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ALLOIMMUNOSENSITIZATION IN LEFT VENTRICULAR ASSIST DEVICE RECIPIENTS AND IMPACT ON POST-TRANSPLANTATION OUTCOME

BY

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

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LIST OF ABBREVIATIONS

ISHLT – International Society for Heart and Lung Transplantation
INTERMACS – International Registry for Mechanically Assisted Circulatory Support
HF – heart failure
MCS – mechanical circulatory support
LVAD – left ventricular assist device
RVAD – right ventricular assist support
TAH – total artificial heart
ECMO – extracorporeal membrane oxygenation
CF – continuous flow
PF – pulsatile flow
MHC – major histocompatibility complex
HLA – human leukocyte antigen
MICA – major histocompatibility class I – related chain
ETAR – endothelin – 1 type-A receptor
AT1R – anti-angiotensin type-1 receptor
GPCR – G protein-coupled receptor
LDH – lactate dehydrogenase
ICAM-1 – intracellular adhesion molecule 1
IL – interleukin
CD – cluster of differentiation
IgG – immunoglobulin G
AP-1 – activator protein 1
Erk ½ - extracellular signal-regulated kinase ½
NF – nuclear factor
CDC – complement dependent cytotoxicity
FCXM – flow cytometry cross-match
ELISA – enzyme-linked immunosorbent assay
IA – immuno-adsorption
PRA – panel reactive antibody
IVIG – intra-venous immunoglobulin
BMI – body mass index
BSA – body surface area
COPD – chronic obstructive pulmonary disease
PRBC – pure red blood cells
FFP – fresh frozen plasma
ACEI – angiotensin-converting enzyme inhibitor
ARB – angiotensin receptor blocker
BP – blood pressure
PGD – primary graft dysfunction
MOF – multi-organ failure
ACR – acute cellular rejection
pAMR – pathology antibody-mediated rejection
CMV – cytomegalovirus
PTLD – post-transplant lymphoproliferative disorder

ABSTRAKT

Úvod: V posledním desetiletí se mechanické srdeční podpory staly nedílnou součástí léčby pacientů v terminálním stádiu chronického srdečního selhání v rámci tzv. přemostění k transplantaci srdce. Jedním z popisovaných vedlejších účinků dlouhodobé mechanické srdeční podpory je zvýšená tvorba autoprotilátek, které mohou na podkladě komplexních imunologických interakcí negativně ovlivnit výsledek následné transplantace.
Cíl: Hlavním cílem naší experimentální prospektivní studie bylo posoudit účinek pozitivity autoprotilátek vůči novějším typům sledovaných receptorů na výsledky chirurgické léčby pokročilého srdečního selhání (implantace mechanické podpory a navazující transplantace srdce).
Metodika: Úvodní část práce tvoří literární přehled současných poznatků o možných mechanismech vedoucích k tvorbě protilátek u pacientů po implantaci mechanické podpory, nové metody detekce protilátek a nejnovější publikace pojednávající o možném negativním vlivu těchto protilátek na výsledky transplantace. Součástí experimentální práce bylo zavedení a praktické osvojení nové metodiky detekce autoprotilátek (Luminex). V první části experimentu jsme nejdříve stanovovali hladiny protilátek proti Angiotensin II Typ 1 Receptoru (AT1R) u 96 pacientů, kterým byla implantována dlouhodobá mechanická srdeční podpora Heart Mate II (HMII) v období 2008-2012. Přežívání na podpoře a morbidita byla porovnána u pacientů s pozitivní a negativní hladinou protilátek. V závěrečné části práce jsme porovnávali vliv hodnoceného primárního parametru na morbiditu, mortalitu a výskyt rejekce vůči kontrolní skupině 64 pacientů, kteří podstoupili transplantaci bez nutnosti přemostění mechanickou srdeční podporou.
Výsledky: Anti AT1R protilátky se vyskytovaly u 13/83 (16%) pacientů již před implantací HMII (AT1R+). Čtyři pacienti (6%) byli rovněž senzitizovaní proti HLA antigenům. Během podpory se sekundárně imunosenzitizovalo dalších 50 pacientů (71%), kteří původně vykazovali negativní AT1R titr protilátek (AT1R+/-). 20 pacientů (29%) zůstalo negativních (AT1R-). Dvouleté přežívání na mechanické srdeční podpoře bylo 78 ± 12% u AT1R-, 60 ± 23% u AT1R+ a 92 ± 6% u AT1R+/- pacientů (p=0.409). Podíl pacientů, u kterých se ve dvouletém sledování nevyskytla žádná z uvedených nežádoucích příhod (selhání mechanické srdeční podpory, infekční, krvácivé a neurologické komplikace) dosáhl ve skupinách s AT1R-, AT1R+ a AT1R+/- hodnot 49 ± 14%, 53 ± 16% a 41 ± 11% (p=0.875). Jedno a pětileté přežívání po transplantaci u AT1R- bylo 88 ± 8% a 76 ± 10% a 87 ± 5% a 81 ± 7% u AT1R+ pacientů (p=0.582). Nepřítomnost akutní celulární rejekce 1 rok po transplantaci dosáhla 68 ± 12% u AT1R- a 75 ± 6% u AT1R+ (p=0.218); u protilátkami zprostředkované rejekce dosáhla 100% u AT1R- a 98 ± 2% u AT1R+ (p=0.198).

Závěr: V analýze jsme nezaznamenali statisticky významný rozdíl v přežívání po implantaci HMII u pacientů mezi pacienty s negativním titrem AT1R autoprotilátek oproti skupinám primárně i sekundárně senzitizovaných pacientů na mechanické srdeční podpoře. Rovněž výskyt komplikací během srdeční podpory nevykazoval signifikantní rozdíly. Navazující srovnání vůči kontrolní skupině neprokázalo statisticky významný rozdíl ve výsledcích po transplantaci a incidenci akutních rejekcí u pacientů s pozitivitou pozorovaných protilátkami oproti pacientům bez jejich přítomnosti. Naše studie podporuje závěr, že u léčby mechanickou srdeční podporou představuje dynamika vzniku sledovaných protilátek biomarker, který je disociovaný s klinickými parametry morbidity, mortality a výskytu rejekce během napojení i po transplantaci srdce.

ABSTRACT

Background: In recent years mechanical circulatory assist devices became an established option in bridging patients with refractory heart failure to heart transplantation. One of the alleged limitations of mechanical devices is a high degree of antibody production with possible deleterious effect on subsequent heart transplantation outcome.
Aim: The main goal of this study is to assess the role of antibodies on the outcome of surgical treatment of patients with end-stage heart failure.
Method: Firstly, we present a literature review on the current state of knowledge of possible immunologic mechanisms involved in antibody production in left ventricular assist device (LVAD) recipients, new methods of antibody detection, desensitization strategies and overview of published evidence assessing the impact of sensitization on post-transplantation outcome. In the experimental part of our study we prospectively evaluated the presence of anti-Angiotensin II Type 1 Receptor (AT1R) antibodies in 83 Heart Mate II (HMII) recipients who were implanted at our institution between 2008 and 2012 and survived the first 60 days. On-device survival and device malfunction, major infection, major bleeding and neurologic dysfunction were compared between antibody positive and antibody negative recipients. Out of a total of 83 patients, 69 eventually underwent heart transplantation between October 2008 and August 2014. Overall survival and post-transplant rejection free survival were compared between both groups.
Results: Anti-AT1R antibodies were observed in 13/83 (16%) of the recipients before HM II implantation (AT1R+). Four of these patients (6%) were also sensitized against HLA antigens. During the support, 50 patients (71%) who were initially anti-AT1R negative became positive (AT1R-/+) and 20 (29%) remained negative (AT1R-). Two year on – device survival was 78 ± 12% in AT1R-, 60 ± 23% in AT1R+ and 92 ± 6% in AT1R-/+ group ($p = 0.409$). Freedom from device malfunction, major infection, major bleeding and neurologic dysfunction at two years for AT1R-, AT1R+ and AT1R-/+ was 49 ± 14%, 53 ± 16% and 41 ± 11% ($p = 0.875$). One and five year post transplant in AT1R- was 88 ± 8% and 76 ± 10% and in 87 ± 5% and 81 ± 7% in AT1R+ ($p = 0.582$). Freedom from ACR at one year was 68 ± 12% for AT1R- and 75 ± 6% for AT1R+ ($p=0.218$). Freedom from pAMR was 100% in AT1R- and 98 ± 2% in AT1R+ ($p = 0.198$).
Conclusions: There was no difference in the post HeartMate II implantation survival among patients who were anti-AT1R antibody positive before device implantation and patients who either became positive or remained negative during the support. The incidence of device malfunction, bleeding, infection and neurological dysfunction was not influenced by the presence of anti-AT1R antibodies. Our data also showed no impact of pre-transplant sensitization against HLA antigens on post-transplant survival.

1. BACKGROUND AND LITERATURE REVIEW

1.1 Heart failure and mechanical assist devices

Heart failure (HF) is a major public health problem with a prevalence of over 23 million worldwide, and rising [1]. Traditionally, heart transplantation is considered a gold standard treatment for patients with end stage heart failure. The discrepancy between the limited availability of donor organs and the increasing number of patients with heart failure has led to the development of left ventricular assist devices (LVADs). LVAD technology has revolutionized the management of refractory heart failure and become an established surgical therapy as a bridge-to-transplantation and for selected group of patients also as a destination therapy

HeartMate II LVAD (Thoratec Corp., Pleasanton, CA, USA) and HVAD LVAD (HeartWare Inter., Framingham, MA, USA) are currently the two most commonly implanted devices worldwide. With the advancements in patient selection, improvements in surgical technique and post-operative management contemporary devices have been proven to provide safe and effective circulatory support with an 80% one year survival.

The number of patients bridged to transplant with MCS has increased from 19% before 2009 up to 35% in 2013 [2]. The widespread use of mechanical devices has led to an increase in the percentage of transplantations of patients from the durable LVADs, reaching 42% in 2013.

Despite the clinical success of these devices, the anatomic and physiologic consequences of long-term LVAD support have yet to be fully clarified. It has been reported that many patients bridged to transplantation with mechanical support develop circulating antibodies both against human leukocyte antigen (HLA) and various non-HLA antigens. Post-transplantation, these newly developed recipient antibodies interact with donor antigens, potentially compromising the outcome. Transplanting against existing or historic donor-specific antibodies is associated with increased risk of antibody-mediated rejection, graft dysfunction, and decreased survival.

1.2 Description of anti-HLA antibodies

The HLA complex is vital in distinguishing self from non-self-proteins (antigens). The HLA genes are the human version of the major histocompatibility complex (MHC) genes that are found in most vertebrates. Foreign antigens presented by MHC class I attract killer T-cells (CD8 positive or cytotoxic T-cells) that destroy cells. MHC class I proteins form a functional receptor on most nucleated cells of the body There are three major (A, B, and C) and three minor (E, F, and G) MHC class I genes in HLA.

HLAs corresponding to MHC class II present antigens from outside of the cell to T-lymphocytes. These antigens stimulate the multiplication of T-helper cells, which in turn stimulate antibody-producing B-cells to produce antibodies. Self-antigens are suppressed by regulatory T cells. There are three major (DP, DQ, and DR) and two minor (DM, DO) MHC class II proteins encoded by the HLA.

1.3 Description of antibodies against non-HLA antigens

Apart from antibodies directed against human leukocyte (HLA), several non-HLA antibodies such as major histocompatibility class I-related chain (MICA), autoantibodies against angiotensin II type 1 receptor (AT1R) and endothelin receptor A (ETAR) as well as antibodies to cardiac self-antigens (Myosin and Vimentin) have been associated with an LVAD use [3-6].

AT1R belongs to type A family of G-protein-coupled receptors (GPCRs) with similar structures to rhodopsin. Agonistic antibodies against AT1R were originally found in women with preeclampsia [7]. Anti-AT1R antibodies have also been associated with systemic sclerosis and malignant hypertension [8, 9]. These antibodies have been shown to be the IgG1 and IgG3 subclasses and have the ability to fix complement.

1.4 Pathogenesis of sensitization in LVAD recipients

Antibodies to HLA and non-HLA antigens do not occur naturally. Commonly recognized risk factors for allosensitization in all transplant candidates include previous allografts, blood product transfusions, and history of pregnancy [10]. Patients who require mechanical support often receive multiple transfusions because of coagulopathy from hepatic congestion and poor hepatic function, bleeding caused by adhesions from previous surgery, or preoperative anticoagulation therapy.

Another mechanism implicated in sensitization of LVAD recipients is the interaction of human body with device biomaterials.

1.5 Impact of Allosensitization on Survival

The true impact of LVAD sensitization on outcome after heart transplantation is controversial. Although the Registry of the International Society for Heart and Lung Transplantation (ISHLT) continues to identify mechanical support as a risk factor for decreased survival after transplantation, experienced centers report survival outcomes of patients with LVAD similar to those of non-bridged patients, despite the significantly higher immunologic risk caused by sensitization.. Regardless of the cause of allosensitization in LVAD-bridged patients, the clinically relevant question is whether VAD-related immune activation is associated with increased rejection rates and mortality after cardiac transplantation. AIMS AND OBJECTIVES

The aims of our project were threefold. First, we assessed the impact of antibodies on outcome of patients implanted with a durable long-term left ventricular assist device HeartMate II. Apart from longer waiting times and associated increased morbidity and mortality, there have been no reports linking anti-HLA antibodies in mechanically bridged recipients to post-LVAD adverse outcomes. While anti-HLA antibodies exert their negative effect via complement activation and antibody – mediated cytotoxicity, antibodies against AT1R, act as a natural allosteric receptor agonist. Given the known potential of these antibodies to activate inflammatory and coagulation cascade we hypothesized that mechanically bridged patients with raised levels of anti-AT1R antibodies may experience increased rate of thromboembolic and infectious complications while on support.

There is sufficient amount of evidence for association of pre-formed anti-HLA antibodies and post-transplant hyper-acute rejection, acute cellular and antibody mediated rejection as well as chronic allograft vasculopathy and organ loss in heart transplant recipients. Little is known about the impact of non-HLA antibodies on post-heart transplantation outcome. Antibodies targeting AT1R have been associated with malignant hypertension, autoimmune diseases and acute rejection and graft loss in kidney transplantation. The objective of the second part of our study was to compare the survival and freedom from acute cellular and antibody mediated rejection in heart transplant recipients bridged with HeartMate II assist device stratified according the pre-transplant presence of anti-AT1R antibodies.

In the third and final part of our analysis, our goal was to evaluate the relationship between pre-transplant alloimmunosensitization against both HLA antigens and AT1R and post-transplantation outcomes in recipients who were either bridged with the durable LVADs or transplanted without prior use of mechanical assist device.

2. METHODS

2.1 Patients

First, we prospectively evaluated the presence of anti-AT1R antibodies in 96 consecutive Heart Mate II recipients at our institution between 2008 and 2012. After excluding 13 patients who died within 60 days of implantation, 83 patients with a mean duration of 375 ± 34 days of support were left for the analysis. On-device survival and various adverse clinical events (device malfunction, major infection, major bleeding and neurologic dysfunction) during the support were compared between antibody positive and antibody negative recipients. Out of a total of 83 patients, 69 eventually underwent heart transplantation, 9 died on support, three were explanted for recovery and two were still alive on support at the last day of follow-up. Sera of all 69 consecutive heart

transplant recipients transplanted between October 2008 and August 2014 were tested for the presence of anti HLA Class 1 and Class 2 antibodies and Angiotensin II type 1 Receptor antibodies before Heart Mate II device implantation and at the time of transplantation. Overall survival and post-transplant rejection free survival were compared between antibody negative and antibody positive recipients.

For the third part of our study we compared the survival and rejection in all first-time heart transplant recipients transplanted at our institution between 2009 and 2010. Seventeen patients who were bridged with Heart Mate II device and survived the first year were compared to 60 non-bridged first-year survivors. The impact of the presence of anti HLA and anti-AT1R antibodies on the post-transplantation survival, rejection and immunosuppression related adverse events was compared between antibody negative and antibody positive recipients.

3. RESULTS

3.1 The impact of Angiotensin II Type 1 Receptor antibodies on morbidity and mortality in Heart Mate II supported recipients

Anti-AT1R antibodies were observed in 13/83 (16%) of the recipients before Heart Mate II implantation (Table 1). Four of these patients (6%) were also sensitized against HLA antigens. During the support, 50 patients (71%) who were initially anti-AT1R negative became positive (AT1R-/+) and 20 (29%) remained negative (AT1R-). Total amount of Heart Mate II support for all 83 patients was 86.7 patients - years. There were no differences in the duration of support or the amount of the blood products used between LVAD recipients who remained negative and those who became positive. There were no differences in basic demographic and clinical characteristics between both patients group Out of 20 patients who remained negative on the mechanical device, 8 became sensitized to HLA antigens. In a cohort of 50 LVAD recipients who developed anti-AT1R antibodies during the support, 15 recipients also developed concurrent anti-HLA antibodies.

3.1.1 Survival

Out of 83 LVAD recipients who survived 60 days post–implantation, 9 additional patients died after a mean duration of support for 462 (minimum 82, maximum 1123) days. Two year estimated on – device survival was 78 ± 12% in AT1R-, 60 ± 23% in AT1R+ and 92 ± 6% in AT1R-/+ group ($p = 0.409$).

3.1.1 Major adverse events

Freedom from device malfunction, major infection, major bleeding and neurologic dysfunction at two years for AT1R-, AT1R+ and AT1R-/+ was 49 ± 14%, 53 ± 16% and 41 ± 11% (p = 0.875)

3.1.2 Device malfunction

Altogether 5 patients (6%) experienced device malfunction in our cohort (0.06 eppy). All episodes were related to pump failure (pump thrombosis in four patients and kinked outflow graft in one patient) and resulted in pump exchange in two patients and death in two patients. One patient with pump thrombosis was successfully treated conservatively and subsequently transplanted. Freedom from device malfunction at 2 years in AT1R+, AT1R- and AT1R-/+ was 100%, 95 ± 5% and 86 ± 8% (p = 0.487).

3.1.3 Major bleeding

Our institutional protocol for patients supported with HeartMate II device is anticoagulation with Warfarin (target INR of 1.8 – 2.2) without antiplatelet therapy. Out of 83, three patients (4%) experienced major bleeding episode after 7 days post implantation (0.03 eppy). The reasons for readmissions for bleeding were epistaxis, retroperitoneal bleeding and GI bleeding. All patients were discharged home following their bleeding episode and all three eventually underwent heart transplantation. Freedom from major bleeding at 2 years in AT1R+, AT1R- and AT1R-/+ was 100%, 100% and 90 ± 5% (p = 0.232).

3.1.4 Major infection

More than one third (27 patients, 33%) of our patients were readmitted due to infection during the course of their mechanical support (0.3). These patients fell into two major categories: infection of a drive - line site (21 patients) and deep sternal wound infection (6 patients). Two patients experienced both drive – line and deep sternal wound infections. One patient with deep sternal wound infection developed sepsis, multi – organ failure and subsequently died as a direct consequence of LVAD infection. Freedom from major infection at 2 years in AT1R+, AT1R- and AT1R-/+ was 54 ± 16%, 62 ± 13% and 51 ± 11% (p = 0.594).

3.1.5 Neurological dysfunction

Altogether six (7%) patients experienced neurological dysfunction. Four patients suffered from hemorrhagic CVA (0.05 eppy) and two from ischemic CVA (0.02 eppy). Two of the patients recovered and were subsequently transplanted, four died as a result of CVA. Freedom from neurologic dysfunction at 2 years in AT1R+, AT1R- and AT1R-/+ was 87 ± 12%, 93 ± 7% and 92 ± 6% (p = 0.997).

3.2 The Impact of Angiotensin II Type 1 Receptor Antibodies on Post – Heart Transplantation Outcome in Heart Mate II Bridged Recipients

Altogether 69 patients were transplanted from the Heart Mate II device at our institution during the study period. The mean time of mechanical support before heart transplantation was 11 months (range 1-53). Anti-AT1R antibodies were present in 8 (11.6%) and anti-HLA antibodies in three (4.3%) patients before Heart Mate II implantation. During the support 44 patients (63.8%) who were initially anti – AT1R negative became positive and 17 (24.6%) remained anti-AT1R antibody negative until transplantation. Out of 67 patients who were not sensitized against HLA antigens before HM II implantation, 6 (9%) developed anti-HLA antibodies during the support. At the time of transplantation there were 13 patients who were antibody negative for both HLA and AT1R antigens (AT1R-HLA-), three patients who were anti-AT1R antibody negative and anti-HLA antibody positive (AT1R-HLA+), 47 patients who were anti-AT1R antibody positive and anti-HLA antibody negative (AT1R+HLA-) and four patients who were sensititized against both AT1R and HLA antigens (AT1R+HLA+). There were no differences in basic demographic and clinical characteristics in patients stratified according to presence of anti-AT1R antibodies.

3.2.1 Survival

Out of 69 transplanted patients 8 did not survive until discharge. Primary graft dysfunction was the leading cause of death, followed by sepsis and neurological complications. Four additional patients died after being discharged from the hospital during the follow-up period.

Survival analysis of recipients stratified according to the presence of anti-AT1R antibodies before transplantation revealed one and five year survival of 88 ± 8% and 76 ± 10% for anti-AT1R antibody negative and 87 ± 5% and 81 ± 7% for anti-AT1R antibody positive patients ($p = 0.582$).

3.2.2 Acute Cellular Rejection

Out of 67 heart-transplant recipients who had biopsy results available, 14 (20.9%) were diagnosed with acute cellular rejection with ISHLT Grade ≥ 2R (12 patients 2R and two patients 3R). Both recipients with grade 3R rejection presented with an associated graft dysfunction. The first patient was successfully treated with 1g of intravenous solumedrol administered daily for three days. The second patient required veno-arterial extracorporeal membrane oxygenation (VA ECMO) implanted centrally for severe bi-ventricular graft dysfunction on top of pulse steroid therapy. After 12 days of support the graft function recovered and ECMO was successfully explanted. The median time to

ACR episode was 147 days (43, 606) in anti-AT1R antibody negative and 46 days (17, 264) in anti-AT1R antibody positive recipients (p = 0.306). Freedom from ACR at one year was 68 ± 12% for anti-AT1R negative and 75 ± 6% for anti-AT1R positive recipients(p=0.218).

3.2.3 Antibody Mediated Rejection

Four patients' endomyocardial biopsy specimens yielded histology and/or immunohistochemistry signs of antibody mediated rejection. Only patient with Grade 3 pAMR was positive for donor specific antibodies against human leukocyte antigen (HLA) and had concomitant graft dysfunction. Acute rejection was treated with a pulse of steroid that consisted of 1 g of intravenous solumedrol administered for three consecutive days, 10 cycles of therapeutic plasma exchange and intravenous immunoglobulins at 100 mg/kg. After multimodality treatment this patient is now symptom free, showing no signs of rejection in the latest endomyocardial biopsies and the graft function assessed with transthoracic echocardiography is satisfactory. None of the anti-AT1R negative patients presented with pAMR at one year post- transplantation, whereas freedom from pAMR in anti-AT1R positive recipients was 98 ± 2% (p = 0.198).

3.3 The impact of anti-HLA and anti-AT1R antibodies on post transplantation outcome in patients stratified by bridging with HeartMate II device

Between 2009 and 2010 altogether 18 patients bridged with HeartMate II device and 68 patients without previous mechanical support underwent first-time orthotopic heart transplantation. One patient from the mechanical support group and 8 patients from the non-supported group died within the first post-transplant year leaving 17 and 60 heart transplant recipients for the final analysis. Median duration of HeartMate II supported patients was 292 days (minimum 59, maximum 736). Apart from the younger age of patients who were transplanted from the HeartMate II device there were no major differences in the baseline demographic and clinical donor and recipient characteristics.

Although there were no differences in the duration of cardiopulmonary bypass time between the groups (135 minutes for HeartMate II versus 143 minutes for patients without prior support, p = 0.475), the use of blood products (packed red blood cells, fresh frozen plasma and platelets) was significantly higher in patients transplanted from HeartMate II device.

Out of 17 patients transplanted from HeartMate II device, 6 (35%) had anti-HLA class I, two (12%) had anti-HLA class II and two (12%) had MICA antibodies before transplantation. Four out of 6 with anti-HLA class I and all two patients with anti-HLA class II antibodies became sensitized during mechanical support. (Table 8). All but one patient with pre-formed anti-AT1R antibodies from the HeartMate II

bridged cohort also became sensitized while on support. When compared to their non-bridged counterparts, recipients transplanted from the device were significantly more sensitized against HLA class I antigens and AT1R.

3.3.1 Survival

Overall one patient from the HeartMate II bridged and 8 patients from the non-supported group died in the late post-transplant period (median 36 months). The post-transplant survival of patients bridged with HeartMate II device at 1, 3 and 5 years was 100%, 94 ± 6% and 94 ± 6%. This was not significantly different from the survival of non-supported heart transplant recipients with 100%, 95 ± 3% and 81 ± 7% (p = 0.398).

There was no difference in survival of patients with pre-transplant anti-HLA class I and class II antibodies in comparison to non-sensitized recipients at 1, 3 and 5 years post-transplantation (100%, 91 ± 9% and 91 ± 9% for sensitized versus 100%, 95 ± 3% and 83 ± 6% for non-sensitized, p = 0.739).

Patients who had antibodies against AT1R before transplantation had survival of 100%, 96 ± 4% and 92 ± 5% at 1, 3 and 5 years. Anti-AT1R negative recipients' survival was 100%, 97 ± 3% and 78 ± 11% (p = 0.489).

3.3.2 Immunosuppression related adverse events

Both HeartMate II bridged and non-bridged recipients experienced the same rate of immunosuppression associated adverse events (opportunistic infection, cytomegalovirus disease and post-transplant lymphoproliferative disorder).

3.3.3 Acute cellular rejection

Freedom from ACR ISHLT Grade ≥ 2R at one year was 88 ± 8% in HeartMate II and 73 ± 6% in non-bridged recipients (p=0.113). There were no differences in the freedom from ACR between patients with and without pre-transplant non-cytotoxic HLA antibodies at one year (71 ± 17% versus 79 ± 5%, p=0.911) (Figure 19).Freedom from ACR ≥ 2R at one year for anti-AT1R antibody positive patients was 75 ± 8%, whereas for anti-AT1R negative recipients it was 80 ± 7% (p = 0.442).

3.3.4 Antibody mediated rejection

Freedom from pAMR ISHLT Grade 1 - 3 was 94 ± 6% in HeartMate II and 95 ± 3% in non-bridged patients (p=0.665). Patients with preformed anti-HLA antibodies experienced significantly less freedom from pAMR than non-sensitized recipients (71 ± 17% for antibody positive versus 96 ± 2% for antibody negative, p = 0.047) (Figure 21). Freedom from pAMR at one year post-transplant was 96 ± 4% in anti-

AT1R antibody and 93 ± 5% in anti-AT1R antibody positive patients (p = 0.460).

4. DISCUSSION

The question of whether antibodies only mark or also mediate immunity remains a challenging one in medicine today. Antibodies against components of nuclei, insulin, and other components of beta cells and even against the surfaces of extra-vascular cells are commonly observed and taken as evidence of autoimmunity. Yet, many people who have autoantibodies do not manifest autoimmune disease and when disease is present the role of autoantibodies can be difficult to determine.

Whether or not antibodies in the circulation of graft recipients damage transplants, they do predict outcome of transplantation

The proportion of heart transplant candidates who are sensitized to HLA with a PRA > 10% is steadily increasing and has reached a 12% mark in 2011. This trend reflects the increased use of mechanical assist devices in bridging patients to transplantation as left ventricular assist devices are a recognized risk factor for sensitization [11-13]. LVAD supported patients now constitute a substantial proportion of the heart transplant recipients. Our results showed that approximately 9% of patients were sensitized against HLA antigens and another 16% were sensitized against AT1R even before LVAD implantation. Anti-HLA and anti-AT1R antibodies develop before LVAD implantation through similar pathways: transfusions, pregnancies and prior transplant. Du et al [14] observed in their previous report an increased titer of anti-AT1R antibodies in the sera of congestive heart failure patients with ischemic cardiomyopathy and hypertension. The authors suggested that these antibodies may play an important role in the pathogenesis and myocardial remodelling of heart failure. We did not find any association between basic demographic and clinical characteristics (female gender/ previous pregnancy, history of surgery) and sensitization against AT1R before LVAD implantation.

The exact mechanism of antibody production in mechanically bridged heart transplant candidates is not known. Avoiding leukofiltered red blood cell transfusions in perioperative period does not prevent alloimmunization in LVAD recipients. Plasma may contain sufficient amount of soluble HLA antigens to cause sensitization. There is evidence that platelet transfusion may be associated with the development of IgG HLA class I antigens but in general there is insufficient evidence to prove causation of blood product use and increased rate of sensitization in LVAD recipients. Studies comparing the rate of sensitization in pulsatile and continuous flow LVADs are of historical value only. By the first half of 2011, more than 99% of LVAD implants were continuous flow devices [15]. In our series we observed that around 24% of previously anti-HLA negative patients became positive during the support.

There is accumulating evidence that LVAD support may be associated not only with an increased anti-HLA but also various anti non-HLA antibodies. Hiemann et al. [16] reported in their pilot study that patients on assist device support before heart transplantation were more likely to develop high anti – AT1R antibody levels (43% of supported versus 18% of non – supported patients, p = 0.021) within 24 hours after heart transplantation, implicating pre – transplant sensitization. Barten el al. [6] found in their study of 29 VAD recipients that 65.5% were positive for anti-AT1R antibodies. Our results confirmed these findings. During the support 71% of the initially negative AT1R patients became positive. There are multiple pathways by which the production of antibodies against AT1R in patients supported with mechanical devices may be initiated. Protein antigenic determinants from targets may become accessible after injury or surgical stress. Inflammatory events might lead to de novo expression of autoantigens [17]. These autoantibodies are generally of the IgG class requiring T cell help [18]. T cell self-tolerance may be broken by an inflammatory event or hypoxia. We observed no association between pre-operative demographics, blood product peri-operative use or duration of mechanical support and conversion of AT1R negative to AT1R positive status.

4.1 Impact of antibodies on LVAD associated complications

Apart from longer waiting times and associated increased morbidity and mortality, there have been no reports linking anti HLA or anti non-HLA antibodies in mechanically bridged recipients to post-LVAD adverse outcomes. Our theory that anti-AT1R antibodies with their pro-inflammatory and pro-coagulation properties and their ability to cause endothelial dysfunction may lead to an increased rate of thromboembolic and infectious complications in LVAD recipients was not borne out in our results. There was no difference in the overall survival among patients who were anti-AT1R antibody negative before Heart Mate II implantation and patients who either became positive or remained negative during the support. The incidence of device malfunction, bleeding, infection and neurological dysfunction was not influenced by the presence of anti-AT1R antibodies. There are several possible explanations for the lack of negative impact of AT1R activating antibodies on survival and adverse LVAD related complications in our cohort. Biological impetus regulating At1R antibody injury is fairly complex. Level of AT1R and induction of specific conformations is dependent on individual genetic polymorphisms and the state of local tissue expression influenced by various stressors. AT1R gene has 14 described polymorphisms, and some of them act as gain or loss of function mutations implicated in receptor activation [19]. The most extensively studied A1166C polymorphism is associated with increased responsiveness to Angiotensin II and various cardiovascular

and renal pathologies [20]. It is plausible that mechanical circulatory support with the continuous flow creates a unique microenvironment resulting in lower AT1R expression, potentially less susceptible to anti-AT1R antibody mediated actions. There is compelling evidence that the AT1R may also be activated by mechanical stress without the involvement of Angiotensin II [21]. The AT1R is the first recognized mechanosensitive GPCR [22]. It is plausible that in the situation when the heart is fully unloaded with mechanical assist device AT1R would be down regulated. There may also be other factors that influence the features of anti-AT1R antibodies, changing their agonistic affinity. The tissue damage caused by certain mechanisms prior to anti-AT1R binding may affect the level of AT1R expression, resulting in different degree of anti-AT1R binding efficiency. Several modifiers have been identified thus far: ischemia, inflammatory events, and microbiome. [23, 24].

4.2 Impact of antibodies on post-transplantation outcome

Our data showed no impact of pre-transplant sensitization against HLA antigens on post-transplant survival. These results are in line with previous reports [25-27]. Although several studies evaluated pre-transplant HLA antibodies as detected by SPA in heart transplantation, there is still conflicting evidence regarding their clinical consequences [28-30]. While there was also no statistically significant difference in the freedom from ACR between anti-HLA positive and negative heart transplant survivors we found that patients with preformed HLA antibodies experienced far less freedom from pAMR than non-sensitized recipients.

Although there is a substantial amount of literature on deleterious effects of anti-AT1R antibodies on post-renal transplantation outcomes, we were only able to find one manuscript in reference to heart-transplantation. Whereas we studied the effect of anti-AT1R antibodies as detected before transplantation, Hiemann et al. [16] evaluated the impact of anti-AT1R antibodies detected immediately post transplantation and during one year of follow-up. The relevant clinical end-points included acute cellular rejection of any grade, antibody mediated rejection and microvasculopathy. Evaluating the results of 30 heart transplant recipients, the authors concluded that elevated post-transplantation levels of anti-AT1R antibodies (cut-off > 16.5 U/ml) are associated with cellular and antibody mediated rejection and early onset of microvasculopathy and should be routinely monitored after heart transplantation. Apart from the difference in the time frame of anti-AT1R antibody evaluation, all our patients were bridged to transplantation with an LVAD and 75% were antibody positive before transplantation. Also, ISHLT standardization of nomenclature of pathologic antibody mediated rejection [31] was only published one year after the study. We believe there are fundamental

differences about how the clinical end points were defined and the results of those two studies are therefore difficult to compare. We nevertheless find the concept of increasing titres of anti-AT1R antibodies after transplantation very intriguing and plan to expand on the results of our study by evaluating the post-transplantation sera of all our patients. Another noteworthy aspect of the study by Hiemann et al. [16] is the suggestion of a potential association between anti-AT1R antibodies and post-transplant microvasculopathy. There is also increasing evidence for the active role of angiotensin II type 1 receptor (AT1R) itself in the pathogenesis of chronic allograft rejection explaining the link between acute rejection and subsequent long-term clinical outcome [32]. Yamani et al. [33] observed an increase in mRNA of AT1R in 14 heart transplant recipients who had recurrent acute cellular rejection in comparison to controls. In our study cohort we only had the results of 41 coronary angiograms available and for that reason we did not include cardiac allograft vasculopathy among the outcome measures in our study. We nevertheless acknowledge the compelling evidence for the immunoregulatory function of the renin-angotensin system and its role in the pathogenesis of chronic allograft rejection. Comparing the incidence of cardiac allograft vasculopathy between groups of patients stratified by the presence of anti-AT1R antibodies and increased expression of AT1 receptor is a challenge for future studies.

Although anti-AT1R antibodies may belong to complement fixing IgG subclasses (IgG1 and IgG3 isotypes), C4d positive staining was found not to be very frequent in biopsies of renal transplant recipients with anti-AT1R antibody mediated rejections [34, 35] implicating complement independent mechanism of injury. This would explain the lack of association between anti-AT1R antibody status and pAMR in our series. Our results also showed no statistically significant difference in the freedom from acute cellular rejection $\geq$ 2R between anti-AT1R antibody negative and positive recipients. Given the putative mechanism of action of these antibodies which primarily act on vascular endothelium causing non-specific, non-complement mediated microvascular damage these results are not surprising. When we stratified the patients not only by the presence of anti-AT1R antibodies but also by the anti-ALA antibodies status our results showed that none of the transplant recipients who were both anti-AT1R and anti-HLA antibody negative experienced pAMR or grade 3R ACR. Conversely, 25% of recipients who were sensitized against both AT1R and HLA antigens presented post-transplantation with high grade ACR with associated graft dysfunction and another 25% with pAMR similarly with graft dysfunction. This leads us to believe that knowing the anti-AT1R antibody status on top of standard evaluation of anti-HLA antibodies pre-transplantation adds an incremental value in a risk stratification of post-heart transplantation immunologic related adverse events.

5. LIMITATIONS

The study has several limitations inherent to the retrospective nature of a single center observational study. Another limitation is a relatively small number of patients with relatively low event rates increasing the probability of Type II error. Another drawback of our study is the fact that all our patients received Heart Mate II device thus limiting the generalization of our results to other types of mechanical devices. Future studies will need to address the question of whether newer generation of devices would show the same high degree of sensitization against HLA and AT1R and asses the role of these antibodies in post-transplantation outcome of mechanically bridged recipients.

6. SUMMARY

The primary finding of our study is that patients who received a long term LVAD developed a high degree on sensitization against both HLA and AT1R antigens after implantation. Our data showed no impact of anti-HLA and anti-AT1R antibodies in Heart Mate II recipients on the overall survival and incidence of LVAD related complications. We found no association between the presence of preformed anti-HLA and anti-AT1R in the pre-transplant sera and acute cellular rejection in the first post-transplant year. Patients with anti-HLA antibodies experienced less freedom from pAMR than patients without preformed antibodies.

7. REFERENCES

1. Bui, A.L., T.B. Horwich, and G.C. Fonarow, Epidemiology and risk profile of heart failure. Nat Rev Cardiol, 2011. 8(1): p. 30-41.
2. Lund, L.H., et al., The Registry of the International Society for Heart and Lung Transplantation: Thirty-first Official Adult Heart Transplant Report - 2014; Focus Theme: Retransplantation. J Heart Lung Transplant, 2014. 33(10): p. 996 - 1008.
3. Askar, M., et al., Comparison of HLA & MICA Allosensitization Patterns among Patients Supported by Ventricular Assist Devices (VAD) and Patients with No Devices. The Journal of Heart and Lung Transplantation, 2013. 32(4): p. S74.
4. Askar, M., et al., HLA and MICA allosensitization patterns among patients supported by ventricular assist devices. J Heart Lung Transplant, 2013. 32(12): p. 1241-8.
5. Banan, B., et al., (47) - Increased Sensitization To HLA and To Cardiac Self-Antigens (Myosin and Vimentin) in Patients

Waiting for Cardiac Transplantation With Left Ventricular Assisting Device (LVAD). The Journal of Heart and Lung Transplantation, 2014. 33(4, Supplement): p. S25.
6. Barten, M.J., et al., 112 Identification of Non-HLA Antibodies in Ventricular Assist Device Recipients. The Journal of Heart and Lung Transplantation, 2012. 31(4, Supplement): p. S46.
7. Wallukat, G., et al., Patients with preeclampsia develop agonistic autoantibodies aganst the angiotensin AT1 receptor. J Clin Invest, 1999. 103(7): p. 945-52.
8. Riemekasten, G., et al., Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis, 2011. 70(3): p. 530-6.
9. Fu, M.L., et al., Autoantibdies against the angotensin receptor (AT1) in patients with hypertension. J Hypertens, 2000. 18(7): p. 945-53.
10. Reed, E.F., et al., Acute antibody-mediated rejection of cardiac transplants. J Heart Lung Transplant, 2006. 25(2): p. 153-9.
11. Drakos, S.G., et al., Prevalence and risks of allosensitization in HeartMate left ventricular assist device recipients: the impact of leukofiltered blood product transfusions. J Thorac Cardiovasc Surg, 2007. 133(6): p. 1612-19.
12. McKenna, D.H., et al., HLA alloimmunization in patients requiring ventricular assist device support. J Heart Lung Transplant, 2002. 21: p. 1218-24.
13. Massad, M.G., et al., Factors influencing HLA sensitization in implantable LVAD recipients. Ann Thorac Surg, 1997. 64(4): p. 1120-25.
14. Du, Q., et al., Perindopril treatment promote left ventricle remodeling in patients with heart failure screened positive for autoantibodies against angiotensin II type 1 receptor. BMC Cardiovasc Disord, 2013. 13: p. 94.
15. Kirklin, J.K., et al., Sixth INTERMACS annual report: A 10,000-patient database. J Heart Lung Transplant, 2014. 33(6): p. 555-64.
16. Hiemann, N.E., et al., Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation. Transpl 2012. 94: p. 919-24.
17. Dragun, D., A. Philippe, and R. Catar, Role of non-HLA antibodies in organ transplantation. Transpl, 2012. 17: p. 440-5.
18. Win, T.S. and G.J. Pettigrew, Humoral autoimmunity and transplant vasculopathy: when allo is not enaugh. Transpl, 2010. 90: p. 113.
19. Mogi, M., M. Iwai, and M. Horiuchi, New insighs into the regulation of angiotensin receptors. Curr Opin Nephrol Hypertens, 2009. 18(2): p. 138-43.

20. van Geel, P.P., et al., Angiotensin II type 1 receptor A1166C gene polymorhism is associated with an incresed response to angiotensin II in human arteries. Hypertension, 2000. 35(3): p. 717-21.
21. Wu, J., et al., Insights into the activation and inhibition of Angiotensin II Type 1 Receptor in the mechanically loaded heart. Circ J, 2014. 78: p. 1283-9.
22. Storch, U., et al., G protein-mediated stretch reception. Am J Physiol Heart Circ Physiol, 2012. 302: p. 1241-9.
23. Dragun, D. and B. Hegner, Non-HLA antibodies post-transplantation: clinical relevance and treatment in solid organ transplantation. Contrib Nephrol, 2009. 162: p. 129.
24. Kaminska, D., et al., Cytokine gene expression in kidney allograft biopsies after donor brain death and ischemia-reperfusion injury using in situ reverse-transcription polymerase chain reaction analysis. Transplantation, 2007. 84(9): p. 1118.
25. Massad, M.G., et al., Does successful bridging with the implantable left ventricular assist device affect cardiac transplantation outcome? J Thorac Cardiovasc Surg, 1996. 112(5): p. 1275-81.
26. Pagani, F.D., et al., Development of anti-major histocompatibility complex class I or II antibodies following left ventricular assist device implantation: effect on subsequent allograft rejection and survival. J Heart Lung Transplant, 2001. 20: p. 646-53.
27. Pamboukian, S.V., et al., Relationship between bridging with ventricular assist device on rejection after heart transplantation. J Heart Lung Transplant, 2005. 24(3): p. 310-15.
28. Smith, J.D., et al., C4d fixing, Luminex binding antibodies - A new tool for prediction of graft failure after heart transplantation. Am J Transplant, 2007. 7: p. 2008-15.
29. Stastny, P., et al., Antibodies against donor human leukocyte antigens and the outcome of cardiac allograft in adults and children. Transpl, 2007. 84: p. 738 - 48.
30. Gandhi, M.J., S.R. DeGoey, and I.C. Bundy, Effect of pretransplant human leukocyte antigen antibody detected by solid phase assey on heart transplant outcomes. Transplant Proc, 2011. 43: p. 3840-6.
31. Berry, G.J., et al., The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. J Heart Lung Transplant, 2013. 32(12): p. 1147-62.

32. Yousufuddin, M., et al., Angiotensin II receptors from peritransplantation through first-year-post transplantation and the risk of transplant coronary artery disease. J Am Coll Cardiol, 2004. 43(9): p. 1565-73.
33. Yamani, M.H., et al., Incresed expression of Angiotensin II Type 1 Receptor (AGTR1) in heart transplant recipients with acute rejection. J Heart Lung Transplant, 2006. 25: p. 1283-9.
34. Dragun, D., et al., Angiotensin II type 1 receptor activating antibodies in renal-allograft rejection. N Engl J Med, 2005. 352(6): p. 558-69.
35. Reinsmoen, N.L., et al., Anti-Angiotensin Type 1 Receptor antibodies associated with antibodynmediated rejection in donor HLA antibody negative patients. Transpl, 2010. 90: p. 1473-7.

8. AUTHOR'S LIST OF PUBLICATIONS
8.1 RELATED TO THE TOPIC WITH IF

Urban M, Pirk J, Dorazilova Z, Netuka I. How does successful bridging with ventricular assist device affect cardiac transplantation outcome? Interact Cardiovasc Thorac Surg. 2011;13(4):405-9. **IF 1.155**

Urban M, Gazdic T, Slimackova E, Pirk J, Szarszoi O, Maly J, Netuka I. Alloimmunosensitization in left ventricular assist device recipients and impact on post transplantation outcome. ASAIO J. 2012;58(6):554-61. **IF 1.516**

Urban M, Slavcev A, Gazdic T, Ivak P, Besik J, Netuka I. The impact of angiotensin II type 1 receptor antibodies on post – heart transplantation outcome in HeartMate II bridged recipients. Interact Cardiovasc Thorac Surg. 2016;22(3):292-7. **IF 1.155**

Urban M, Slavcev A, Gazdic T, Ivak P, Netuka I. The impact of Angiotensin II Type 1 Receptor antibodies on morbidity and mortality in HeartMate II supported recipients. Biomed papers. Available online: April 27,2016. **IF 1.200**

Pitha J, Dorazilova Z, Melenovsky V, Kralova Lesna I, Stavek P, Stepankova J, Urban M, Maly J, Netuka I. The impact of left ventricle assist device on circulating endothelial microparticles – pilot study. Neuro Endocrinol Lett. 2012;33(Supp2):68-72. **IF 0.935**

Svobodova E, Gazdic T, Kubanek M, Vymetalova J, Voska L, Kmet M, Lanska V, Kolesar L, Urban M, Netuka I, Pirk J, Melenovsky V, Kautzner J, Slavcev A, Malek I. Novel insights into pre-transplant allosensitization

in heart transplant recipients in the contemporary era of immunosuppression and rejection surveillance. Transplant Int. 2016;29(1):63-72. **IF 2.599**

8.2 NON-RELATED TO THE TOPIC WITH IF

Urban M, Pirk J, Szarszoi O, Besik J, Netuka I. Post – heart transplantation outcome of HeartMate II – bridged recipients requiring unplanned concomitant temporary right ventricular mechanical support. Interact Cardiovasc Thorac Surg. 2015;20(3):372-8. **IF 1.155**

Urban M, Besik j, Szarszoi O, Voska L, Netuka I. Right ventricular outflow tract obstruction caused by ectopic thyroid gland. Ann Thorac Surg. 2014;98(1):345. **IF 3.849**

Urban M, Szarszoi O, Pirk J, Netuka I. What is the optimal mode of mechanical support in transplanted patients with acute graft failure? Interact Cardiovasc Thorac Surg. 2013;16(4):517-9. **IF 1.155**

Urban M, Pirk J, Szarszoi O, Skalsky I, Maly J, Netuka I. Mitral valve repair versus replacement in simultaneous aortic and mitral valve surgery. Exp Clin Cardiol 2013;18(1):22-6. **IF 0.758**

Urban M, Novotny J, Kovac J, Netuka I. Giant right coronary artery aneurysm presenting as cardiac tamponade. EurJ Cardiothorac Surg. 2011;40(5):1267. **IF 3.304**

Urban M, Pirk J, Turek D, Netuka I. In patients with concomitant aortic and mitral valve disease is aortic valve replacement with mitral valve repair superior to double valve replacement? Interact Cardiovasc Thorac Surg.2011;12(2):238-42. **IF 1.155**

Kacer P, Urban M, Kautznerova D, Szarszoi O. Coronary – coronary bypass with patency validated by 64 – slice multidetector computer tomography. Tex Heart Inst J. 2012;39(5):766-7. **IF 0.649**

Skalsky I, Urban M, Malouskova J, Szarszoi O, Pirk J, Netuka I. Aortic dissections following heart transplantations. Card Surg. 2012;27(1):125-7. **IF 0.89**

Kacer P, Urban M, Jonak M, Adamkova V. Extensive devastating prosthetic aortic valve endocarditis. Case Report. Neuro Endocrinol Lett. 2011 21;32(Suppl2):9-12. **IF 0.935**

Netuka I, Stepankova P, Urban M, Maly J, Szarszoi O, Dorazilova Z, Kotulak T, Pirk J. Is severe cardiac dysfunction a contraindication for

complex combined oncotherapy of Hodgkin's lymphoma? Not anymore. ASAIO J. 2013;59(3):320-1. **IF 1.516**

Netuka I, Ivak P, Szarszoi O, Urban M, Novotny J, Besik J, Maly J. Minimally invasive removal of a temporary RVAD. ASAIO J. 2015;61(2):202-4. **IF 1.516**

Pirk J, Maly J, Szarszoi O, Urban M, Kotulak T, Riha H, Neuzil P, Netuka I. Total artificial heart support with two continuous-flow ventricular assist devices in a patient with an infiltrating cardiac sarcoma. ASAIO J. 2013;59(2):178-80. **IF 1.516**

Szarszoi O, Maly J, Turek D, Urban M, Skalsky I, Riha H, Malouskova J, Pirk J, Netuka I. Implantation of left ventricular assist device complicated by undiagnosed thrombophilia. Tex Heart Inst J. 2012;39(5):615-7. **IF 0.649**

Netuka I, Maly J, Szarszoi O, Kautznerova D, Urban M, Pirk J. Novel treatment of an infiltrating cardiac fibrosarcoma. Tex Heart Inst J. 2014;41(2):248-9. **IF 0.649**

Szarsoi O, Besik J, Smetana M, Maly J, Urban M, Maluskova J, Lodererova A, Hoskova L, Tucanova Z, Pirk J, Netuka I. Biomarkers of cellular apoptosis and necrosis in donor myocardium are not predictive of primary graft dysfunction. Physiol Res. 2015 Oct 8 [Epub ahead of print]. **IF 1.293**

Besik J, Szarszoi O, Bartonova A, Netuka I, Maly J, Urban M, Jakabcin J, Pirk J. Intracardiac Ectopic Thyroid (Struma Cordis). Card Surg. 2014;29(2):155-8. **IF 0.89**

Besik J, Pirk J, Netuka I, Szarszoi O, Marek T, Urban M, Honsova E, Laco J. Aortic and mitral valve replacement due to extensive inflammatory immunoglobulin G4 – related pseudotumor. Ann Thorac Surg 2015;100(4):1439-41. **IF 3.849**

Netuka I, Szarszoi O, Maly J, Adamira M, Turek D, Besik J, Urban M, Riha H, Kotulak T, Pirk J. Comprehensive management of severe intestinal bleeding in a patient supported for 94 days by the biventricular Levitronix Centrimag System. Heart Surg Forum. 2010;13(6):E 409-10. **IF 0.392**

8.3 NO RELATIONS TO THE TOPIC WITHOUT IF

Szarszoi O, Netuka I, Janak D, Urban M, Maly J, Besik J, Turek D, Kacer P, Skalsky I, Pirk J. Cardiosurgical procedures in patients refusing blood transfusions: IKEM outcomes. Rozhl Chir. 2011;90(2):102-5

Urban M, Pirk J, Skalsky I, Szarszoi O, Netuka I. Outcomes of combined mitral valve repair surgery and aortic valve replacement in IKEM Praha. Rozhl Chir. 2011;90(2):95-101

Netuka I, Szarszoi O, Maly J, Riha H, Turek D, Urban M, Skalsky I, Kotulak T, Dorazilova Z, Pirk J. Technique of implantation and experience with temporary mechanical cardiac support in right ventricular failure . Rozh Chir. 2011;90(2):88-94

Turek D, Netuka I, Maly J, Szarszoi O, Besik J, Urban M, Pirk J, Petkov V. Management of deep device related infection in patient implanted with mechanical cardiac support device using VAC system. Rozhl Chir. 2009;88(12):693-6

Urban M, Netuka I, Parikova A, Maly J, Szarszoi O, Pirk J. Impact of mechanical circulatory support on renal function in a patient with end stage heart failure. Cas Lek Cesk. 2010;149(3):129-32

Urban M, Pirk J, Szarszoi O, Vodickova J, Netuka I. Transmyocardial laser revascularization. Rozh Chir. 2009;88(9):489-92