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The role of donor-specific anti-HLA antibodies in the development of humoral rejection

L.K. Tkhatl'[1,2], I.A. Pashkova[1,2], E.D. Kosmacheva[1,2]

Research Institute – Regional Clinical Hospital No 1

n.a. Prof. S.V. Ochapovsky,

167 1 May str., Krasnodar 350086 Russia;

[2]*Kuban State Medical University,*

4 Sedin str., Krasnodar 350063 Russia

Correspondence to: Laura K. Tkhatl', Cardiologist, Cardiology Department No 3 at Research Institute – Regional Clinical Hospital No 1 n.a. Prof. S.V. Ochapovsky, e-mail: laura_namitokova@mail.ru

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Humoral rejection belongs to severe complications of the post-transplant period. Mechanisms of humoral rejection proceed through antibody formation. Donor specific anti-HLA antibodies (*anti-HLA DSA) damage transplant tissues by activating the complement system, leading to the dysfunction and loss of the transplanted organ. This article describes the clinical case of humoral rejection development with the identification of anti-HLA DSA, their impact on the risk of coronary artery disease of the transplanted heart, and the graft loss.*

Keywords: transplantation of heart, humoral rejection, donor specific anti-HLA antibodies

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Ao, aorta

CAG, coronary angiography

CHF, chronic heart failure

CI, cardiac index

CPAP, calculated pulmonary artery pressure

CTCAD, cardiac transplant coronary artery disease

DCMP, dilatation cardiomyopathy

DSA, donor-specific antibodies

Echo-CG, echocardiography

EDD, end-diastolic dimensions

EDV, end-diastolic volume

EF, ejection fraction

EMB, endomyocardial biopsy

HLA, Human Leukocyte Antigens

HR, heart rate

HT, heart transplantation

IHC, immunohistochemistry

ISHLT, International Society for Heart and Lung Transplantation

IVS, interventricular septum

LA, left atrium

LV PW, left ventricle posterior wall

LV, left ventricle

MHC, major histocompatibility complex

MV, mitral valve

non-DSA, non-donor-specific antibodies

PA, pulmonary artery

PAWP, pulmonary artery wedge pressure

PCI, percutaneous coronary intervention

PVR, pulmonary vascular resistance

RV, right ventricle

TPG, transpulmonary gradient

Heart transplantation (HT) is a radical treatment for patients with end-stage chronic heart failure (CHF) [1, 2].However, the success of surgical treatment is limited due to a number of complications of the postoperative period [3]. Graft rejection reactions leading to loss of the transplanted organ are a dangerous complication of the post-transplant period. Despite the improvements in immunosuppressive therapy for heart transplant recipients, immune responses against alien graft cells still actively occur triggering the cellular and humoral mechanisms of transplanted graft rejection. One of the targets of the immune response includes donor leukocyte antigens (Human Leukocyte Antigens - HLA) [4]. The HLA system antigens are encoded by the genes of the major histocompatibility complex (MHC). These genes are responsible for the coordination of cellular and humoral immunity. In solid organ transplantation, the HLA class I (HLA-A, HLA-B), and class II (HLA-DR) genes are of importance. Depending on the path of antigen recognition, this or that type of rejection is dominant. Mechanisms of humoral response proceed through antibody formation mediated by B lymphocytes that are transformed into plasma cells producing antibodies that in turn damage graft tissues by activation of the complement system or antibody-dependent cellular cytotoxicity [4]. The complement system proteins do not recognize specific antigens themselves, however, the activation of the complement cascade requires the antigen-antibody complex formation, which results in triggering the classical pathway of complement

activation mediating most of the biological responses of the humoral immune response. The cascade of reactions leads to C4b production, C4d being its fragment. For already 10 years, the role of the complement C4d component in the development of humoral rejection and the relationship with antibodies against leukocyte donor antigens (anti-HLA) has remained controversial [5, 6]. The use of immunofluorescence in endomiocardial biopsy (EMB) for the diagnosis of humoral rejection made it possible to present more precisely the morphological pattern of early stages of this process, where the vessels of the microcirculatory bed are the main target for antibodies. Due to a wide implementation of immunohistochemical (IHC) techniques for detecting the C4d fixation on the capillary endothelium, the criteria of humoral rejection were revised and extended in 2015 Classification Edition. The International Society for Heart and Lung Transplantation (ISHLT) recommends a routine detection of C4d after HT. Currently the humoral rejection diagnosis is made in cases of present histological signs of rejection, IHC, the presence of antibodies against donor organ antigens in the recipient, and the transplanted organ dysfunction [7]. However, EMB is an invasive diagnostic method and is associated with a number of complications; therefore an active search for non-invasive methods to diagnose the graft rejection is going on. In modern transplantation, the serum tests for anti-HLA raise much interest. The relevant anti-HLA tests prior to transplantation, the identification of donor-specific antibodies (DSA) in the postoperative period may be referred to promising noninvasive methods of diagnosing and predicting the risk of humoral rejection development [8-10]. A number of studies have indicated that the DSA emerging after HT (de novo) increase the risk of poor outcomes associated with graft loss. Patients with DSA have higher rates of

poor outcomes, severe vasculopathy, and early development of the cardiac transplant coronary artery disease (CTCAD), compared to the recipients without DSA [11-15]. M.J.O'Connor, B.C.Keeshan et al. described also the adverse effect of non-donor-specific antibodies (non-DSA) on the prognosis, their association with a high risk of death and late cellular and humoral rejection [9]. The available data demonstrate that the association of anti-HLA de novo, in particular DSA, with clinical results has been still insufficiently proved and therefore further research is necessary [16-18]. We deemed it advisable to present a clinical Case Report of the humoral rejection with de novo-identified DSA that was seen in the Research Institute – Regional Clinical Hospital (RI RCH) No.1, in Krasnodar.

Clinical Case Report

In July 2010, Patient A., 43 years old, turned to the polyclinic of the Center for Thoracic Surgery of RI RCH No.1 with complaints of dyspnea at minimal physical exertion that increased in the horizontal position, of dry cough, blood streaks in sputum, asphyxia attacks at night, abdominal pain, lower extremity swelling, palpitations and weakness. The above complaints appeared in March 2010, for which the patient was hospitalized in a local hospital at his place of residence with the diagnosis of "Ischemic heart disease, old myocardial infarction (without specifying the date)". The patient received the therapy with beta-blockers, diuretics, anti-aggregants without much improvement of his condition. For the additional examinations and diagnosis clarification, the patient was referred to the Center for Thoracic Surgery of RI RCH No.1 that resulted in hospital admission. In-hospital examination diagnosed the dilatation cardiomyopathy (DCMP); a symptomatic therapy for heart failure was administered, including diuretics,

angiotensin-converting enzyme inhibitors, beta-blockers, aldosterone in therapeutic doses. The patient was discharged for outpatient treatment and follow-up. The therapy proved to be ineffective, the patient's condition progressively deteriorated, his dyspnea increased, the exercise tolerance went down. In October 2010, the patient was re-hospitalized to RI RCH No. 1 for investigations aimed at his preparation to HT.

The patient's condition on hospital admission was severe. Skin surface, mucous membranes were pale. There was swelling in the legs and feet. He showed coarse breathing in lungs, occasional dry wheezes in the lower sections of the lungs, the respiration rate was 24-26 breaths per minute. The boundaries of obtusio cardiaca relativa were extended to the left by 2 cm. There were muffled heart sounds, tone I at the apex was weakened, tone II on the pulmonary artery was split, the rhythm was regular. Systolic murmur was detected at the apex and mitral valve areas. The arterial blood pressure was 85/65 mm Hg, the heart rate (HR) was 95 per min. The liver was enlarged by 4 cm, being dense, painless on palpation. No dysuriac symptoms were noted. The physical examinations and tests gave the following findings: sinus tachycardia with a heart rate of 95 per min on electrocardiography, the horizontal position of the electric axis, left His bundle-branch block, impaired intra-atrial conduction. There were signs of enlargement of both atria and ventricles. Spirography demonstrated obstructive abnormalities. The 6-minute walk test result was 210 m. Transthoracic echocardiography (Echo-CG) showed the dilated cardiac chambers: with the left ventricle (LV) end-diastolic dimension (EDD) of 78 mm, the LV end-diastolic volume (EDV) of 369 ml, the LV ejection fraction (EF) of 10%, the left atrium (LA) being 55 mm, the right ventricle (RV) 37 mm, the interventricular septum (IVS) 9 mm; the LV posterior wall (PW)

was 9 mm; there was the LV wall diffuse hypokinesis, the calculated pulmonary artery pressure (CPAP) of 42 mm Hg, the mitral valve regurgitation of grade 2-3 (MV ++/+++) (Table 1).

Table 1. Transthoracic echocardiography

EDD, mm	EDV, ml	EF, %	LA, mm	Pancreas, mm	ISV, mm	LV PW, mm	CPAP, mmHg.	Regurgitation on MV	Impaired contractility
↑ 78	↑ 369	↑ 10	↑ 55	↑ 37	9	9	↑ 42	++ / +++	*Diffuse hypokinesis*

Coronary angiography (CAG) showed no hemodynamically significant obstructions to the blood flow in the left and right coronary arteries, and their main branches. Simultaneously, left ventriculography with cardiac catheterization and pressure measurement was performed yielding the following results: EF 15.1%, EDV 479.2 ml, dilated LV cavity, reduced overall contractility due to diffuse hypokinesia, LV pressure 120/65/20 mm Hg, the pressure in the aorta (Ao) 110/95/86 mm Hg, an increased pressure in the pulmonary artery (PA) 55/44/36 mm Hg. (Table 2).

Table 2. Left ventriculography and cardiac catheterization

EDV, ml	LV Systolic pressure, mmHg	Diastolic pressure in LV, mm Hg	LV Systolic pressure, mm Hg	Systolic pressure in Ao, mm Hg	Diastolic pressure in Ao, mm Hg	Mean pressure in Ao, mm Hg	Systolic pressure in PA, mm Hg	Diastolic pressure in PA, mm Hg	Mean pressure in PA, mm Hg
↑ 479.2	120	↑ 62	↓ 20	110	↑ 95	86	↑ 55	↑ 44	↑ 36

Central hemodynamics parameters were also evaluated using the Swan-Ganz catheter (Table 3).

Table 3. Central hemodynamics parameters

Mean pressure in PA, mm Hg.	Mean pressure in PA after nitric oxide	PAWP, mm Hg	TPG, mm Hg	CI, l/m^2 x min	PVR, Wood units	Reaction to nitric oxide
↑ 41	↑ 46	↑ 34	7th	↓ 1.7	165	positive

On October 20, 2010, the patient was placed on the waiting list for heart transplantation with the final clinical diagnosis of "DCMP. Mitral, tricuspid valve insufficiency. Pulmonary hypertension. CHF classified as CF-2B-3, NYHA FC III-IV. Acute left ventricular failure attacks. UNOS Status 1b". On November 26, 2010, the orthotopic HT was performed on vital indications. The donor was a 29-year-old man who died of injuries sustained as a result of a traffic accident. The donor and the recipient were AB0-group compatible. Before HT, HLA typing was performed, anti-HLA antibodies were screened (no antibodies were detected); a standard complement-dependent cytotoxicity cross-match test showed negative cross-match. After HT, a standard immunosuppressive therapy was administered including mycophenolic acid 1440 mg/day, tacrolimus 4 mg/day maintaining the concentration of 10-15 ng/ml, methylprednisolone 125 mg with a dose reduction to 4 mg/day. In the first month, EMB was performed weekly showing no signs of humoral rejection (AMR0). A control Echo-CG demonstrated EDD 48 ml, right atrium 38 x 57 mm, LA 44 mm, RV 24 mm, EF 62%. The patient was discharged home in a satisfactory condition a month after HT. After discharge from the hospital, he followed all the recommendations. A year after HT, the first CAG was performed showing no signs of a hemodynamically significant coronary artery constriction. The anti-HLA antibodies were screened (Luminex, IMMUCOR, Lifecodes

Deluxe) at 6 months, 1 year, 1.5 years, and 2 years, the results of the tests were negative.

In December 2013 (3 years after HT), the patient displayed complaints of recurrent dyspnea, reduced tolerance to physical exertion, and a drop in blood pressure. At a scheduled visit to the clinic on 02.12.2013, the Cardiologist of RI RCH No.1, taking into account the complaints, hospitalized the patient for CAG and EMB. EMB revealed the signs of humoral rejection (AMR1), IHC-expression of C4d, the signs of Grade 1 cellular rejection (R1), according to ISHLT classification. Simultaneously, the blood serum was assayed for the presence of anti-HLA antibodies (by Luminex methodology, IMMUCOR, Lifecodes LSA). HLA class I, and class II antibodies were identified: PRAI 4% (non-DSA); PRAII 20% (DSA). Specificity of DSA anti-DRB*07; DQA; DQB (Table 4).

Echo-CG yielded the following: IVS thickening up to 19 mm (estimated as myocardial edema), a preserved EF of 63%. CAG revealed insignificant stenosis of the circumflex artery of 40% in the middle section. Considering the increased myocardial edema (IVS up to 19 mm), the appeared stenoses in the coronary arteries, high titres of DSA (determined by Luminex methodology with diluting the recipient's serum), the diagnosis of the heart transplant humoral rejection was documented. The patient was given pulse therapy with methylprednisolone at a dose of 1000 mg for 3 days and 5 sessions of plasma filtration performed every other day (15 liters of plasma were replaced with fresh frozen donor plasma in 5 procedures) under the control of anti-HLA and EMB. While the patient being on therapy, a decrease in anti-HLA was achieved: class I non-DSA, PRA 0%; class II DSA, PRA 1% (Table 4), the specificity of anti-HLA DQA; DQB. A control endomiocardial biopsy showed no signs of humoral rejection; there were

signs of Grade 1 cellular rejection (R1, AMR0) requiring no therapy. Considering the improvement of clinical and laboratory parameters improvement, the patient was discharged from hospital, in a satisfactory condition on December 31, 2013, for outpatient follow-up by the Cardiologist of the Center for Thoracic Surgery.

Table 4. Screening of antibodies after heart transplantation

Date	Class I	Class II
06.12.2013-3 years	4% non-DSA	20% DSA
20. 12.013	7% non-DSA	27% DSA
27.12.2013	0%	1% DQB, DQA

In 2014-2015, the patient's condition was satisfactory, there were no complaints. Planned CAG revealed moderate lesion of the coronary bed (the anterior descending artery stenosis of 50% in the distal department) requiring no endovascular treatment (percutaneous coronary intervention; PCI). In antibody screening and identification, HLA panel reactive antibodies class II *(*PRAII) were detected comprising 9%, specificity of anti-HLA DQA; DQB.

On March 31, 2016, the complaints of dyspnea, lower extremity swelling resumed. The patient was urgently hospitalized in the RI RCH No.1. The Echo-CG showed a decreased myocardium contractility (EF of about 48%), LV wall edema (the thickness up to 20 mm). The performed EMB failed due to the pancreas fibrosis development; IHC demonstrated C4d expression in individual capillaries. The immunological study results for DSA HLA class I antibodies PRA was 17%, the specificity of anti-A*02; for DSA class II antibodies PRA was 34%, the specificity of anti-DRB*07; DQA; DQB. Given the clinical pattern of heart failure, the IHC data, and the

presence of anti-HLA antibodies, the condition was regarded as humoral rejection crisis, and the patient started receiving the pulse therapy with methylprednisolone at a dose of 1000 mg for 3 days. The tacrolimus dose was increased from 5 to 6 mg/day (tacrolimus concentration on hospital admission was from 8.7 to 15 ng/ml). Daily sessions of plasma filtration with monitoring the anti-HLA titer were given. After 4 sessions of plasma filtration (each session comprising the infusion of 3400 ml of donor fresh frozen plasma, the exfusion of 3400 ml of patient plasma), the anti-HLA titer (assessed by serum dilution on the Luminex platform) was without changes 1 : 32. On April 3, 2016, thymoglobulin was administered in a single dose of 2 mg/kg. The control EMB revealed morphological and IHC signs of humoral rejection, no signs of cellular rejection: R0, AMR1 (C4d expression). Given no significant positive effect of the given therapy, the EMB-confirmed humoral rejection, the patient was administered the therapy with anti-CD20 (rituximab) at a dose of 500 mg intravenously once a week, twice. Two hemodialysis sessions were performed for progressing renal failure. Thanks to the administered therapy, the complaints regressed; Echo-CG revealed positive dynamics: EF 50%, a decreased myocardial edema (from 20 to 16-17 mm in IVS, from 18 to 14-15 mm in LV PW). The 6-minute walk test result showed the distance of 480 m. However, a high DSA titer persisted (1 : 32) despite the clinical improvement. The patient's management was discussed at several consultations with the clinical center's main experts: the conservative treatment was recommended to be continued with high doses of glucocorticosteroids and the maintenance of tacrolimus high concentrations (12 ng/ml); the administration of anti-CD20 agents was recommended on necessity. Given the positive clinical dynamics, the patient

was discharged home in a satisfactory condition to continue receiving the treatment on an outpatient basis.

On May 16, 2016, the patient was hospitalized again with complaints of dyspnea in a horizontal position, a "lack of air" sensation, lower extremity swelling up to the lower third of the thigh, an increased body temperature to subfebrile levels. Echo-CG showed IVS thickening up to 22-23 mm, with a preserved contractility (EF 63%). CAG demonstrated multiple vessel lesions of the coronary arteries of the heart transplant with the involvement of the distal vascular bed due to which the PCI was contraindicated. EMB displayed no convincing data on cellular and humoral rejection. Echo-CG repeated after 3 days demonstrated the decrease in myocardium contractility (EF of 33-35%) with diffuse hypokinesis of the walls and IVS thickening up to 25 mm. Immunology study revealed high titres of DSA anti-HLA class I that was 1% and 26% of DSA class III antibodies. The patient received plasmapheresis sessions every other day (7 sessions with the total replaced plasma volume of 21 liters), a single dose of rituximab 500 mg. The patient's clinical condition improved, there were no complaints of dyspnea, swelling regressed. The patient was presented to the collegium consultation of the clinical center leading experts. Given the inefficacy of conservative treatment, a surgical treatment of heart retransplantation was recommended. However, the patient refused further treatment in the hospital (for family reasons) and was discharged on May 27, 2016, with recommendations for therapy and the scheduled date of the follow-up visit. On June 16, 2016, his condition worsened dramatically, there was dyspnea at rest, ascites, hyperthermia up to 38.0° C, which necessitated an emergency hospitalization in the Intensive Care Unit of the RI RCH No. 1, where inotropic support (adrenaline, norepinephrine) and hemodialysis sessions

were initiated. According to Echo-CG, EF was 25% with diffuse hypokinesis of LV walls and dilated heart chambers. Despite receiving the ongoing complex therapy, the patient's condition progressively deteriorated, and resulted in death. Cardiopulmonary resuscitation for 40 minutes was unsuccessful.

The post-mortem autopsy revealed diffuse fibrosis of coronary artery intima without atheromatous plaques, with a total replacement of media and adventitia with fibrosis in small epicardial branches (CTCAD manifestations), and the ischemic injury of myocardium with the area of 7 x 5 cm that involved the apex and posterior wall of the left ventricle (type 2 myocardial infarction of 10 days old, in phase of organization).

Discussion

Humoral rejection occurs in approximately 10% of HT recipients and is accompanied by the signs of CHF decompensation refractory to treatment [19]. The treatment of acute humoral rejection involves the removal of circulating antibodies (plasmapheresis) and the suppression of newly formed anti-HLA antibodies (immunoglobulins, monoclonal antibodies). The humoral rejection crisis requires therapy with high doses of glucocorticoids, administration of antilymphocyte antibodies, the patient's conversion from cyclosporine to calcineurin inhibitors, increased doses of mycophenolates and calcineurin inhibitors. Given the risks of severe toxicity of calcineurin inhibitors, and malignancies, the conversion to everolimus may be possible, but in this case, there is a risk of developing hyperlipidemia. The effect of therapy is assessed by repeated EMB of the transplanted heart with the IHC-study, as well as by monitoring the DSA content in recipient's blood. If the

therapy is ineffective, the consideration of heart retransplantation is recommended.

We have presented here the clinical case report demonstrating that DSA de novo identification was a prognostic factor of the graft loss, which is comparable to the data presented by of a number of researchers. So, in 2009-2013, K.L.Wong et T.Taner examined the heart transplant recipients who were regularly monitored for anti-HLA antibodies at the time points per protocol: before HT, at 1 week, at 4 months, and at 1 year post-transplant. We considered the role of DSA de novo, their impact on the acute cellular rejection, humoral rejection, and CTCAD. The authors revealed a prognostically high risk of adverse outcomes in recipients having DSA, the role of DSA in the development of cellular rejection, and the relationship of humoral rejection with the CTCAD development [9, 20]. A 15-year survival in the recipients without DSA was the highest compared to those who had DSA after HT (70% vs. 47%, respectively); and the patients with DSA de novo identified at one year or more after HT had the lowest survival rate [21]. The role of complement C4d component in the humoral rejection development and the relationship with anti-HLA have remained debatable for already 10 years, as well as the impact on the CTCAD development. In the period from 2004 to 2014, A.N.Husain et al. [22] studied heart transplant recipients using the IHC test, antibody content identification, and assessing the relationship with graft dysfunction; and he found that 33% of C4d-positive patients had anti-HLA antibodies, 10% had CTCAD, in most patients the C4d emerging at a later stage (a year or more) after transplantation. The study showed the C4d correlation with the CTCAD development and death (68%). We administered the therapy for humoral

rejection in accordance with the accepted national clinical recommendations "Heart Transplantation" of 2013.

Conclusion

Perhaps, the continuation of plasma filtration, the early administration of anti-CD20 preparations and their weekly dosing, as well as adding everolimus to the treatment in this patient would produce comparable results and extend the time to cardiac retransplantation. In patients with humoral rejection in whom the therapy has no significant effect, the prospect of performing heart retransplantation should be considered in the shortest possible time.

It is necessary to continue studying the role of donor-specific antibodies identified in de novo heart recipients, their effect in the development of humoral rejection and CTCAD in the late-term after transplantation, to determine the management tactics of patients with circulating anti-HLA antibodies without other clinical, morphological, and immunohistochemical signs of graft dysfunction, and to reveal a correlation between the anti-HLA antibody presence and the expression of complement C4d in order to reduce the frequency of endomyocardial biopsy.

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