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CLINICAL SIGNIFICANCE OF ANTI-HLA ANTIBODIES ASSOCIATED WITH VENTRICULAR ASSIST DEVICE USE IN PEDIATRIC PATIENTS: A UNITED NETWORK FOR ORGAN SHARING DATABASE ANALYSIS

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Running head: Anti-HLA Antibodies Associated with VAD Use

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Clinical Significance of Anti-HLA Antibodies Associated with Ventricular Assist Device Use in Pediatric Patients: A United Network for Organ Sharing Database Analysis

Pediatr Transplant

Abstract:

Background: While ventricular assist device (VAD) use in pediatric patients has previously been associated with anti-human leukocyte antigen (HLA) antibody production, the clinical significance of these antibodies is unclear. We investigated the clinical impact of anti-HLA antibodies associated with VAD use in a large cohort of pediatric heart transplant (HTx) recipients.

Methods: From 2004-2011, pediatric cardiomyopathy patients post-HTx (N=1,288) with pre-HTx panel reactive antibody (PRA) levels were identified from the United Network for Organ Sharing database. PRA levels were compared between VAD patients and those with no history of mechanical circulatory support (MCS). Incidence of rejection and overall survival were compared between VAD and non-MCS groups after stratification by PRA and age.

Results: VAD recipients were more likely to produce anti-HLA antibodies than non-MCS patients (25.5% *vs.* 10.5% had PRA>10%, $p<0.0001$). Sensitized VAD patients (PRA>10%) had a higher incidence of rejection within 15 months of HTx compared to sensitized non-MCS patients (57.1% *vs.* 35.9%, $p=0.02$). There was no intergroup difference in 15-month mortality.

Conclusion: Among pediatric cardiomyopathy patients supported with a VAD, the presence of anti-HLA antibodies prior to HTx is associated with an increased risk of rejection. The mechanism of the association between VAD-associated antibodies and early rejection is unclear and warrants further investigation.
Keywords: Pediatric heart transplant, anti-HLA antibodies, PRA, ventricular assist device, rejection

Introduction:

Over the past decade, the use of ventricular assist devices (VADs) to support pediatric patients awaiting heart transplantation has increased dramatically (1). Survival outcomes among pediatric patients supported by VADs have generally been quite good, with waitlist and post-transplant outcomes equivalent to or better than the general pediatric heart transplant (HTx) population (2-5). One concern associated with the increased use of VADs is the potential for increased sensitization, or anti-HLA antibody production, among HTx candidates. There is evidence from both adult and pediatric populations that VAD use is associated with increased production of anti-HLA antibodies (6-8).

The pathogenicity of the anti-HLA antibodies produced in association with VAD support is unclear, however. In the adult population, most studies have found no increased rejection in VAD sensitized patients (9-12), however a few studies show an increased risk of rejection in patients with VAD associated antibodies (13, 14). Large studies looking specifically at the effect of anti-HLA antibodies formed in association with VAD support on post-HTx outcomes in children are lacking.

The purpose of this study was to investigate the clinical impact of anti-HLA antibodies associated with VAD use in a large cohort of pediatric cardiomyopathy patients undergoing HTx. We hypothesized that anti-HLA antibodies associated with the use of VAD support were less likely to be associated with rejection compared to non-MCS associated antibodies, as the preponderance of adult literature has shown. In order to maximize the likelihood that antibodies measured prior to HTx were associated with VAD support and not due to other exposures, we looked exclusively at patients with a diagnosis of cardiomyopathy, who had no prior history of cardiac surgery and were less likely to be previously sensitized than patients with congenital heart disease.

Patients and Methods:

A retrospective cohort study was performed using the United Network for Organ Sharing (UNOS) database. Pediatric HTx recipients with a primary diagnosis of cardiomyopathy (restrictive, hypertrophic, dilated, noncompaction or combined) were identified in the database from 2004 to 2011. Patients with a history of congenital heart disease were excluded, as were repeat transplants, patients undergoing multi-organ transplantation, or patients who were identified as being on ECMO prior to transplant (in order to try to limit sensitization due to other factors). The following patient and clinical characteristics were collected: VAD support prior to transplant, type of VAD support (BIVAD vs. LVAD), age, sex, race, primary diagnosis, most recent PRA prior to transplant (if both Class I and Class II PRA available, the highest of the two was used), and results of crossmatch. Due to the high frequency of missing data (68%), history of previous transfusions at the time of listing was not included. Patients were divided into two groups: those who received VAD support prior to transplant vs. those with no history of mechanical circulatory support (MCS; VAD or ECMO). Patients within each group were stratified as PRA 0, PRA 1 to 10%, PRA > 10%, and PRA > 50%.

The primary endpoints were rejection prior to discharge ("hospital rejection") and within 15 months post-HTx; secondary outcomes were mortality due to rejection at 15 months post-transplant and overall survival at 15 months post-transplant. Rejection was defined as a drug treated episode of acute rejection prior to discharge or hospitalization or treatment for rejection within 15 months of heart transplant. Biopsy was not a criterion for the diagnosis of rejection in this study; rejection could be diagnosed by biopsy, clinically or by echocardiogram. It was therefore a composite outcome that included both cellular, antibody mediated and mixed rejection. Primary graft failure was excluded from the definition of rejection. Mortality was defined as death or retransplantation. All causes of death were included in the analysis. Incidence of post-HTx rejection and overall survival were compared between VAD and non-MCS groups after stratification by PRA and age.

Statistical analysis:

Data were reported as N (%) for categorical variables and median (interquartile range) for continuous variables. Patient characteristics and incidences of sensitization and rejection (in overall groups and after stratification by PRA levels) were compared between the VAD and non-MCS groups using Chi-square test or Fisher's exact test. To examine the effect of increasing PRA on rejection within the VAD support group, Chi-square test for trend was used. Mortality due to rejection and overall survival at 15 months of post-HTx were calculated using Kaplan-Meier estimates and compared between the VAD and non-MCS groups using log-

rank test. All analyses were performed using SAS Version 9.4 (SAS Institute Inc., Cary, NC). A p-value of <0.05 was considered statistically significant.

Results:

Patient characteristics

Patient characteristics are presented in Table 1. The entire patient cohort included a total of 1,288 patients of whom 51.5% were male, 46.7% were Caucasian and 27% were less than 2 years old. Dilated cardiomyopathy (84.2%) was the most common diagnosis, and 21.4% of the patients were supported by a VAD prior to HTx. The VAD group was slightly older, with a higher percentage of non-Caucasians and patients with a diagnosis of dilated cardiomyopathy in comparison to the non-MCS group.

Anti-HLA antibodies

PRA data was available for 83.5% of the patients. The incidence of anti-HLA antibody production prior to HTx was higher in patients 2-18 years old than in those 0-1 year old (30.8% vs. 21.7%, p=0.003). Anti-HLA antibody production did not appear to be influenced by race. Overall, 11.5% of patients had PRA (class I or class II) >10% prior to HTx (table 2A). In this cohort overall, the incidence of a high degree of sensitization (PRA >50%) was uncommon, with only 44 patients (3.1%) with PRA >50%. VAD recipients were more likely to have PRA >10 % than patients without a history of mechanical support (25.5% *vs.* 10.5%, p<0.0001; table 2B).

Crossmatch results

Crossmatch results were available for 81.2% of patients. There was no difference in the incidence of positive crossmatch between VAD and non-MCS patients. Among those with a PRA >0%, 15.8% of patients with no mechanical support had a positive crossmatch compared to 23.2% of VAD patients (p=0.12). In the PRA >10% group, 24.4% of those without MCS had a positive crossmatch compared to 31.6% of those supported by a VAD (p=0.35).

Rejection and survival

The overall incidences of rejection prior to hospital discharge and at 15 months post-transplant were 14.8% and 31.4% respectively (table 3). VAD support pre-HTx was associated with increased post-HTx rejection, but only among those patients who had developed anti-HLA antibodies (figures 1a and 1b). Among patients without anti-HLA antibodies, there was no difference in rejection either by hospital discharge or at 15 months post-HTx between VAD and non-MCS patients (9.2% vs. 13.2% prior to discharge and 33.3% vs. 34.8% at 15 months, p=0.21 and p=0.78, respectively). In comparing VAD and non-MCS sensitized patients (PRA >10%) there was no difference in the incidence of rejection prior to discharge (25% in the VAD group vs. 18.2% in the non-MCS group, p= 0.32), however at 15 months post-HTx, a history of VAD support pre-HTx was associated with increased rejection (57.1% vs. 35.9%, p=0.02). Only 44 patients were highly sensitized (PRA > 50%), and while the rates of rejection prior to hospital discharge (26.3% vs 5.6%) and within 15 months of HTx (61.5% vs 33.3%) were higher among the VAD group than the non-MCS group, these differences were not statistically significant (p=0.18 and p=0.16, respectively). Primary graft failure was rare, and there was no difference in the incidence of primary graft failure between the sensitized VAD and sensitized non-MCS group.

To more carefully examine the effect of higher levels of PRA within the VAD and non-MCS groups, we looked at PRA levels of 0, 1 to 10%, 11-50%, and >50%. Among VAD supported patients, higher PRA was associated with an increased incidence of rejection prior to hospital discharge and within 15 months of transplant (figure 2a). Incidence of rejection prior to hospital discharge increased from 9.2% for those with PRA 0, to 26.3% for PRA >50% (p= 0.004). Incidence of rejection at 15 months increased from 33.3% for those with PRA of 0, to 61.5% for a PRA >50% (p= 0.02). Increasing PRA was not associated with increased rejection among patients who had not received MCS prior to transplant (figure 2b). Within the non-MCS group, the incidence of rejection at 15 months was relatively static at approximately 35% regardless of PRA level.

Despite increased rejection among sensitized VAD patients, there was no difference in rejection-related mortality or overall survival between VAD and non-MCS patients regardless of PRA (table 4). Overall survival was 92.8% for VAD and 93.3% for non-MCS patients at 15 months (p=0.64). Increasing PRA did not affect overall survival for either group. Among VAD patients, 15 month survival was 90.9% for those with a PRA of 0, 94.9% for those with a PRA 1 to 10%, and 92.6% for those with a PRA >10% (p=0.68). Similarly among non-MCS patients, 15 month survival was 93.3%, 93.7% and 91.1% for those with a PRA of 0, 1 to 10% and >10%, respectively (p=0.39).

DISCUSSION:

This analysis found that among pediatric cardiomyopathy patients, a history of VAD support both increases the likelihood of anti-HLA antibody production and increases the risk of early rejection in those patients who do become sensitized. Among VAD patients, the risk of early rejection increased as PRA became more elevated, from 33.3% in patients with a PRA of 0 to 61.5% in patients with a PRA >50%. In contrast, the incidence of early rejection remained steady at approximately 35% regardless of PRA among patients who did not receive MCS prior to transplant. VAD support by itself was not associated with increased rejection, as there was no difference in rejection between VAD and non-MCS patients with a PRA of 0. The increased risk of rejection in the sensitized VAD group did not appear to be influenced by a higher incidence of a positive crossmatch, as there was no difference in the incidence of a positive crossmatch between the sensitized VAD and non-VAD patients. Mortality did not appear to be impacted by PRA in either group.

Anti-HLA antibodies have been associated with inferior post-HTx outcomes in adults (15, 16) and children, with increased graft failure (17) and increased mortality (18). The reasons for these inferior outcomes are unclear; possibilities include increased rejection, primary graft dysfunction, and early coronary allograft vasculopathy (17). In pediatric patients, some studies have shown an increased risk of rejection with elevated PRA (19, 20), while others have not (18, 21). The use of VADs is associated with an increased incidence of sensitization (7, 8, 22), however it is unclear if antibodies associated with VAD exposure have the same clinical significance as antibodies formed as a result of other sensitizing events, such as exposure to human allograft material or blood products (23, 24).

In this study, we aimed to assess the clinical impact of anti-HLA antibodies produced in the setting of VAD support. In order to decrease the likelihood that antibodies measured in VAD patients were present prior to VAD exposure, we chose to limit the study population to patients diagnosed with cardiomyopathy, a population with a lower likelihood of prior sensitizing events than patients with congenital heart disease. The 25.5% incidence of sensitization seen in the VAD supported patients in this study is lower than prior reports of 35-69% sensitization in the general pediatric VAD population (7, 8), likely reflecting the exclusion of many pre-sensitized patients with congenital heart disease and eliminating confounding etiologies of PRA sensitization that may have different pathogenicity. Interestingly, the incidence of sensitization found in this study was similar to the 22% incidence of new sensitization

after VAD placement in an adult cohort supported by continuous flow VADs (13). Furthermore, only 10.5% of the non-VAD group was sensitized, suggesting that the majority of anti-HLA antibodies in the VAD group were likely associated with VAD exposure.

The data regarding pathogenicity of anti-HLA antibodies produced by pediatric VAD patients are limited. One small single center study did appear to show a trend towards increased risk of rejection among sensitized pediatric VAD patients compared to non-sensitized VAD patients, though this difference did not reach statistical significance, possibly due to limitations in sample size (7). The data regarding VAD associated antibodies in adults are conflicting, with some studies showing an increased the risk for allograft rejection (13, 14) and others finding no increased risk of rejection in those with VAD associated antibodies (9-11). There are several potential explanations for these disparate results, including variability in the method of evaluating anti-HLA antibodies among different centers, and potential differences in antibody reduction strategies. Information regarding PRA technique and approach to densensitization is not available in the UNOS database and could not be controlled for in our study. Interestingly, in the largest adult study showing no difference in rejection between VAD sensitized and non-VAD sensitized patients, immunotherapy information was similarly lacking (11). However, in a smaller, single center adult study, sensitized untreated VAD patients did have an increased risk of rejection compared to sensitized but treated, as well as non-sensitized, VAD patients (14). Data on antibody reduction strategies utilized in our patient population would shed further light on potential management strategies that might lead to differences in post-transplant outcome.

The mechanism by which sensitized VAD patients appear to have increased early rejection compared to sensitized non-MCS patients is not clear, and attempts to generate likely hypotheses are limited by the lack of data on antibody specificity available in the UNOS database. While one interpretation of our data could be that VAD-associated antibodies have increased pathogenicity, the lack of a difference in positive crossmatch frequency between groups suggests that the antibodies present may not necessarily have been donor specific, and may not have been directly responsible for the early rejection seen. An alternative hypothesis is that the anti-HLA antibodies measured by PRA may not be directly involved in rejection, but instead could be a marker of a generalized activation of the immune system caused by VAD exposure in some patients. LVAD implantation has been shown to initiate immune and inflammatory responses, at least in part due to the formation of pseudointima (composed of T cells, macrophages and monocytes) on the surface of these devices (25, 26). T-cell dysregulation, B-cell hyperreactivity and abnormal antibody production

have been reported in LVAD recipients (27). In this study, the finding of increased rejection prior to discharge among VAD patients with very low level antibody production (PRA 1 to 10%) is consistent with this antibody production being a marker for an inflammatory state. If the HLA antibodies detected are really a marker for a generalized activation of the immune system, then it may be that the patients were at increased risk for cellular as well as antibody mediated rejection. This hypothesis is supported by a recent article by Ko *et al*, which showed that sensitized VAD patients had an increased risk of both cellular and antibody mediated rejection (28). An additional hypothesis to explain our findings could be that different antibody reduction strategies or immunosuppression were used for VAD and non-MCS sensitized patients. If that were the case, then the increased risk of rejection seen among the VAD patients in this study may be modifiable.

Despite the increased risk of rejection in VAD sensitized patients, this group had a similar survival at 15 months when compared to other sensitized patients. There are no pediatric studies regarding survival in sensitized VAD patients compared with sensitized non-VAD patients, and data regarding post-transplant survival in the overall population of sensitized pediatric patients are conflicting (17, 18). Adult studies which specifically investigated VAD associated antibodies support our findings, with no difference in overall survival seen in VAD versus non-VAD patients with PRA >10 (9, 11, 12, 29), even when increased rejection was found (28).

Limitations:

This study had several limitations. The overall rate of sensitization in this cohort was low (11.5%) which reduces the power of the study and potentially limits the conclusions that can be drawn. As this was a retrospective study using a large database (UNOS), we were limited to the information collected in the database. Specifically, there was no information regarding pre- or post-transplant immune modulation or antibody reduction strategies. Transfusion history was lacking in a large percentage of patients so this could not be analyzed as a variable influencing the incidence of sensitization between the groups. We were unable to determine what percent of rejection episodes were proven by biopsy, as that variable was only part of the UNOS database until 2007. Therefore, we do not know the incidence of antibody mediated rejection or cellular rejection, and it is possible that true number of rejection episodes may have been either more or less than captured by this study. Information regarding the way in which PRA was calculated was not available in the database, and we did not have access to the antigen-specific data available from newer solid phase single antigen

bead techniques of PRA calculation. The lack of data regarding antibody specificity, in combination with the lack of information about whether the reported rejection was antibody mediated or cellular, complicates the attempt to hypothesize on likely mechanisms for the increased rejection in sensitized VAD patients. Antibody levels likely vary at different points in time after VAD implantation (30), and the dataset only captures the most recent PRA. Furthermore, information regarding timing of VAD implantation and most recent PRA level was not available; it is possible that the PRA reported could have been drawn prior to VAD implantation. We are also unable to differentiate between antibodies formed before and after VAD placement, although we did make an effort to minimize the number of pre-VAD antibodies by restricting the population to cardiomyopathy patients. Antibody class (I or II) was not analyzed due to lack of adequate data. Finally, we did not have enough data regarding VAD type to include this in our analysis.

Conclusion:

In the pediatric cardiomyopathy population, the combination of VAD support and anti-HLA antibody production appears to be associated with an increased incidence of early rejection. Sensitized VAD patients have increased rates of early rejection compared to both non-sensitized VAD patients and comparably sensitized non-VAD patients. The mechanism of increased rejection in this population, and the potential for modifying the risk of rejection through peri- and post-transplant antibody reduction therapy, are unknown and warrant further investigation.

Authorship statement:

H.Sonali Magdo, Department of Pediatrics, University of Utah, Salt Lake City, UT: concept/design, data analysis and interpretation, drafting article, approval of the submitted and final version.

Kurt R. Schumacher, Department of Pediatrics, University of Michigan, Ann Arbor MI: concept/design, data interpretation, critical revision of article, approval of the submitted and final version.

Sunkyung Yu, Department of Pediatrics, University of Michigan, Ann Arbor MI: study design, statistics, data analysis, critical revision of article, approval of the submitted and final version.

Robert J. Gajarski, Department of Pediatrics, Nationwide Children's Hospital, Columbus OH: study concept, critical revision of article, approval of the submitted and final version.

Joshua M. Friedland-Little, Department of Pediatrics, Seattle Children's Hospital, Seattle WA: concept/design, data analysis and interpretation, critical revision of article, approval of the submitted and final version.

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Figure legends:

Figure 1a and 1b:

*Including deaths and retransplants due to rejection

Abbreviations: VAD, ventricular assist device; MCS, mechanical circulatory support; PRA, panel reactive antibody

P-value from Chi-square test or Fisher's exact test, as appropriate

Figure 2a and 2b:

*Including deaths and retransplants due to rejection

Abbreviations: HTx, heart transplant; VAD, ventricular assist device; PRA, panel reactive antibody

P-value from Chi-square test for trend

Table 1. Comparison of patient and clinical characteristics

	Overall (n= 1,288)	VAD (n=275)	No MCS (n= 1,010)	p-value[§]
Median age at transplant, years	10 (1-14)			
Age at transplant, years				
0-1	350 (27.2)	60 (21.8)	290 (28.7)	0.02
2-18	938 (72.8)	215 (78.2)	720 (71.3)	
Male sex	663 (51.5)	155 (56.4)	507 (50.2)	0.07
Race				
Caucasian	601 (46.7)	117 (42.5)	482 (47.7)	
African American	343 (26.6)	87 (31.6)	256 (25.3)	
Hispanic	227 (17.6)	37 (13.5)	189 (18.7)	0.01
Asian	74 (5.7)	25 (9.1)	49 (4.9)	
Other	43 (3.3)	9 (3.3)	34 (3.4)	
Primary diagnosis				
Dilated cardiomyopathy	1085 (84.2)	268 (97.5)	814 (80.6)	
Restrictive cardiomyopathy	146 (11.3)	4 (1.5)	142 (14.1)	<0.001
Hypertrophic cardiomyopathy	57 (4.4)	3 (1.1)	54 (5.3)	

Data are presented as N (%); median age is presented as age (interquartile range)

Abbreviations: VAD, ventricular assist device; MCS, mechanical circulatory support

[§]P-value from Chi-square test; comparison is between VAD vs. no MCS group

Table 2. Incidence of sensitization

A. Overall sensitization

Most recent PRA prior to transplant, %[1]	
0	772 (59.9)
1-10	156 (12.1)
>10	148 (11.5)
>50	44 (3.1)
Unknown	212 (16.5)

B. Sensitization by type of support pre-transplant

	Incidence		
	PRA >10%	PRA >50%	p-value[§]
Type of support pre-transplant			
No mechanical support	88 (10.5)	18 (2.1)	<.0001
VAD only (LVAD or BiVAD)	60 (25.5)	19 (8.1)	<.000

Abbreviations: PRA, panel reactive antibody; VAD, ventricular assist device; LVAD, left ventricular assist device; BiVAD, biventricular assist device

Data are presented as N (%) for categorical variables and median (interquartile range) for continuous variables

[1]If both class I and class II PRA were available, the highest PRA was used

Data are presented as N (%)

[§] P-value from Chi-square test

Table 3. Outcome measures	
Acute rejection episode(s) prior to hospital discharge	191 (14.8)
Rejection[1] within 15 months of HTx	404 (31.4)
Death within 15 months of HTx due to rejection	27 (2.1)
Re-HTx within 15 months of HTx due to rejection	2 (0.2)
Time from HTx to discharge, days	15 (11-23)

Abbreviations: HTx, heart transplant

Data are presented as N (%) for categorical variables and median (interquartile range) for continuous variables

[1]Including deaths and retransplants due to rejection

Table 4. Mortality due to rejection and overall survival at 15 months post-transplant

			Mortality due to rejection		Overall survival	
PRA levels	Type of support	# of patients available	Rate	p-value[§]	Rate	p-value[§]
PRA= 0	No MCS	640	2.4	0.08	93.3	0.27
(n= 772)	VAD	130	5.1		90.9	
PRA 1-10%	No MCS	110	2.2	0.40	93.7	0.87
(n= 304)	VAD	45	0		94.9	

PRA >10%	No MCS	88	3.3	0.68	91.1	0.77
(n= 148)	VAD	60	4.2		92.6	
PRA >50%						
(n= 37)	No MCS	18	15.4	0.58	84.6	0.63
	VAD	19	7.7		82.0	

Abbreviations: PRA, panel reactive antibody; MCS, mechanical circulatory support; VAD, ventricular assist device

Rates were derived from the Kaplan-Meier method

[§]P-value from log-rank test

Figure 1a.

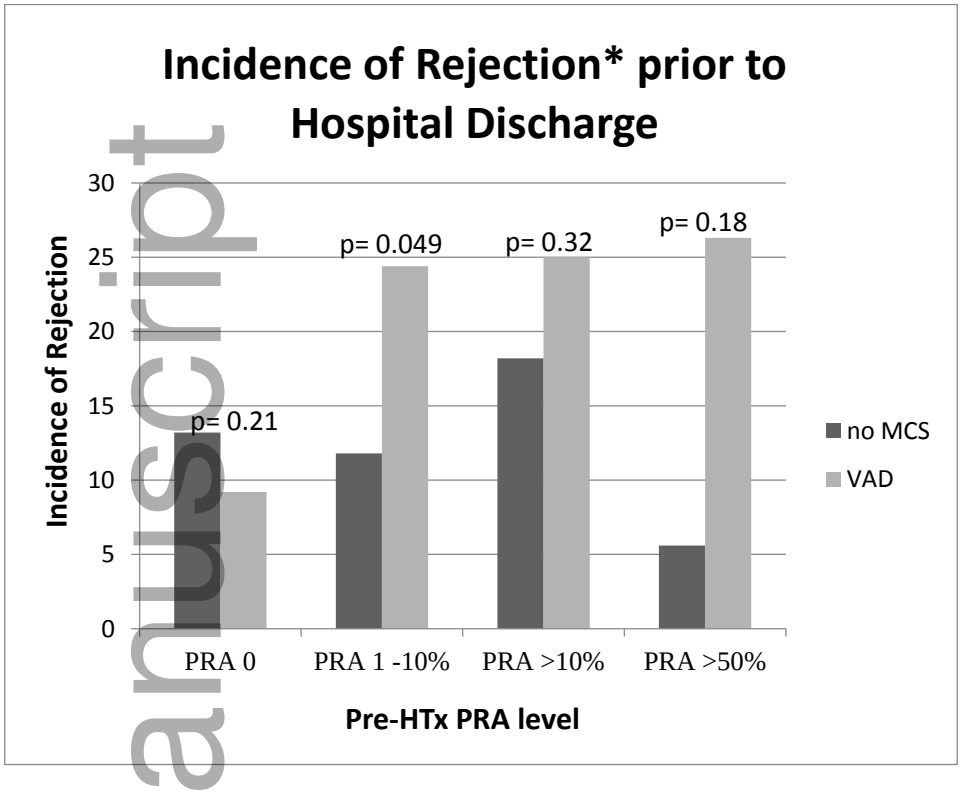

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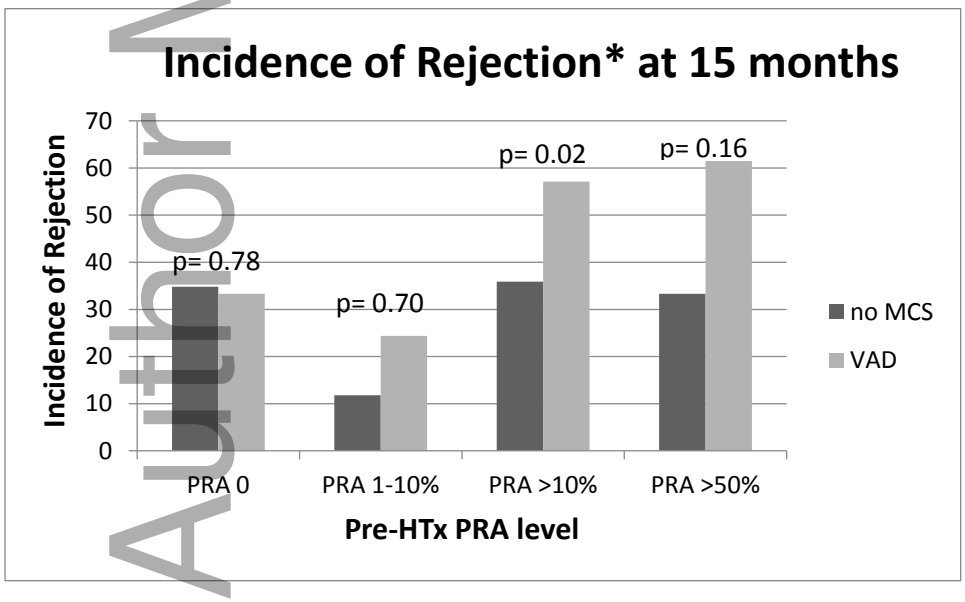
Figure 1b.



*Including deaths and retransplants due to rejection
Abbreviations: VAD, ventricular assist device; MCS mechanical circulatory support; PRA, panel reactive antibody
P-value from Chi-square test or Fisher's exact test, as appropriate

Figure 2a.

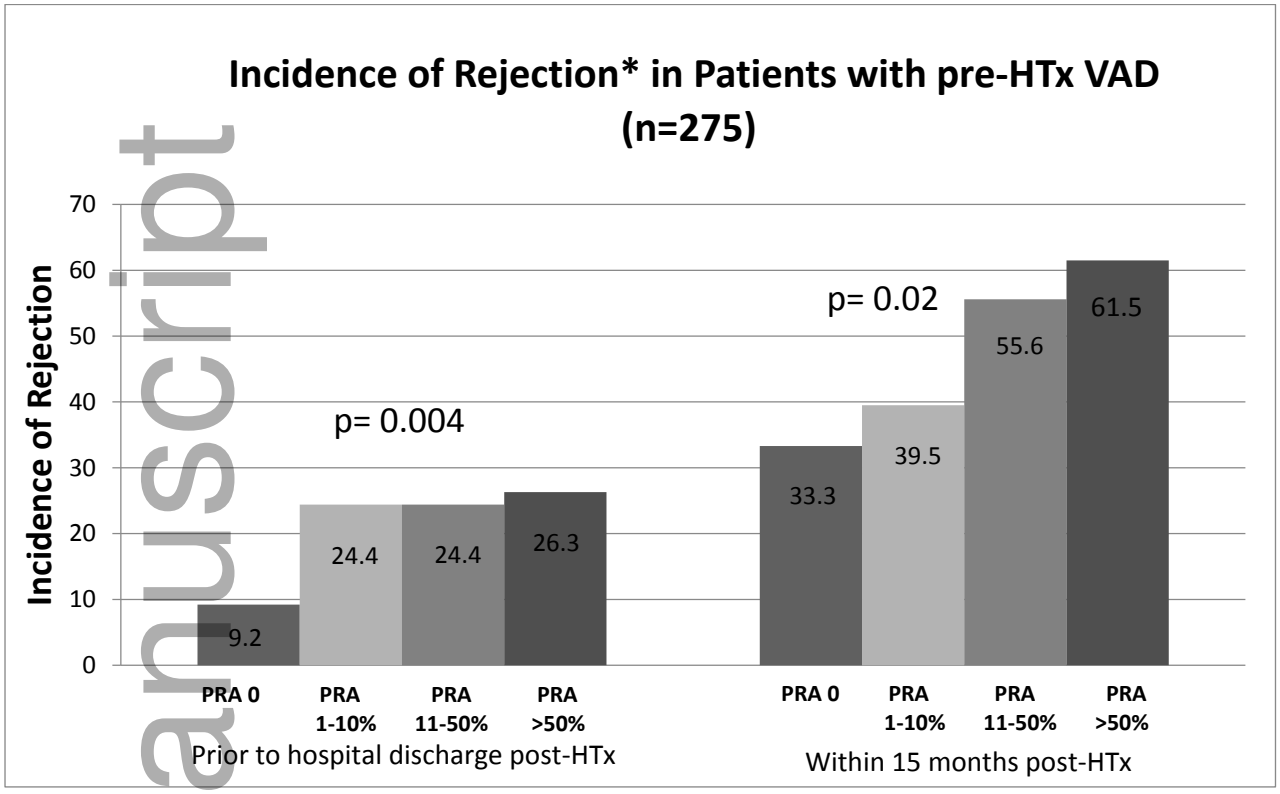
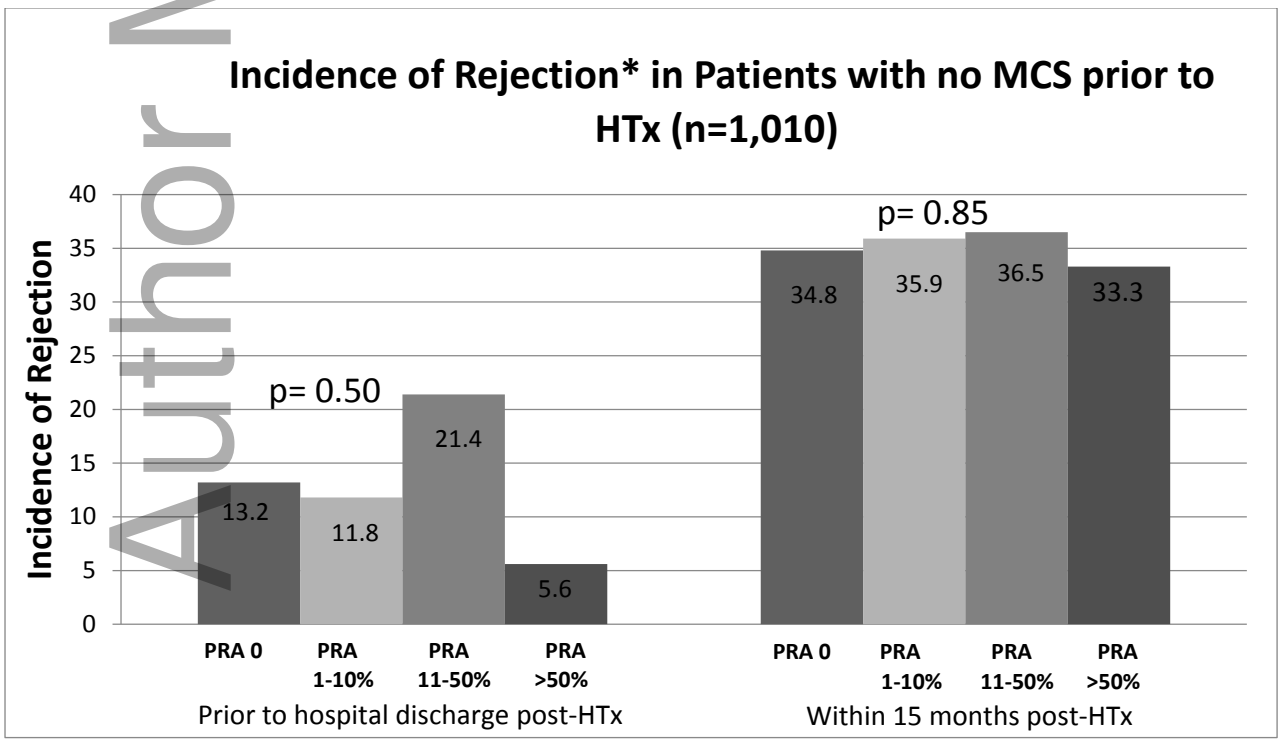


Figure 2b.



*Including deaths and retransplants due to rejection

Abbreviations: HTx, heart transplant; VAD, ventricular assist device; PRA, panel reactive antibody
P-value from Chi-square test for trend