DSA) as well (Granger and Senchenkova, 2010; Arnold et al., 2018).

Human leukocyte antigens (HLA) determination and specifity is

* Corresponding Author at: Adnan Menderes Bulvari, Vatan Caddesi, 34093, Fatih, Istanbul, Turkey. Tel.: +90 212 523 22 88 / ext.1465; Fax: +90 212 453 18 70.
E-mail address: eyucesan@bezmialem.edu.tr (E. Yucesan).
[1] Present address: Yeditepe University, Faculty of Medicine, Department of General Surgery, Istanbul, Turkey.

https://doi.org/10.1016/j.imbio.2019.05.007
Received 22 April 2019; Received in revised form 18 May 2019; Accepted 28 May 2019
Available online 10 June 2019
0171-2985/

enhanced by DNA-based typing techniques. In transplantation, reliable and feasible understanding of HLA typing enabled matching and mismatching HLA allele between donor and recipient is needed. The impact of this pre-evaluated HLA-identity has been contributed with a decreased risk of graft failure (Garces et al., 2017; Montgomery et al., 2018). The matching and mismatching allel criteria depends on the graft sources in transplantation. In addition Panel Reactive Antibody (PRA) screening provides an approximate score of circulating HLA class I and II antigens in patient's serum. Acceptable PRA score differs between each laboratory and cut-off value changes depending on the transplanted graft type. On the other hand, flow cytometric cross match tests (CXM) identified with an increased sensitivity and challenged the HLA antibody detection in serum of recipients while showing that HLA antibodies rise and fall overtime via blood transfusion, transplantation and even pregnancy (Garces et al., 2017; Montgomery et al., 2018; Lee et al., 2011). Furthermore, the microlymphocytotoxicity cross-match (CDC) screening denotes the presence of cytotoxic alloreactive immunoglobulin (Ig) G antibodies against the potential donor HLA antigens. CXM is more sensitive than CDC and cut-off values vary between laboratories (Garces et al., 2017; Montgomery et al., 2018; Ju et al., 2018; Leto Barone et al., 2013). DSA test following transplantation allows for the detection of positive antibodies in recipients serum against donor cells. DSA presence in blood could trigger AMR overtime (Garcia de Mattos Barbosa et al., 2018; Platt et al., 2017). The limitation of this assay is the requirement of donor cells which can be diffucult to obtain if the donor is deseased (Sood and Testro, 2014).

There were no common consensus between most of the immune monitoring tests for kidney, liver, heart, lung, islet or parathyroid transplantations. Each transplantation type includes different pre-op and follow-up criteria. Pre- or post-assessment cannot be graded as a determining factor for which test is more reliable or provides a better success rate is not certain. Each transplantation case should be considered on its own facts.

In parathyroid allotransplantation (PA), there are no reports that have been indentified for immune monitoring. Therefore, the aim of this study is to evaluate the significant role of pre-determination of HLA typing, PRA, CXM tests and as well as post-transplant follow up with PRA and *de novo* DSA's for PA.

2. Materials and methods

This study was conducted after receiving approval from the Local Human Ethics Committee and all of the protocols conformed to the ethical guidelines of the Helsinki Declaration, and written informed consent was obtained from all subjects

2.1. Pre-evaluation of donor and recipients

One donor and four recipients were evaluated for this study. The donor was a 44 year-old 0 Rh (+) male. He had parathyroid hyperplasia secondary to chronic renal failure. Written informed consent was obtained and screened for viral markers; anti-HIV, anti-HCV, anti-HBc,anti-HBs, anti-Hbe antibodies, HBV antigen and cytomegalovirus IgG and IgM antibodies, Epstein-Barr virus IgG, IgM, and venereal disease research laboratory (VDRL) tests. After review of the results, the patient underwent a parathyroidectomy.

Recipient #1 (R#1) was a 48 year old female. She had undergone total thyroidectomy seven years ago. Permanent hypoparathyroidsm (PH) had occurred in the postoperative early stage and she was treated for two years with 2000 mg/d oral calcium (Calcimax-D3®; Basel Ilac Co, Istanbul, Turkey), calcitriol 1.5 mg/d (Rocaltrol®; Deva Ilac Co, Istanbul, Turkey), and levothyroxine sodium 200 mg/d (Levotiron® 100 mg; Abdi Ibrahim Ilac Co, Istanbul, Turkey) to relieve symptoms.

Recipient #2 (R#2) was a 41 year old female. She had undergone total thyroidectomy 17 years ago. She was diagnosed with PH in the early postoperative stage and was also treated for 11 years with 2000 mg/d oral calcium (Calcimax-D3®; Basel Ilac Co, Istanbul, Turkey), and levothyroxine sodium 100 mg/d (Levotiron® 100 mg; Abdi Ibrahim Ilac Co, Istanbul, Turkey) to relieve symptoms.

Recipient #3 (R#3) was a 45 year old female. She had undergone total thyroidectomy 12 years ago. She was diagnosed with PH in the early postoperative stage and was also treated for 7 years with 2000 mg/d oral calcium (Calcimax-D3®; Basel Ilac Co, Istanbul, Turkey), calcitriol 1 mg/d (Rocaltrol®; Deva Ilac Co, Istanbul, Turkey), and levothyroxine sodium 150 mg/d (Levotiron® 100 µg; Abdi Ibrahim Ilac Co, Istanbul, Turkey) to relieve symptoms.

Recipient #4 (R#4) was a 58 year old female. She had undergone total thyroidectomy 21 years ago. She was diagnosed with PH in the early postoperative stage and was also treated for 16 years with 3000 mg/d oral calcium (Calcimax-D3®; Basel Ilac Co, Istanbul, Turkey), calcitriol 1.5 mg/d (Rocaltrol®; Deva Ilac Co, Istanbul, Turkey), and levothyroxine sodium 100 mg/d (Levotiron® 100 mg; Abdi Ibrahim Ilac Co, Istanbul, Turkey) to relieve symptoms.

After obtaining approval and informed consents from the donor and all the recipients, we performed a PA. We evaluated HLA typing, CXM tests for donor and each recipient and PRA was screened for the four recipients as well.

Blood samples were placed into vacutainer tubes. DNA isolation was performed according to the manufacturer's protocol with QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). The HLA genotyping was performed by sequence-specific oligonucleotide primed PCR (PCR-SSO) method by using LABType® SSO typing kit (One-Lambda, CA, USA). The procedure was performed according to the manufacturer's instructions with using LABScan™ 100 analyzer (One-Lambda, CA, USA).

Class I and class II HLA antibodies were screened with a LABScreen® PRA Kit (One Lambda, CA, USA) according to manufacturer's protocol. Positive samples were further tested for the specificity of antibodies for HLA-A, -B, -C, -DR, -DP, -DQ and fluorescence intensity was measured with a Luminex100 flow analyzer. Data was analyzed using LABScan 100 software (One Lambda, CA, USA). Median fluorescence intensity (MFI) of the PRA bead reactions was obtained from the output file generated by the flow analyzer and adjusted for the background signal. Recipients were defined as anti-HLA antibody-positive when they had HLA antibodies with MFI > 2000. In addition, CXM tests for T and B cells (T-FCXM, B-FCXM respectively) and microlymphocytotoxicity (CDC) tests were performed. The donor's and the four recipient's peripheral blood mononuclear cells (PBMC) were isolated. The CDC screening for HLA antibodies was performed and each recipients' serum was treated with dithiothreitol (DTT—an agent that removes IgM) and mixed with the donors' lymphocytes. In addition 5×10^5 PBMCs were incubated in duplicates with serum of the recipients; negative control serum and positive control serum, respectively (mixture of sera with PRA of > 95%). Then, incubated groups were labeled with anti-CD3 antibody for the T-FCXM and anti-CD20 antibody for the B-FCXM, then measured by flow cytometry. The cut-off values were 5% for T cells and 8% for B cells by fluorescence index (FI) which is the percentage shift in the test serum as compared to the positive control ((test serum FI—negative control FI)/(positive control—negative control FI) × 100).

Serum samples from the four recipients were analyzed for Class I and Class II IgG HLA antibodies using the commercially available LABScreen SAB assay kit (One Lambda, Inc., CA, USA) on a LABScan™ 100 (Luminex® 100/200, One Lambda, Inc., CA, USA). The procedure was performed according to the manufacturer's instructions, and samples were analyzed using Luminex 100 IS v 2.3 software (Luminex Corporation, USA).

2.2. Parathyroid cell isolation and cryopreservation

After parathyroidectomy and histopathologic confirmation of hyperplasia, the remaining parts of the glands were transported to the parathyroid cell culture laboratory unit in an ice cold AmnioMAX II

Complete Medium (Thermofisher Scientific,MA USA) supplemented with 20% heat inactivated fetal bovine serum (FBS) (Thermofisher Scientific, MA USA) and 1% penicillin-streptomycin (Thermofisher Scientific, MA USA). Remaining parathyroid tissue pieces were mechanically disintegrated and filtered with a cell strainer (70 µm, BD Biosciences, NJ, USA) in 1X PBS (pH 7.4) then supplemented with 5% heat inactivated FBS and 0.4 µM Dnase I (AppliChem, Gatersleben, Germany). Subsequently cell suspension was centrifuged at 270 *g-forces* for seven minutes at room temperature. The supernatant was removed and the cells re-suspended in 1 mL of culture medium. The cell viability was assessed with a Muse™ Cell Analyzer (Merck Millipore, Darmstadt, Germany) and then the cells were cryogenically stored with 10% DMSO (AppliChem, Darmstadt, Germany) using a isopropanol-based freezing container, then stored in the nitrogen tank.

2.3. Immunosuppression treatment

Each recipient received 250 mg methylprednisolone one hour before the transplantation time. Methylprednisolone was administered at a dose of 125 mg and 60 mg on the second and third day of post-PA respectively, to minimize the host's reaction.

2.4. Preparation of cells for transplantation

The cells in the nitrogen tank were removed from cryopreservation and cultivated in 175 cm^2 T-flasks with AmnioMAX II Complete Medium and placed in an incubator (ESCO, Singapore) at 37 °C with 5% CO_2 humidified atmosphere. After cultivation for approximately 24–30 hours, cells were collected and centrifuged at 270 *g-forces* for seven minutes at room temperature. Supernatant were removed and cell viability was assessed using a Muse Cell Analyzer (Merck Millipore, Darmstadt, Germany) with a Muse Count & Viability Assay Kit (Merck Millipore, Darmstadt, Germany). The 50×10^6 parathyroid cells were re-suspended in each recipient serum and cells were injected into the left deltoid muscle of the four recipients using a 14-gauge needle. Wound dressings were applied to the injection sites, and an arm sling was used to immobilize and rest the deltoid muscles.

2.5. Monitoring after transplantation and statistical analysis

Circulating PTH and calcium levels were measured weekly for 3 months and monthly for one year then followed for four years biannually after transplantation, and all data during the given period were compared with pre-op values. Unpaired *t*-test (GraphPad Software, Inc., CA, USA) was used for analysis, and $p < 0.05$ was considered statistically significant.

In addition, PRA changes and *de novo* post-transplant DSA of the donor after four years of follow-up were evaluated.

3. Results

All of the recipients were screened for PRA, HLA typing and crossmatch tests before transplantation. Donor and recipient's were ABO compatible. The T-FCXM, B-FCXM, and CDC crossmatch tests, as well as PRA screenings were negative for four recipients before transplantation.

HLA locus matching analysis indicated R#1 HLA-DQB1 was fully matched, R#2 HLA-A one allele matched, R#3 HLA-B one allele matched, R#4 HLA-DQB1 one allele matched. HLA matching/mismatching allele are indicated in Table 1.

According to the analyzes, PTH levels were found statistically significant for R#1 and R#2 ($p = 0.0005$ and $p = 0.0004$, respectively) after four years. When we evaluate R#3, no statistically significant was found after four years ($p = 0.2750$). Interestingly, during two years of post-transplantation PTH level was increased for R#4, however after the second year of transplantation, PTH level is decreased dramatically. At the end of the year four PTH level could not be detected, and at year four was not found statistically significant when compared with pre-op levels ($p = 0.2750$).

Recipients were discharged after transplantation from the hospital without any complications on day 3. Calcium and calcitrol were discontinued on post-transplantation day 10 for all recipients. Three recipient's (R#1, R#2 and R#4) PTH levels were increased at day 10 and medication ceased steadily and hypocalcemic symptoms diminished. Although R#3 lowered the current calcium and calcitriol dose to one half at day 10 even the PTH levels increased rapidly, her symptoms remained moderate. In addition, R#4 started using her medications to the pre-transplantation level after three years. Changes in PTH and calcium levels during the four year follow-up are included in Table 2.

PRA levels at pre-transplantation, two and four years after transplantation were determined and these results are presented in Table 3. R#4 PRA levels in HLA class I at year two after transplantation were positive. In addition, R#1 PRA levels in HLA class II at year four after transplantation were positive. R#2 and R#3 PRA levels remained negative on subsequent post-transplant monitoring (up to four years).

de novo DSA were evaluated in R#1, R#2, R#3 and positivity was only detected in R#1 and R#3 at the fourth year (Table 4).

4. Discussion

In the pre-transplant setting, the donor must be compatible with the recipient. Therefore, monitoring the immune system for HLA typing, PRA and CXM tests are the major standard screening for graft survival and even elimination of acute rejection. Another important influence is immunosuppression type and dosing following transplantation. In particular, many transplantation cases reported assays for immune monitoring with their advantages and disadvantages (Sood and Testro, 2014). Monitoring for immune status of a recipient can be broadly divided into two distinct groups; antigen-specific and antigen-non-specific (Townamchai et al., 2013; Montgomery et al., 2018; Leto Barone et al., 2013; Sood and Testro, 2014). The donor-specific immune response can be determined with antigen-specific screening tests such as PRA, DSA, Single Antigen Bead (SAB), Mixed Lymphocyte Reaction (MLR), and the Enzyme-linked ImmunoSpot Assay (ELISPOT) (Townamchai et al., 2013; Sood and Testro, 2014).

Different transplantations show heterogeneous patterns, thus every single tissue should be evaluated by itself. In kidney transplantation, clinically significant HLA antigens are -A, -B, -DR (Khan et al., 2016) and as a post-transplant biomarker as well; serum creatinine level, PRA, DSA and renal biopsies are widely used for the detection of circulating HLAs against donor cells (Townamchai et al., 2013; Khan et al., 2016). The liver is known as an immunotolerant organ, however in liver transplantation, rejection rate is 30–40%. Neither recipient's age nor ABO incompatibility has an effect on liver transplant outcome significantly (Wei et al., 2018; Chae et al., 2018). In heart transplantation, pre-transplant evaluation of ABO compatibility and PRA screening tests and as a post-transplant determination of rejection endomyocardial biopsy is the gold standard follow-up test (Mangini et al., 2015). In lung transplantation, diagnostic criteria of rejection revised in 2016 and HLA-A and –DR matching, PRA screening, CXM tests and SAB evaluation before transplantation are the most valuable tests as well as during post-transplantation follow-up (Ju et al., 2018). In order to achieve higher transplantation success, it is first necessary to have a compatible donor and recipient by pre- and post- determination of antibody characteristics via immunologic monitoring tests for each graft separately.

In PA, immunologic monitoring has no standard criteria yet. In the literature, several authors reported different methods to restore parathyroid function via transplantation, particularly without immunosupression (Barczynski et al., 2017; Nawrot et al., 2007; Yucesan et al., 2017; Aysan et al., 2016a; Aysan et al., 2016b; Agha et al., 2016; Torregrosa et al., 2005). From a clinical point of view, the side effects of immunosuppressants are more destructive than the use of medication for hypoparathyroidism and this is why the use of immunosuppression

Table 1
Matching/mismatching HLA alleles between donor and four recipient. (HLA: Human Leukocyte Antigens, N/A:Not available).

	Class I			Class II	
	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1
Donor	A*26 / A*32	B*14 / B*44	C*05 / C*08	DRB1*01 / DRB1*04	DQB1*03 / DQB1*05
Recipient 1	A*02 / A*02	B*51 / B*51	C*07 / C*15	DRB1*11 / DRB1*16	DQB1*03 / DQB1*05
Recipient 2	A*01 / A*32	B*08 / B*57	C*06 / C*07	DRB1*03 / DRB1*03	DQB1*02 / DQB1*02
Recipient 3	A*11 / A*24	B*44 / B*51	C*04 / C*07	DRB1*13 / DRB1*13	DQB1*06 / DQB1*06
Recipient 4	A*02 / A*68	B*18 / B*51	C*07 / C*15	DRB1*11 / DRB1*11	DQB1*03 / DQB1*03

Table 2
Determination of PTH and calcium levels of each recipient's throughout four year. PTH and calcium levels shown as pg/mL and mg/dl respectively. (PTH: Parathormone, pre-PA: before parathyroid allotransplantation, post-PA:after parathyroid allotransplantation).

		R#1		R#2		R#3		R#4	
		PTH	Calcium	PTH	Calcium	PTH	Calcium	PTH	Calcium
pre-PA	**day 0**	0.1	12.5	8.8	6.6	0.1	8.3	0.1	9.2
post-PA	**day 3**	4.3	9.5	13.1	6.4	0.1	7	0.01	7.9
	day 10	10.2	9	11.2	8.7	3.83	8.4	3.52	10.3
	month 1	39.4	8.8	5.9	6.9	5.54	6.4	8.99	7.8
	month 2	13.99	9.8	8	7.1	0.1	6.5	9.23	7.3
	month 3	20.45	9.2	14.6	6.6	5.68	6.6	11.33	7
	year 1	31.5	9	11.39	7.2	7.49	7.5	8	7.3
	year 2	43.1	9.1	10.7	6.8	0.9	8.9	9.5	9
	year 3	22	9.3	N/A	N/A	1.7	7.8	2.5	11.8
	year 4	42	9.2	14.4	7.5	0.5	8.9	N/A	N/A

Table 3
Comparision of pre-transplantation, post-transplantation (second and fourth year respectively) PRA levels of four recipient's (HLA: Human Leukocyte Antigen, PRA: Panel Reactive Antibody, MFI: Median Fluorescence Intensity, N/A: Not available).

	Recipient 1		Recipient 2		Recipient 3		Recipient 4	
	HLA Class I	HLA Class II	HLA Class I	HLA Class II	HLA Class I	HLA Class II	HLA Class I	HLA Class II
Pre-transplantation	negative	negative	negative	negative	negative	negative	negative	negative
Year 2	negative	negative	N/A	N/A	N/A	N/A	B35 (MFI:2476) B46 (MFI:2476) B53 (MFI:2184) B54 (MFI:917) B58 (MFI:843)	negative
Year 4	negative	DQ3 (MFI:866) DR1 (MFI:1321) DR10 (MFI:1504) DR16 (MFI:1284) DR52 (MFI:1504) DR53 (MFI:1297)	negative	negative	negative	negative	N/A	N/A

Table 4
Evaluation of post-transplant *de novo* DSA at year four (HLA: Human Leukocyte Antigen, DSA:Donor Specific Antibody, MFI: Median Fluorescence Intensity, N/A: Not available).

	DSA	
	HLA Class I	HLA Class II
Recipient 1	negative	positive (MFI:1095)
Recipient 2	negative	negative
Recipient 3	negative	positive (MFI:540)
Recipient 4	N/A	N/A

was ruled out.

Flechner et al., performed a redo-PA between zero-HLA mismatched deseased donor and a kidney transplant recipient receiving treatment with immunosuppressants, and during 26 weeks follow-up, the recipient did not experience rejection (Flechner et al., 2010). Another study reported that simultaneous transplantation with kidney and healthy parathyroid tissue was maintained successfully and still continues after four years, with a HLA-haploidentical and ABO compatible donor. Besides, the recipient was already receiving immunosuppressive drugs for the kidney transplantation (Garcia-Roca et al., 2016). Several cases have been published about patients with a kidney and a parathyroid transplanation and therefore it is easy to achieve transplantation because of receiving immunsupression medication (Torregrosa et al., 2005; Flechner et al., 2010; Alfrey et al., 1992). In another study, Yucesan et al. carried out parathyroid transplantation without immunsuppression on two unrelated recipients and before transplantation the PRA MFI value < 2000 and negative CXM results were determined (Yucesan et al., 2017).

Initial results are encouraging, however we still are unable to determine pre-transplantation, post-transplantation follow-up criteria for PA. In this study we pre-evaluated matching/mismatching HLA allele, PRA and CXM tests between ABO compatible, donor and four unrelated recipients. During post-transplantation, the outcomes of the four recipients with PRA, DSA tests were analyzed.

During the follow-up the PRA level for R#2 and R#3 remained negative Only for R#1, PRA became positive for HLA class II after four years. Although MFI is less than 2000 and graft functionality was stable. On the other hand, R#4's PRA levels only changed for HLA class I after two years (MFI < 2500). Surprisingly, R#4 PRA level of HLA class I positivity was accompanied by the function loss of the graft after two years of the PA and began her medications. This outcome, particularly for HLA class I antigens, has been known to cytotoxic activation and leads the recipient for risk of rejection (Butler et al., 2017). According

to these results, PRA levels may become positive either for HLA class I or class II with 20% however this alteration may seem significant only for HLA class I.

Throughout the post-PA, R#2 was negative for DSA but R#1 and R#3 were positive for HLA class II. However, even the DSA was positive for R#1 and she did not experience any rejection. In addition, R#2 remained negative for PRA, DSA and also the allograft was still functioning. Moreover, R#3 had graft dysfunction and continued her medications after post-PA at year one, despite negative PRA. Also, DSA was positive after four years and we assumed that this could be the reason for rejection after one year. Yet we are unable to predict how harmful DSA will be, further follow-up with other PA recipient's is required. Intriguingly, a couple of patients following DSA in solid organ transplantations did not have any rejection even with the stable DSA positivity (Townamchai et al., 2013; Montgomery et al., 2018; Garcia de Mattos Barbosa et al., 2018; Butler et al., 2017). The presence of DSA is a critical question, in our case R#1 should be followed for the persistancy of DSA.

In conclusion pre-evaluation of compatible donor and recipient for PA should require PRA, CXM screenings both T, B, and CDC as well. Concurrently the graft function follow-up should be evaluated with more cases.

References

Jung, H.Y., Kim, Y.J., Choi, J.Y., Cho, J.H., Park, S.H., Kim, Y.L., Kim, H.K., Huh, S., Won, D.I., Kim, C.D., 2017. Increased circulating t lymphocytes expressing HLA-DR in kidney transplant recipients with microcirculation inflammation. J. Korean Med. Sci. 32 (6), 908–918.

Cravedi, P., Heeger, P.S., 2012. Immunologic monitoring in transplantation revisited. Curr. Opin. Organ Transplant. 17 (1), 26–32.

Moreau, A., Varey, E., Anegon, I., Cuturi, M.C., 2013. Effector mechanisms of rejection. Cold Spring Harb. Perspect. Med. 3 (11).

Garces, J.C., Giusti, S., Staffeld-Coit, C., Bohorquez, H., Cohen, A.J., Loss, G.E., 2017. Antibody-mediated rejection: a review. Ochsner J. 17 (1), 46–55.

Townamchai, N., Safa, K., Chandraker, A., 2013. Immunologic monitoring in kidney transplant recipients Kidney. Diabetes Res. Clin. Pract. Suppl. 32 (2), 52–61.

Marin, E., Cuturi, M.C., Moreau, A., 2018. Tolerogenic dendritic cells in solid organ transplantation: where do we stand? Front. Immunol. 9 (274).

Granger, D.N., Senchenkova, E., 2010. Inflammation and the Microcirculation. San Rafael (CA).

Arnold, M.L., Kainz, A., Hidalgo, L.G., Eskandary, F., Kozakowski, N., Wahrmann, M., Haslacher, H., Oberbauer, R., Heilos, A., Spriewald, B.M., Halloran, P.F., Böhmig, G.A., 2018. Functional Fc gamma receptor gene polymorphisms and donor-specific antibody-triggered microcirculation inflammation. Am. J. Transplant.

Montgomery, R.A., Tatapudi, V.S., Leffell, M.S., Zachary, A.A., 2018. HLA in transplantation. Nat. Rev. Nephrol.

Lee, J., Romero, R., Xu, Y., Kim, J.S., Park, J.Y., Kusanovic, J.P., Chaiworapongsa, T., Hassan, S., Kim, C.J., 2011. Maternal HLA panel-reactive antibodies in early gestation positively correlate with chronic chorioamnionitis: evidence in support of the chronic nature of maternal anti-fetal rejection. Am J Reprod Immunol. 66 (6), 510–526.

Ju, L., Suberbielle, C., Li, X., Mooney, N., Charron, D., 2018. HLA and lung transplantation. Front. Med.

Leto Barone, A.A., Sun, Z., Montgomery, R.A., Lee, W.P., Brandacher, G., 2013. Impact of donor-specific antibodies in reconstructive transplantation. Expert Rev. Clin. Immunol. 9 (9), 835–844.

Garcia de Mattos Barbosa, M., Cascalho, M., Platt, J.L., 2018. Accommodation in ABO-incompatible organ transplants. Xenotransplantation 25 (3), e12418.

Platt, J.L., Kaufman, C.L., Garcia de Mattos Barbosa, M., Cascalho, M., 2017. Accommodation and related conditions in vascularized composite allografts. Curr. Opin. Organ Transplant. 22 (5), 470–476.

Sood, S., Testro, A.G., 2014. Immune monitoring post liver transplant. World J. Transplant. 4 (1), 30–39.

Khan, A., Nasr, P., El-Charabaty, E., El-Sayegh, S., 2016. An insight into the immunologic events and risk assessment in renal transplantation. J. Clin. Med. Res. 8 (5), 367–372.

Wei, Q., Wang, K., He, Z., Ke, Q., Xu, X., Zheng, S., 2018. Acute liver allograft rejection after living donor liver transplantation: risk factors and patient survival. Am. J. Med. Sci. 356 (1), 23–29.

Chae, M.S., Lee, N., Choi, H.J., Chung, H.S., Park, C.S., Lee, J., Choi, J.H., Hong, S.H., 2018. Comparison of liver graft regeneration between ABO-Compatible and ABO-Incompatible living donor liver transplantation: a propensity score matching analysis. Ann. Transplant. 23, 507–519.

Mangini, S., Alves, B.R., Silvestre, O.M., Pires, P.V., Pires, L.J., Curiati, M.N., Bacal, F., 2015. Heart transplantation: review. Einstein (Sao Paulo) 13 (2), 310–318.

Barczynski, M., Golkowski, F., Nawrot, I., 2017. Parathyroid transplantation in thyroid surgery. Gland Surg. 6 (5), 530–536.

Nawrot, I., Wozniewicz, B., Tolloczko, T., Sawicki, A., Gorski, A., Chudzinski, W., Wojtaszek, M., Grzesiuk, W., Sladowski, D., Karwacki, J., Zawitkowska, T., Szmidt, J., 2007. Allotransplantation of cultured parathyroid progenitor cells without immunosuppression: clinical results. Transplantation 83 (6), 734–740.

Yucesan, E., Goncu, B., Basoglu, H., Ozten Kandas, N., Ersoy, Y.E., Akbas, F., Aysan, E., 2017. Fresh tissue parathyroid allotransplantation with short-term immunosuppression: 1-year follow-up. Clin. Transplant. 31 (11).

Aysan, E., Altug, B., Ercan, C., Kesgin Toka, C., Idiz, U.O., Muslumanoglu, M., 2016a. Parathyroid allotransplant with a new technique: a prospective clinical trial. Exp. Clin. Transplant. 14 (4), 431–435.

Aysan, E., Kilic, U., Gok, O., Altug, B., Ercan, C., Kesgin Toka, C., Idiz, U.O., Muslumanoglu, M., 2016b. Parathyroid allotransplant for persistent hypocalcaemia: a new technique involving short-term culture. Exp. Clin. Transplant. 14 (2), 238–241.

Agha, A., Scherer, M.N., Moser, C., Karrasch, T., Girlich, C., Eder, F., Jung, E.M., Schlitt, H.J., Schaeffler, A., 2016. Living-donor parathyroid allotransplantation for therapy-refractory postsurgical persistent hypoparathyroidism in a nontransplant recipient - three year results: a case report. BMC Surg. 16 (1), 51.

Torregrosa, N.M., Rodriguez, J.M., Llorente, S., Balsalobre, M.D., Rios, A., Jimeno, L., Parrilla, P., 2005. Definitive treatment for persistent hypoparathyroidism in a kidney transplant patient: parathyroid allotransplantation. Thyroid 15 (11), 1299–1302.

Flechner, S.M., Berber, E., Askar, M., Stephany, B., Agarwal, A., Milas, M., 2010. Allotransplantation of cryopreserved parathyroid tissue for severe hypocalcemia in a renal transplant recipient. Am. J. Transplant. 10 (9), 2061–2065.

Garcia-Roca, R., Garcia-Aroz, S., Tzvetanov, I.G., Giulianotti, P.C., Campara, M., Oberholzer, J., Benedetti, E., 2016. Simultaneous living donor kidney and parathyroid allotransplantation: first case report and review of literature. Transplantation 100 (6), 1318–1321.

Alfrey, E.J., Perloff, L.J., Asplund, M.W., Dafoe, D.C., Grossman, R.A., Bromberg, J.S., Holland, T., Naji, A., Barker, C.F., 1992. Normocalcemia thirteen years after successful parathyroid allografting in a recipient of a renal transplant. Surgery 111 (2), 234–236.

Butler, C.L., Valenzuela, N.M., Thomas, K.A., Reed, E.F., 2017. Not all antibodies are created equal: factors that influence antibody mediated rejection. J. Immunol. Res. 2017, 9.