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의학박사 학위논문

한국인 심장이식 환자 예후 예측에 대한 연구

- 비 HLA 자가항체에 대한 고찰 -

Non-HLA Autoantibodies Supplement Donor Specific Antibodies in Predicting Graft Survival after Heart Transplantation

2023년 2월

서울대학교 대학원

의학과 내과학 전공

오 규 철

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- 비 HLA 자가항체에 대한 고찰 -

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Abstract

Predicting Prognosis of Korean Patients Undergoing Heart Transplantation

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Introduction

Pre-sensitization has been known to be a risk factor associated with poor outcomes after heart transplantation (HTx). With increasing prevalence of advanced heart failure (HF), waiting time for HTx has also increased, raising the risk of pre-sensitization. In addition to presence of donor-specific human leukocyte antigen (HLA) antibodies (DSA), non-HLA antibodies have been also reported to have associations with adverse outcomes in HTx. The current study aimed to evaluate the combined effect of DSA and non-HLA antibodies on graft outcome in Korean patients undergoing HTx.

Methods

Data of patients undergoing HTx from January 2014 to December 2016 in 4 nationwide large transplant centers in Korea were prospectively collected. All analyses were performed using data from the Korean Organ Transplantation Registry (KOTRY), an organization established in 2014 to collect data on allograft transplant patients from participating centers. Presence of non-HLA antibodies were analyzed in a subset of patients who consented to donate serum

samples. Association of pre-sensitization, DSA, and non-HLA antibody status and early (≤ 1 year) and late (> 1 year) graft failure were assessed.

Results

A total of 290 patients were enrolled in the KOTRY HTx database. Among 288 patients with available panel reactive antibody (PRA) results, 104 (36.1%) had a screening value of 10% or more, and were defined as being pre-sensitized. Risk factors associated with pre-sensitization were female sex (adjusted HR 3.73, 95% CI 2.17-6.42, p<0.001) and previous HTx (HR 7.36, 95% CI 1.75-30.96, p=0.006), while diabetes mellitus had a protective effect (HR 0.44, 95% CI 0.22-0.88, p=0.020). There were no significant difference in outcomes of 1-year graft failure (HR 1.87, 95% CI 0.93-3.78, p=0.118) and all-cause mortality (HR 1.76, 95% CI 0.84-3.69, p=0.197) according to pre-sensitization. There was also no significant association with pre-sensitization and biopsy confirmed acute cellular rejections (log-rank p=0.700). Patients with DSA+ had a significantly increased risk of 1-year graft failure, with most of the difference occurring in the first month following transplantation. Analysis on late graft failure according to pre-sensitization and DSA status showed similar results with early outcomes (HR 1.42, 95% CI 0.65-3.10, p=0.378 for pre-sensitization, HR 2.47, 95% CI 1.16-5.27, p=0.019 for DSA+).

Pre-transplant non-HLA antibodies were assessed in 192 patients. In cox regression analysis, anti-vimentin antibody (AVA) (HR 2.73, 95% CI 1.21-6.16, p=0.016) and anti-collagen II antibody (ACA) (HR 2.76, 95% CI 1.12-6.81, p=0.027) were significantly associated with outcomes. AVA was present in 98 (51.0%) patients and was

more prevalent in males (p=0.042). Pre-transplant AVA+ was an independent predictor of 1-year graft failure (log-rank p<0.001), but not for long-term graft failure (log-rank p=0.120). However, AVA+ stratified 1-year graft outcomes in patients with DSA+, with AVA+ increasing the risk of graft failure compared to AVA- (log-rank p = 0.002). Use of AVA also increased the prediction model for 1-year graft survival in addition to traditional risk factors (IDI=11%, p=0.002, NRI=23%, p=0.047). Pre-transplant ACA was an independent predictor for both early (log-rank p=0.009) and late (log-rank p<0.001) graft failure. Compared to those with AVA-/ACA-, AVA+/ACA+ patients had significantly poor graft survival both in the early and long-term periods (all log-rank p<0.001).

Conclusion

Analysis of Korean patients undergoing HTx showed that pre-sensitization was not associated with a significant increase in risk of graft failure and death at 1 year. However, using the non-HLA antibody AVA stratified early outcomes in patients also having DSA, and ACA was associated with both early and late graft outcomes after HTx. Pre-transplant assessment of non-HLA antibodies could help predict outcomes and tailor graft allocation and post-HTx immunotherapy in specific patients.

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Keywords : heart transplantation; pre-sensitization; donor-specific human leukocyte antigen antibody; non-human leukocyte antigen antibody; anti-vimentin antibody; anti-collagen II antibody

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List of Abbreviations

ACA	Anti-collagen II antibody
AMR	Antibody-mediated rejection
AVA	Anti-vimentin antibody
CAV	Cardiac allograft vasculopathy
CKD	Chronic kidney disease
ECMO	Extracorporeal membrane oxygenation
DM	Diabetes mellitus
DSA	Donor-specific human leukocyte antigen antibody
HF	Heart failure
HLA	Human leukocyte antigen
HTx	Heart transplantation
KOTRY	Korean Organ Transplant Registry
LVAD	Left ventricular assist device
MFI	Mean fluorescence intensity
PRA	Panel reactive antibody
RRT	Renal replacement therapy
VAD	Ventricular assist device

Introduction

Heart transplantation (HTx) in Korea has steadily increased over the years since its first case in 1992,[1] with over 100 annual cases being performed since 2012.[2] With advances in medicine, the number of advanced heart failure (HF) is on the rise. Early detection, successful coronary revascularization, and guideline-directed medical treatment have increased the life expectancy of patients, but on the other hand, these patients are more likely to develop HF in the following years. As more and more countries are entering aged and super-aged societies, patients needing HTx are increasing, and even with utilization of marginal heart donors, the waiting time for organ allocation for HTx is getting longer.[3]

Most patients requiring HTx are admitted in the hospital and given continuous intravenous (IV) medications. For patients with more advanced disease, ventricular assist devices (VAD) such as extracorporeal membrane oxygenation (ECMO) support or left ventricular assist devices (LVAD) are considered. Low cardiac output may also lead to deteriorating renal function, requiring them to undergo renal replacement therapy (RRT) before transplantation. However, most of these procedures stimulate the recipients'

immune system, increasing the risk of developing antibodies to human leukocyte antigens (HLA),[4, 5] known as pre-sensitization.

Pre-sensitization has been known to be associated with poor outcomes not only in kidney transplantations,[6, 7] but also in HTx.[8, 9] The waiting period is also longer for pre-sensitized patients, as they are more likely to have a positive cross-match result with a random donor.[9] Pre-sensitization is assessed by panel reactive antibody (PRA) screening, which detects the percentage of patients with antibodies to HLA of the general population.[10] Racial difference has been known to affect the prevalence of pre-sensitization and its association with outcome, with Asians showing higher levels of PRA, but having better outcomes compared with other ethnic groups.[11, 12] However, the association has not been so consistent.[13]

Studies on the importance donor-specific HLA antibodies (DSA) has allowed early detection, treatment, and desensitization. However, patients without DSA may also experience early, acute deterioration after transplantation. Recent reports have suggested non-HLA antibodies as a factor associated with outcomes in HTx.[14] The search for significant non-HLA antibodies have shown positive results in the field of kidney transplantation,[15] but have produced mixed results regarding HTx. Detecting non-HLA

antibodies has not been routine practice, and measurement methods have not been standardized. Antibodies to angiotensin II receptor type 1 (AT_1), which is one of the most widely studied non-HLA antibody in HTx, have been associated with both negative and positive results, and is currently not routinely used to predict outcomes after HTx.[16, 17]

Using a nationwide prospective registry of HTx patients, the status and risk factors of pre-sensitization were assessed and the effect of DSA and non-HLA antibodies on early (≤ 1 year) and late (> 1 year) graft outcomes after HTx were investigated. This study aimed to disclose a novel biomarker to aid in personalizing organ allocation and post-HTx immunotherapy.

Methods

Data collection

Patients undergoing HTx in 4 nationwide representative transplant centers were prospectively enrolled after written informed consent. Patient data was submitted to the Korean Organ Transplant Registry (KOTRY), as described in a previous report.[18] The current analysis was conducted in accordance with the Declaration of Helsinki and study protocols were reviewed and approved by the institutional review board of Seoul National University Hospital (IRB No. E-1709-091-887, E-1805-001-941).

From January 2014 to December 2016, 290 patients were consecutively enrolled and data from follow-up visits were recorded at 1, 6, 12 months, and yearly thereafter. A subset of 192 patients had consented to donate pre-transplant serum to the database. The design of the analysis is shown in Figure 1.

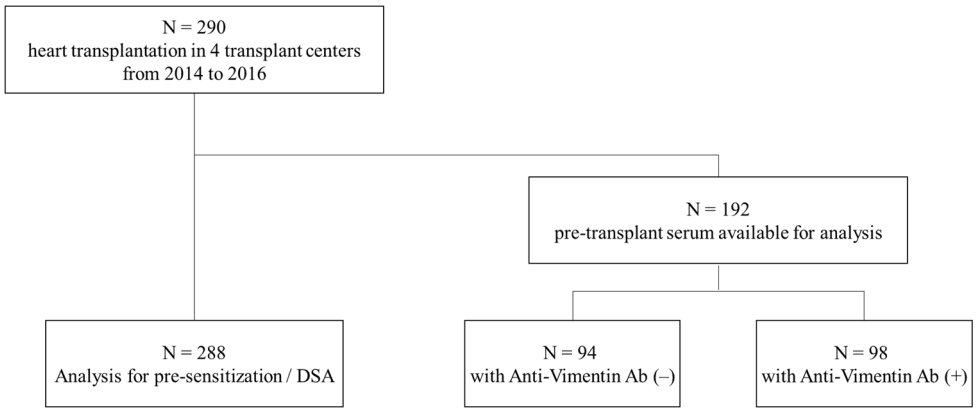

Figure 1 Study design

Variable definition

Pre-sensitization was defined as having a PRA-1 or PRA-2 value of 10% or more.[19] Positivity for DSA was defined according to results of PRA identification or single-bead assays. Non-HLA antibodies were assessed using a commercially available kit. A total of 39 non-HLA antibodies were measured by the Luminex method (LABScreen Autoantibody, One Lambda, CA, United States). The mean fluorescence intensity (MFI) of each antibody was calculated by subtracting sample-specific fluorescence values of negative control beads from sample-specific fluorescence value for non-HLA antigen beads. Positivity of non-HLA antibodies were stratified according to 2 methods: 1) using manufacturer-recommended 95% cutoff values and 2) using 75% cutoff values of the total study subjects, which could be determined as the 75% cutoff values of the

Korean HTx population.

Outcomes

The primary outcome was graft failure, a composite of all-cause mortality and re-transplantation at 1-year follow-up. Secondary outcomes were all-cause mortality, re-transplantation, and acute rejection at 1-year, and long-term graft failure and all-cause mortality (median follow-up of 5.1 years [IQR 4.8 – 6.0]). Acute cellular rejection (ACR) was defined when grade 1R+ histopathologic rejection was confirmed on endomyocardial biopsy. Antibody mediated rejection (AMR) was clinically determined, when a decrease in left ventricular (LV) systolic function from baseline was observed without evidence of ACR on endomyocardial biopsy.

Statistical analysis

Continuous variables were assessed using the Student's t-test or Wilcoxon rank sum test and categorical variables using chi-square test or Fisher' s exact test. Univariate and multivariate logistic regression analysis was performed to assess factors associated with sensitization and presence of non-HLA antibodies. Outcomes according to pre-sensitization, DSA, and non-HLA antibody status were compared using Kaplan-Meier analysis and risk was assessed

using Cox proportional hazard regression analysis. Landmark analyses were performed at 3 months to assess the role of non-HLA antibodies during follow-up. All analyses were two-sided, and p value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 25 (IBM, Chicago, Illinois) and R version 4.1.1 [20] using the survival, survIDINRI, rms, and dcurves packages.[21-24]

Results

Sensitization and outcome

Baseline characteristics

From a total of 290 patients enrolled in KOTRY during 2014 to 2016, 288 patients had results of pre-transplant PRA screening tests available for analysis. Pre-sensitization, defined as having a PRA level of 10% or higher, was observed in 36.1% (104/288) of the total study population. Comparison of baseline characteristics showed that pre-sensitized patients were more likely to be female, be non-smokers, have a previous cardiac transplant history, or have hypertrophic cardiomyopathy as the etiology of advanced HF, while less likely to be prevalent with DM. Patients who had been on the waiting list for a longer period also had a higher risk of being pre-sensitized (Figure 2). Detailed baseline characteristics, including comorbidities, HF etiology, and in-hospital treatments are described in Table 1.

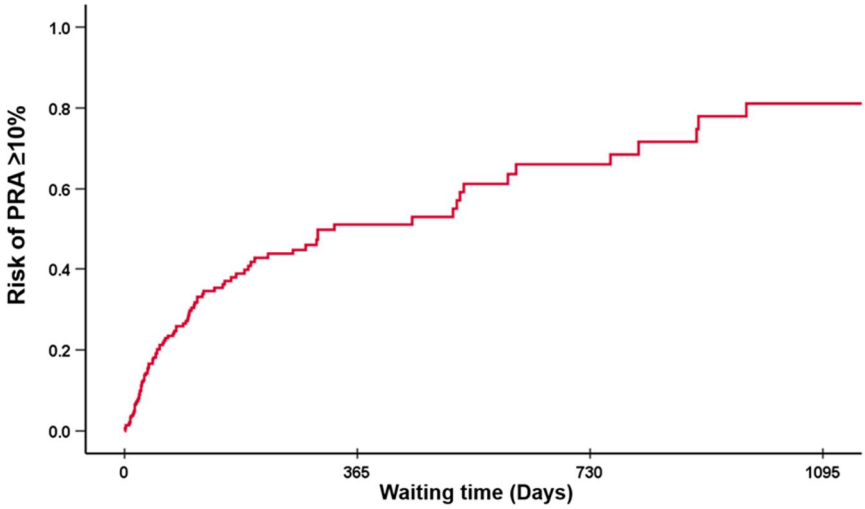

Figure 2 Risk of pre-sensitization increased as waiting time increased

Table 1 Baseline characteristics of study population according to pre-sensitization

	Total (n=288)	Pre-sensitized (n=104)	Non-sensitized (n=184)	P
Donor-recipient sex mismatch	93 (32.3)	42 (40.4)	51 (27.7)	0.027
HLA mismatches, n (%)				0.276
1-2	16 (5.7)	8 (8.1)	8 (4.4)	
3-4	119 (42.5)	44 (44.5)	75 (41.4)	
5-6	145 (51.8)	47 (47.5)	98 (54.1)	
Crossmatch, n (%)				
CDC T-cell	3 (1.0)	2 (1.9)	1 (0.5)	0.068
Flow T-cell	6 (2.1)	6 (5.8)	0	0.001
CDC B-cell	4 (1.4)	4 (3.8)	0	0.001
Flow B-cell	2 (0.7)	2 (1.9)	0	0.001
Solid phase assay	44 (16.7)	42 (40.4)	2 (1.3)	<0.001
Waiting time,	62.0	66.0	68.5	0.741

days (median)	[1,3556]	[1,1561]	[1, 3556]	
Surgical management				
Cold ischemic time, min	91.5 [20, 277]	81.5 [30, 261]	104.5 [20,277]	0.053
Warm ischemic time, min	54.0 [19, 165]	50.5 [27, 120]	57.0 [19, 165]	0.384
ACC time, min	55.6 [48, 303]	118.0 [56, 272]	105.5 [48, 303]	0.005
CPB time, min	142.5 [66, 420]	143.5 [66, 376]	140.5 [67, 420]	0.918

CMP, cardiomyopathy; IV, intravenous; ECMO, extracorporeal membrane oxygenation; LVAD, left ventricular assist device; HLA, human leukocyte antigen; CDC, complement dependent cytotoxicity; ACC, aortic cross clamp; CPB, cardiopulmonary bypass.

Risk of pre-sensitization

According to multivariate logistic regression analysis, female sex (OR 3.73, 95% CI 2.17-6.42, p<0.001) and previous transplantation (OR 7.36, 95% CI 1.75-30.96, p=0.006) were significant risk factors for pre-sensitization. On the contrary, history of DM (HR 0.44, 95% 0.22-0.88, p=0.020) was protective against pre-sensitization. Results of the univariate and multivariate regression analyses are shown in Table 2.

Table 2 Risk factors for pre-sensitization

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p

Sex (female)	3.89 (2.31 – 6.54)	<0.001	3.73 (2.17 – 6.42)	<0.001
Age (every 5-year increase)	1.05 (0.95 – 1.15)	0.303		
BMI (every 1kg/m2 increase)	0.97 (0.90 – 1.03)	0.316		
Current smoking	0.31 (0.12 – 0.79)	0.014		
DM	0.45 (0.24 – 0.84)	0.012	0.44 (0.22 – 0.88)	0.020
Pre-TPL MV	1.13 (0.63 – 2.03)	0.687		
Pre-TPL ECMO	0.94 (0.52 – 1.68)	0.824		
Pre-TPL RRT	1.57 (0.81 – 3.03)	0.185		
Re-TPL	5.03 (1.30 – 19.39)	0.019	7.36 (1.75 – 30.96)	0.006
Waiting time (per 30-days)	1 (0.97 – 1.03)	0.709		

HR, hazard ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus; TPL, transplant; MV, mechanical ventilation; ECMO, extracorporeal membrane oxygenation; RRT, renal replacement therapy.

Pre-sensitization and graft failure

In Kaplan-Meier analysis assessing for primary outcome of graft failure, pre-sensitization was not associated with a significant difference in 1-year graft survival (log-rank p=0.118). Most events were due to deaths, with only 3 cases of re-transplantation recorded in the study population. Figure 3 exhibits the Kaplan-Meier survival curve for 1-year graft survival and all-cause mortality according to presence of pre-sensitization. Complete in-hospital and 1-year outcomes according to sensitization status are given in Table 3.

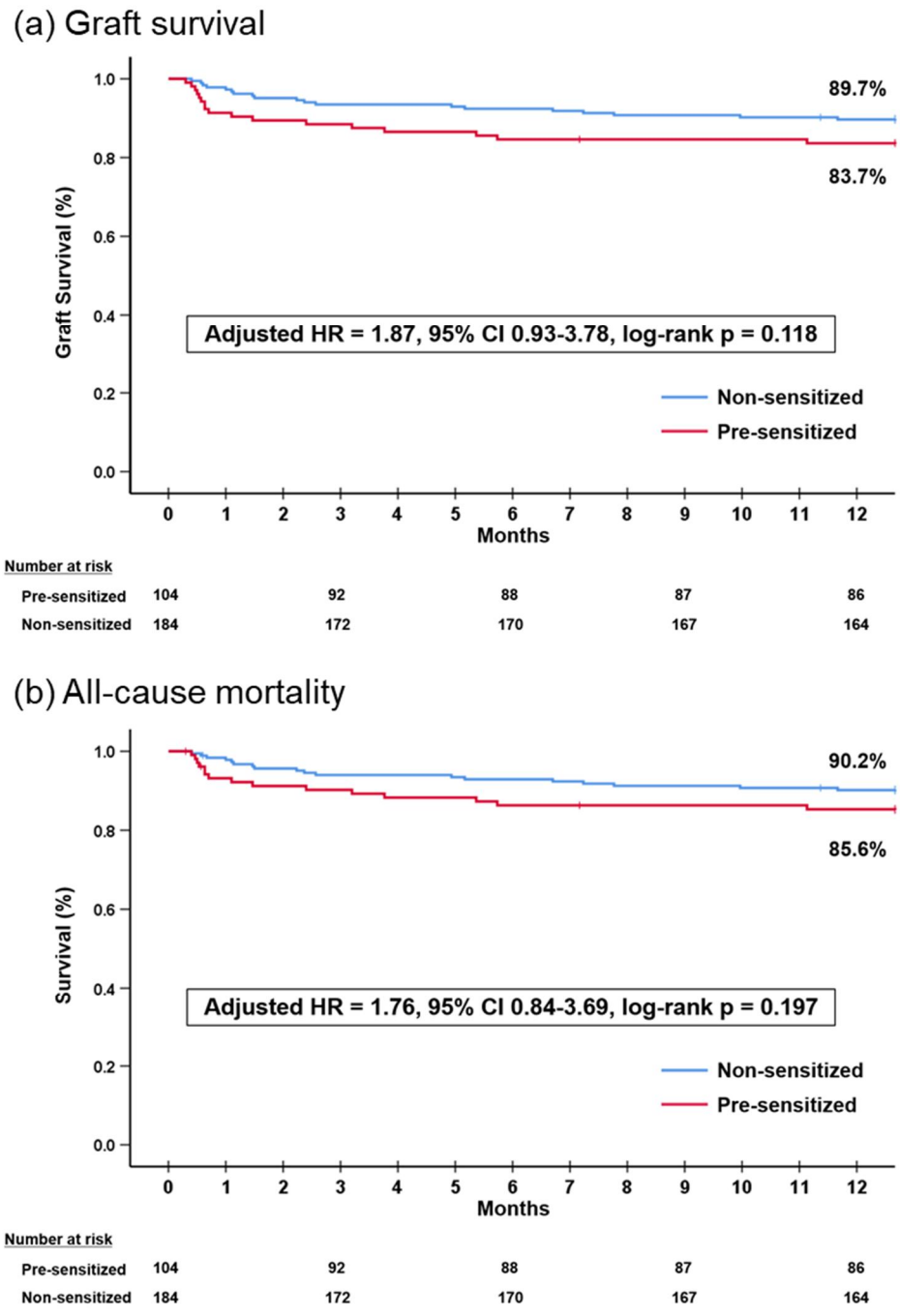

Figure 3 Kaplan-Meier curve according to pre-sensitization. (a) Graft survival. (b) All-cause mortality.

Table 3 In-hospital and 1-year outcomes according to pre-sensitization

	Pre-sensitized (n=104)	Non-sensitized (n=184)	Logrank p
In-hospital outcomes			
Hospital stay (days)	45.1 ± 36.2	40.3 ± 39.9	0.287
Graft failure	14 (13.5)	14 (7.6)	0.181
Mortality	12 (11.5)	13 (7.1)	0.307
Re-transplantation	2 (1.9)	1 (0.5)	0.262
Mechanical ventilation	1 [0.4, 88]	1 [0.2, 201.0]	0.302
ECMO	18 (17.3)	22 (12.0)	0.207
RRT	30 (28.8)	40 (21.7)	0.177
1-year outcomes			
Graft failure	17 (16.3)	19 (10.3)	0.118
Mortality	15 (14.4)	18 (9.8)	0.197
Re-transplantation	2 (1.9)	1 (0.5)	0.258
ACR	58 (55.8)	105 (57.1)	0.700
AMR	0	1 (0.5)	0.452

ECMO, extracorporeal membrane oxygenation; RRT, renal replacement therapy; ACR, acute cellular rejection; AMR, antibody-mediated rejection.

Results of Kaplan-Meier analysis for long-term graft failure also showed comparable results. Being pre-sensitized did not significantly increase the risk of long-term graft failure (HR 1.25, 95% CI 0.63-2.45, p=0.523) (Figure 4).

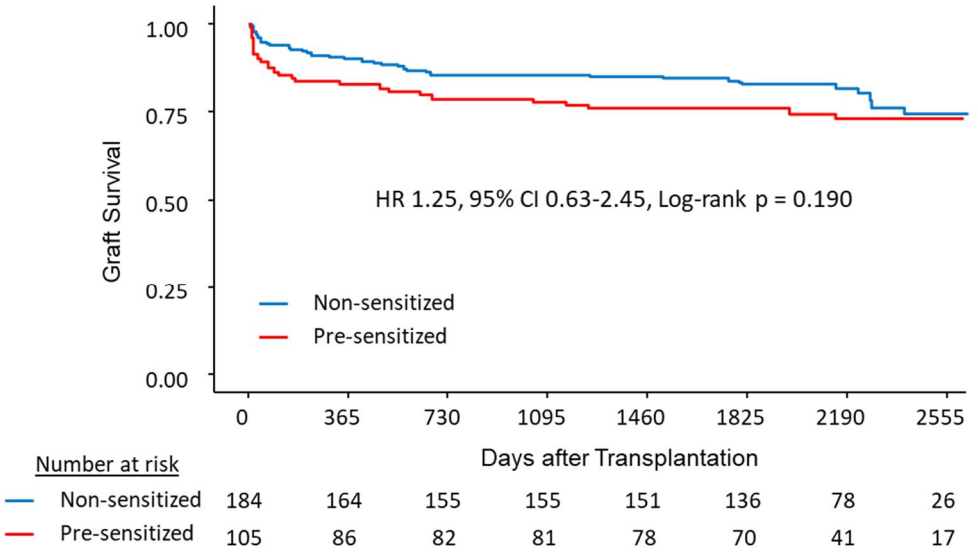

Figure 4 Kaplan-Meier curve for long-term graft failure according to pre-sensitization

Donor specific antibody and outcome

Among 264 patients with data for status of DSA, 44 (16.7%) were DSA+, which was targeted at HLA class I, class II, or both class I/II in 16, 18, and 10 patients. During follow-up, DSA+ patients had a significantly increased rate of graft failure at 1-year compared to non-sensitized patients, especially in the early period after transplantation (Figure 5). Risk factors associated with DSA+ were female sex (OR 6.52, 95% CI 2.25-18.91, p=0.001), re-transplantation (OR 13.75, 95% CI 1.72-110.13, p=0.014), and ≥4 HLA mismatch (OR 3.39, 95% CI 1.04-11.00, p=0.043) in multivariate logistic regression analysis. Presence of DSA was also

associated with significantly increased rate of long-term graft failure (HR 2.23, 95% CI 1.23-4.14, p=0.009) (Figure 6).

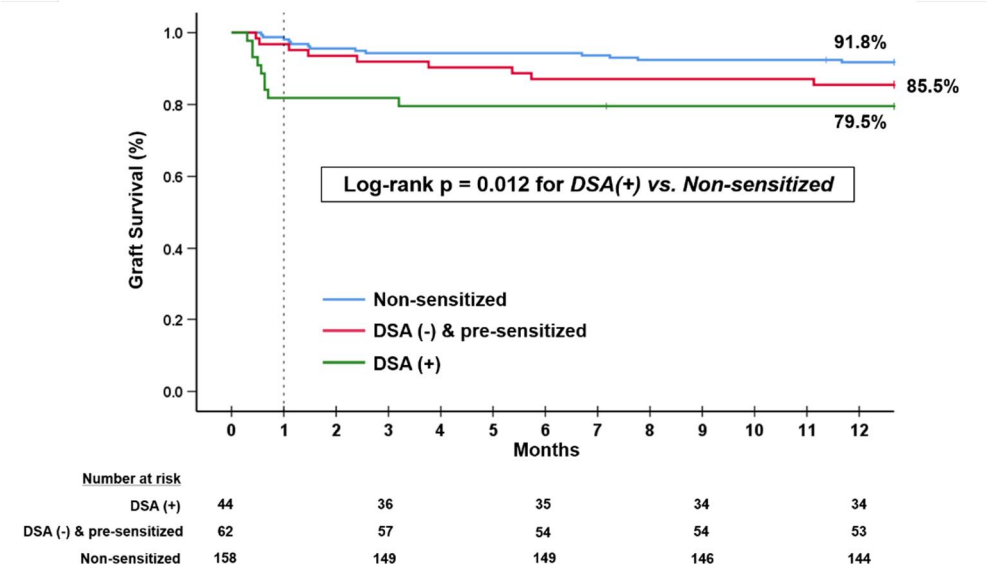

Figure 5 1-year graft survival according to presence of donor specific antibodies and pre-sensitization

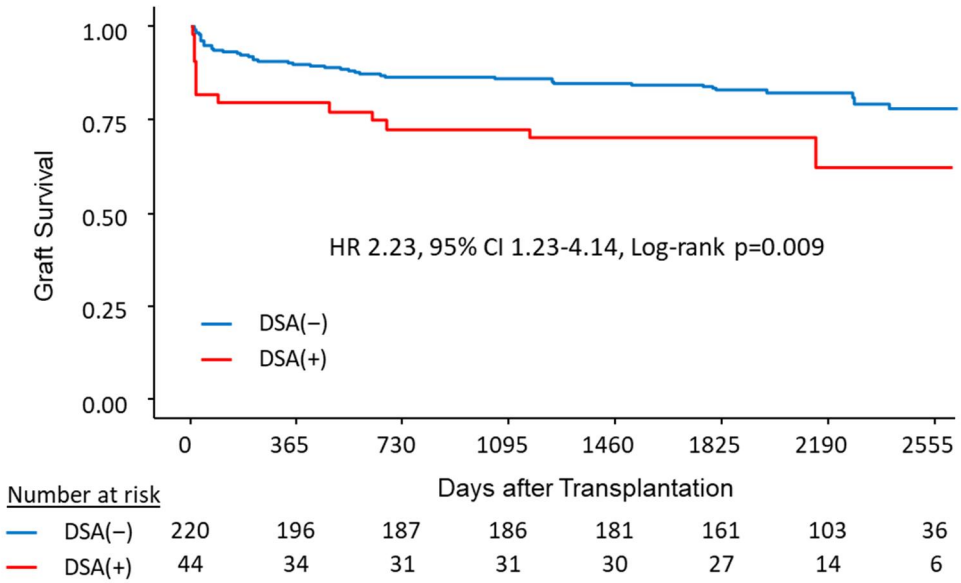

Figure 6 Kaplan-Meier curve for long-term graft failure according to presence of DSA

Predictors of graft failure

Univariate and multivariate cox regression analysis was performed to assess risk factors associated with graft failure. In multivariate analysis, diabetes mellitus, DSA+, pre-transplant VAD and RRT were significant predictors of graft failure at 1-year. Results of cox regression analyses are shown in Table 4.

Table 4 Results of cox regression analyses

	Univariate analysis			*Multivariate analysis*		
	HR	95% CI	p	HR	95% CI	p
Sex (male)	0.83	0.42-1.64	0.588			

	Univariate analysis			*Multivariate analysis*		
	HR	95% CI	p	HR	95% CI	p
Age (every 1year)	1.01	0.99-1.04	0.289			
Re-transplant	1.59	0.38-6.61	0.525			
BMI (every 1 kg/m^2)	1.03	0.95-1.13	0.471			
Current Smoker	0.75	0.23-2.44	0.632			
HTN	1.53	0.79-3.00	0.211			
DM	2.75	1.43-5.32	0.002	3.15	1.48-6.70	0.003
Insulin use	3.33	1.46-7.61	0.004			
CKD	2.35	1.07-5.15	0.034			
VAD	3.81	1.98-7.34	<0.001	2.35	1.05-5.24	0.037
RRT	4.98	2.56-9.67	<0.001	3.20	1.43-7.15	0.005
DSA	2.34	1.08-5.09	0.031	2.58	1.14-5.82	0.023
PRA	1.74	0.90-3.38	0.102			

Desensitization

Pre-transplant desensitization treatment was performed in 19 patients, with reasons for desensitization being positive complement dependent cytotoxicity (CDC) B-cell crossmatch (n=2), positive B- and T-cell cross match (n=1), positive flow cytometry T-cell cross-match (n=5), and presence of DSA+ (n=12). All patients who performed desensitization had a PRA value of 10% or more.

Frequently used desensitization methods were IV rituximab (n=14), plasmapheresis (n=14), and IV immunoglobulins (n=2).

Acute rejection

The median time to first acute rejection was 11.8 ± 0.9 months, and 56.4% of patients experienced biopsy-proven rejection episodes during 1-year follow-up. There was no significant difference in risk of ACR according to presence of pre-sensitization (adjusted HR 1.07, 95% CI 0.77-1.47, p=0.700) or pre-formed DSA (adjusted HR 1.24, 95% CI 0.76-2.03, p=0.117) (Figure 7).

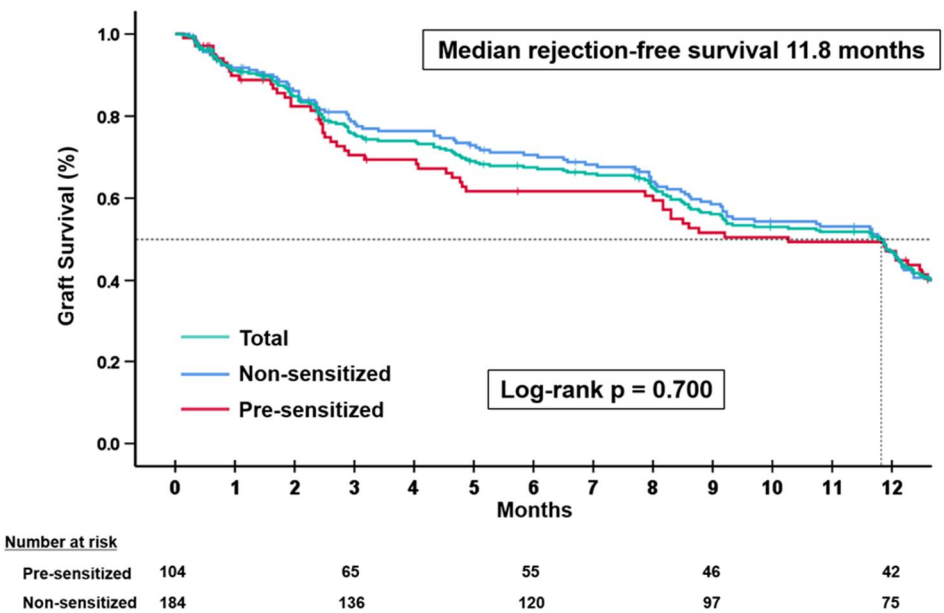

Figure 7 Acute rejection episodes according to pre-sensitization

status

Subgroup analysis

Exploratory subgroup analysis was performed to assess whether the effect of pre-sensitization was different according to baseline patient characteristics. There were no significant differences in risk of pre-sensitization according to sex, age, or baseline comorbidities (Figure 8).

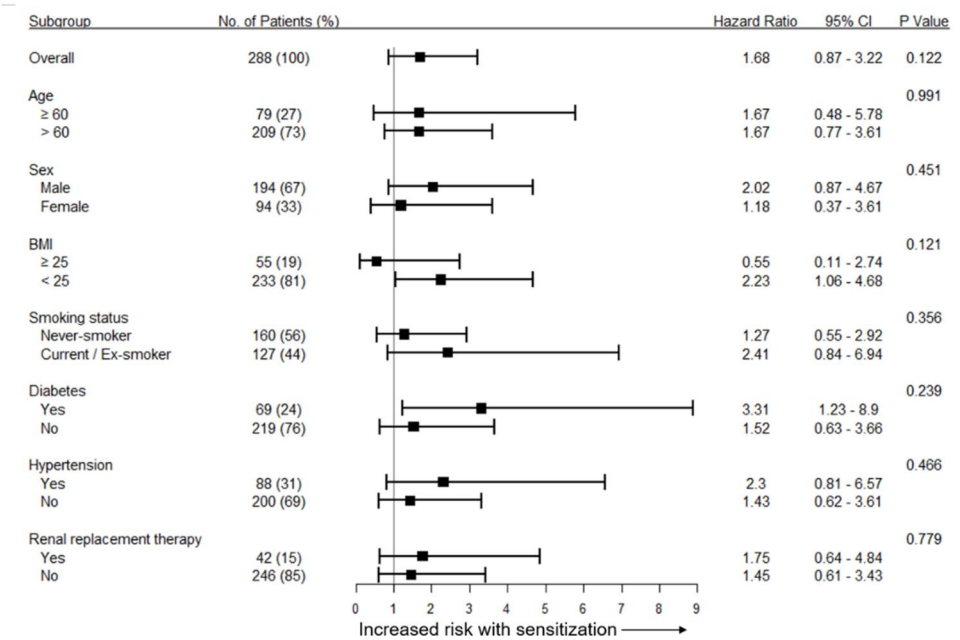

Figure 8 Subgroup analysis of risk of pre-sensitization on graft survival

Non-HLA antibodies and outcome

Baseline characteristics

Among the study population, pre-transplant serum was available in 192 patients. Mean age was 50.2 ± 13.4 years, 66.1% (127/192) were male, 24.5% (47/192) had diabetes, the etiology of HF was dilated cardiomyopathy in 57.3% (110/192), and 3.6% (7/192) were re-transplantations. The average waiting time after registration was 180 ± 62 days, 19.3% (37/192) were on ECMO and 13.5% (26/192) were on maintenance RRT before transplantation. Non-HLA antibody titers were analyzed, and their association with early (≤ 1 year) and late (> 1 year) outcomes were assessed.

Prognostic value of non-HLA autoantibodies

Non-HLA autoantibodies were analyzed using pre-transplant recipient serum using manufacturer recommendations. A total of 33 non-HLA autoantibodies were assessed in 192 patients, and 6 more autoantibodies were assessed for a subset of 171 patients with available serum. The prognostic value of each autoantibody was assessed using univariate Cox proportional hazards regression analysis for graft survival, and results are shown in Table 5. Among 39 autoantibodies, high titers of anti-vimentin Ab (AVA+) (HR 2.73, 95% CI 1.21-6.16, p=0.016) and anti-collagen II antibodies (ACA+)

(HR 2.76, 95% CI 1.12-6.81, p=0.027) showed significant correlation with 1 year graft survival. The results of multivariate Cox regression analyses for the total 39 autoantibodies are provided in Figure 9.

Table 5 Univariate Cox PH regression analysis using 29 non-HLA autoantibodies

Autoantibody	HR	Lower CI	Higher CI	p-value
ENO1	0.303	0.072	1.273	0.1030
FLRT2	1.109	0.524	2.348	0.7870
VM	2.730	1.209	6.163	0.0157
TUBA1B	0.371	0.051	2.730	0.3304
CD36	0.807	0.110	5.931	0.8329
IFIH1	0.630	0.150	2.650	0.5287
AGT	0.987	0.437	2.228	0.9745
PTPRN	0.655	0.156	2.755	0.5637
AURKA	2.312	0.700	7.642	0.1694
CHAF1B	3.720	0.884	15.663	0.0732
PPIA	1.109	0.423	2.906	0.8337
GSTT1	0.880	0.266	2.908	0.8341
LMNA	2.081	0.724	5.979	0.1737
PRKCZ	0.370	0.088	1.557	0.1753
PECR	1.243	0.474	3.259	0.6577
PRKCH	0.977	0.398	2.399	0.9591
LMNB	1.045	0.399	2.740	0.9280
CXCL11	1.059	0.500	2.242	0.8815
CXCL10	0.959	0.463	1.986	0.9097
AGRIN	1.905	0.576	6.296	0.2906
GDNF	0.839	0.341	2.059	0.7009

HNRNPK	1.161	0.540	2.498	0.7017
IFNG	1.027	0.418	2.521	0.9543
GAPDH	0.679	0.205	2.243	0.5252
PLA2R	2.558	0.774	8.456	0.1236
LG3	0.545	0.130	2.292	0.4075
COLL2	2.761	1.120	6.809	0.0274
COLL3	1.375	0.579	3.266	0.4704
COLL4	0.915	0.114	7.317	0.9331

*Positivity of 10 non-HLA autoantibodies according to manufacturer-provided MFI values were less than 10%, and were not used in Cox regression analyses.

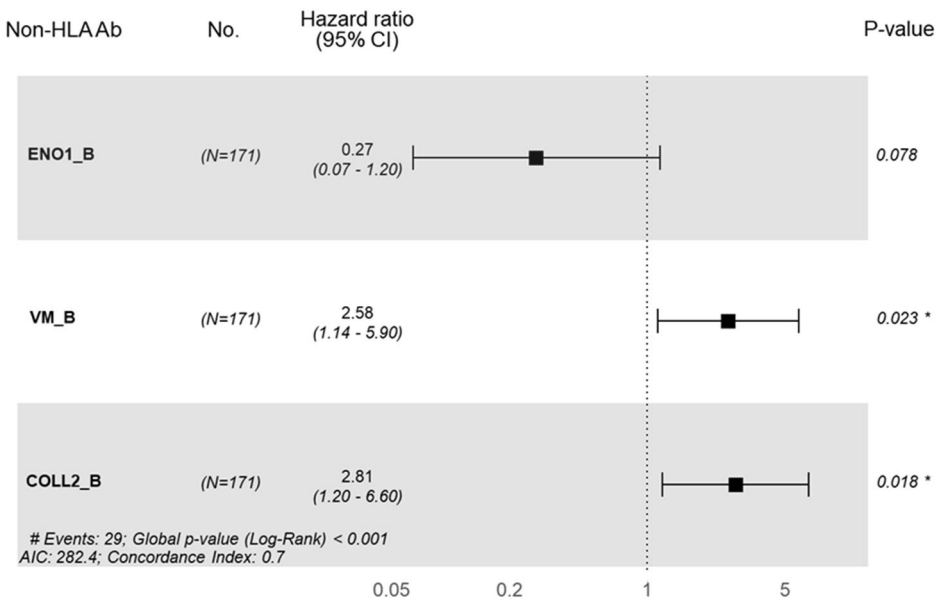

Figure 9 Multivariate Cox PH regression analysis using 39 non-HLA autoantibodies.

Role of anti-vimentin antibodies

Among 192 patients, 98 patients (51.0%) had high AVA titers (AVA+)before transplantation. There was a higher proportion of males with AVA+ (73.5% vs. 58.5%, p = 0.042), but no other

significant differences were observed according to presence of AVA+. The baseline characteristics of the study population according to baseline AVA status are provided in Table 6.

Table 6 Baseline characteristics according to AVA status

	AVA (-) (N=94)	AVA (+) (N=98)	p-value
Sex (male), n (%)	55 (58.5%)	72 (73.5%)	0.042
Age (years), mean ± SD	50.9 ± 13.5	49.5 ± 13.5	0.461
BMI, kg/m^2	22.5 ± 3.5	22.6 ± 3.6	0.835
Etiology			
- ischemic heart disease	12 (12.8%)	18 (18.4%)	0.384
- valvular heart disease	4 (4.3%)	2 (2.0%)	0.641
- idiopathic	58 (61.7%)	52 (53.1%)	0.287
- re-transplantation	2 (2.1%)	5 (5.1%)	0.475
Comorbidities			
-Hypertension	30 (31.9%)	31 (31.6%)	1
-Diabetes	23 (24.5%)	24 (24.5%)	1
-Insulin use	5 (5.3%)	8 (8.2%)	0.619
-History of malignancy	9 (9.6%)	2 (2.0%)	0.053
-Chronic kidney disease	13 (13.8%)	11 (11.2%)	0.743
Pretransplant management			
-Mechanical ventilation	17 (18.1%)	18 (18.4%)	1
-Ventricular assist device	19 (20.2%)	22 (22.4%)	0.84

--ECMO	17 (18.1%)	20 (20.4%)	0.822
--LVAD	2 (2.1%)	2 (2.0%)	1
-Renal replacement therapy	12 (12.8%)	14 (14.3%)	0.923
--Hemodialysis	1 (1.1%)	3 (3.1%)	0.643
--CRRT	11 (11.7%)	12 (12.2%)	1
Serology			
-CMV	83 (95.4%)	91 (98.9%)	0.332
-EBV	82 (96.5%)	88 (97.8%)	0.948
-HIV	1 (1.1%)	0 (0.0%)	0.974
-HBsAg (+)	6 (6.6%)	2 (2.1%)	0.252
PRA >10%	37 (39.8%)	39 (41.5%)	0.93
Donor specific antigen*	17 (18.1%)	11 (11.2%)	0.259
Blood type			0.306
-A	37 (39.4%)	29 (29.6%)	
-B	26 (27.7%)	27 (27.6%)	
-O	20 (21.3%)	22 (22.4%)	
-AB	11 (11.7%)	20 (20.4%)	

*Donor specific antigens were available in 87 patients in the AVA(-) group, and 90 patients in the AVA(+) group

There was a significant difference in 1-year graft survival and all-cause mortality according to pre-transplant AVA+, as shown in Figure 10 and Figure 11a. No significant differences were observed in occurrence of acute rejections according to AVA status (Figure 11b). Additionally, graft survival was analyzed according to

AVA positivity by pre-transplant DSA status. There were no differences in graft survival according to AVA+ in patients with DSA- (log-rank p = 0.150), but among patients with DSA+, those with AVA+ had worse outcomes compared with patients with AVA- (log-rank p = 0.002) (Figure 12).

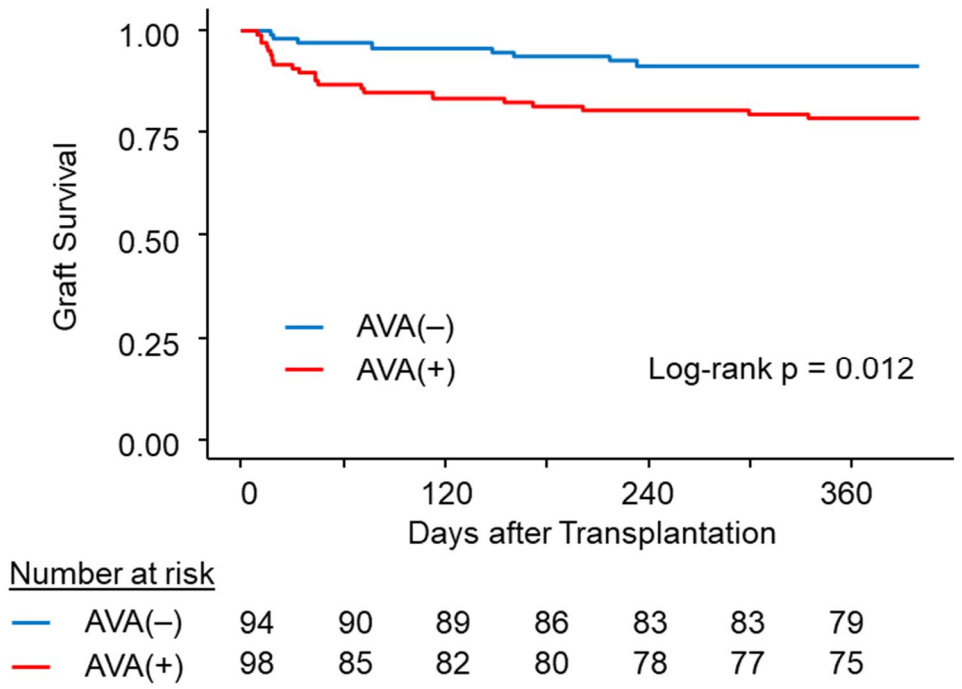

Figure 10 Kaplan-Meier curve for 1-year graft survival according to AVA status

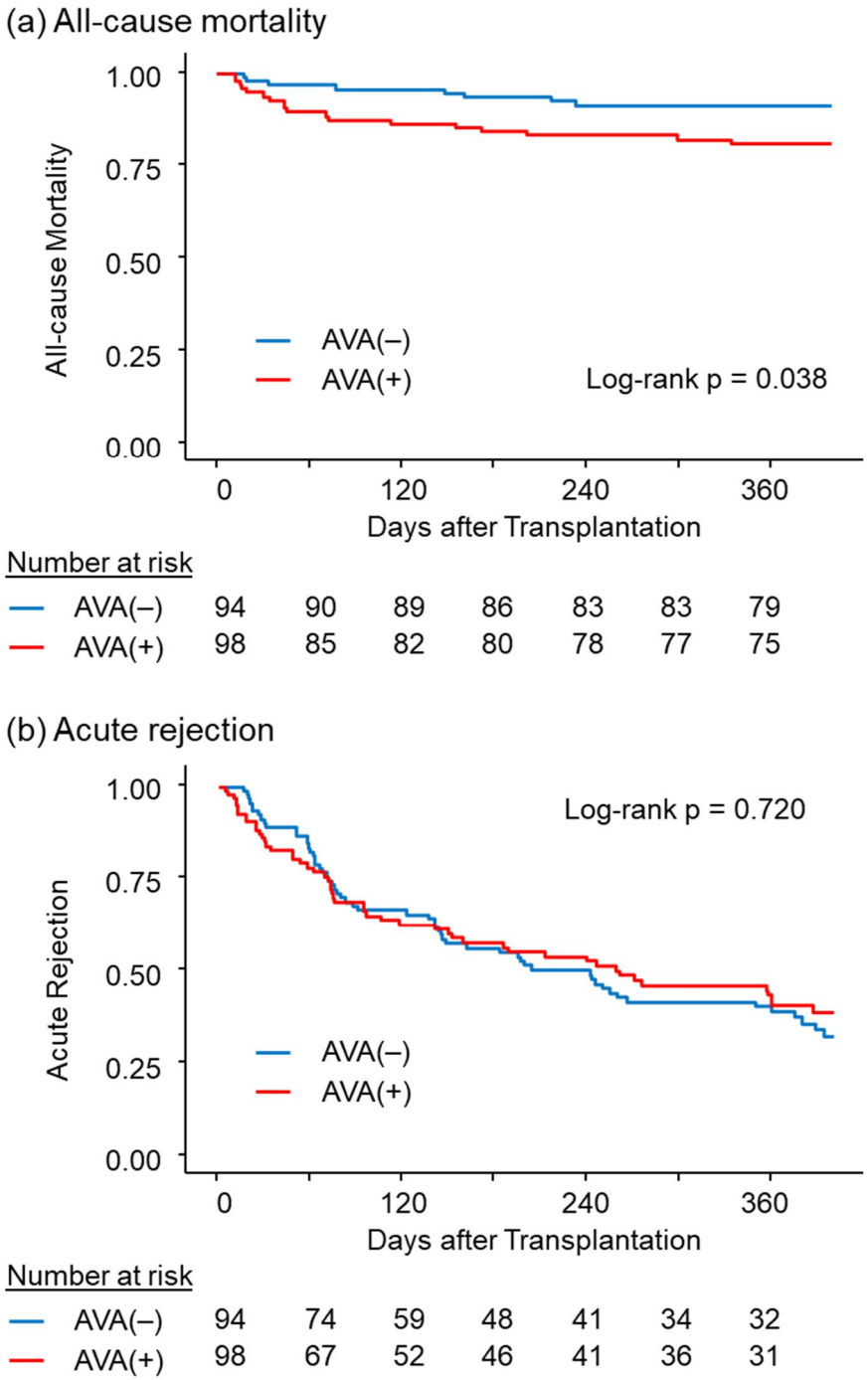

Figure 11 Kaplan–Meier curve according to AVA status. (a) All-cause mortality. (b) Acute rejection.

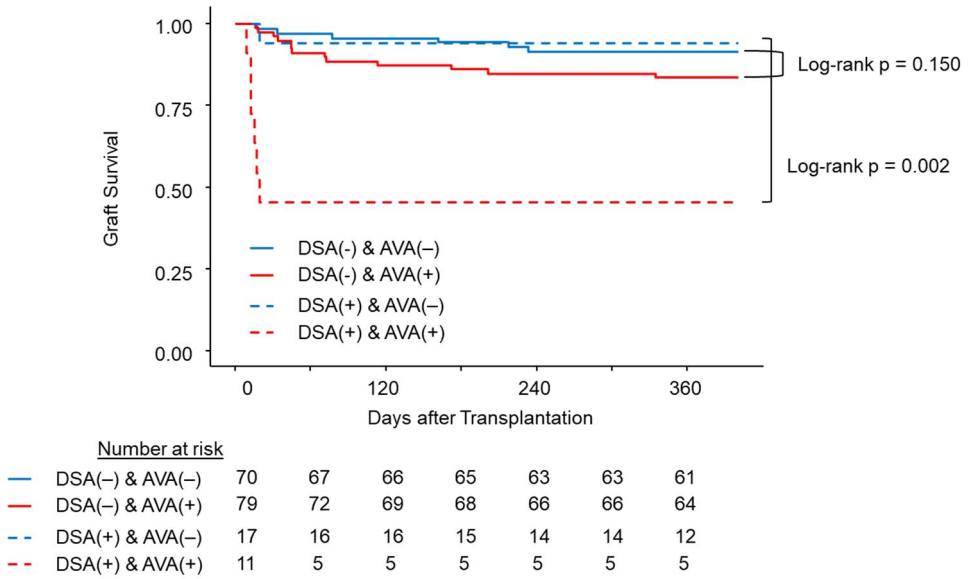

Figure 12 Kaplan–Meier curve for 1–year graft survival according to AVA x DSA status

In analysis for long–term outcomes, AVA+ was not associated with a significant difference in long–term graft failure (log–rank p=0.120) (Figure 13). However, landmark analysis performed at 90 days showed significant differences in graft failure up to 3 months (log–rank p=0.010), whereas no differences were observed in graft failure after 3 months (log–rank p=0.840) (Figure 14).

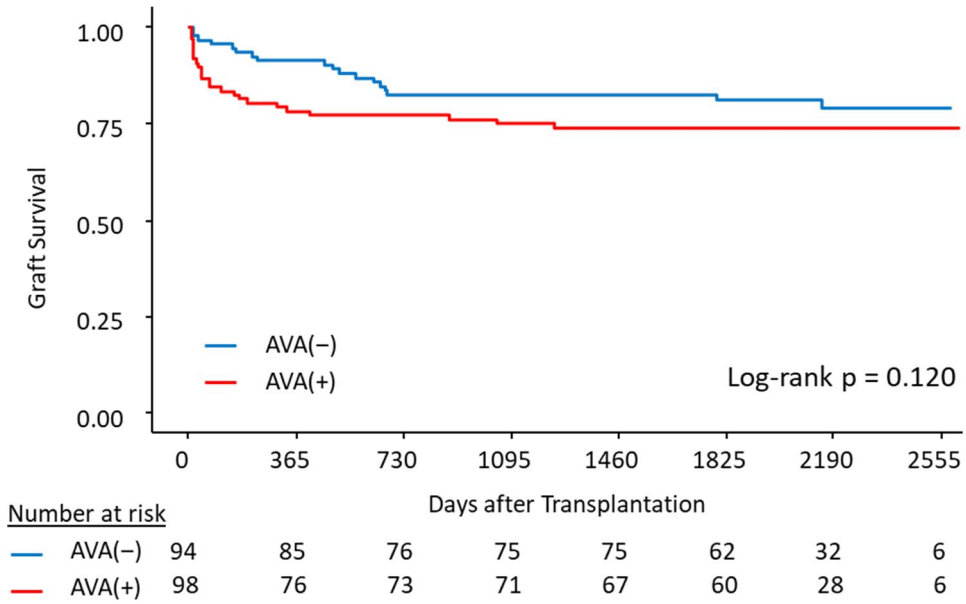

Figure 13 Kaplan−Meier curve for long−term graft survival according to AVA.

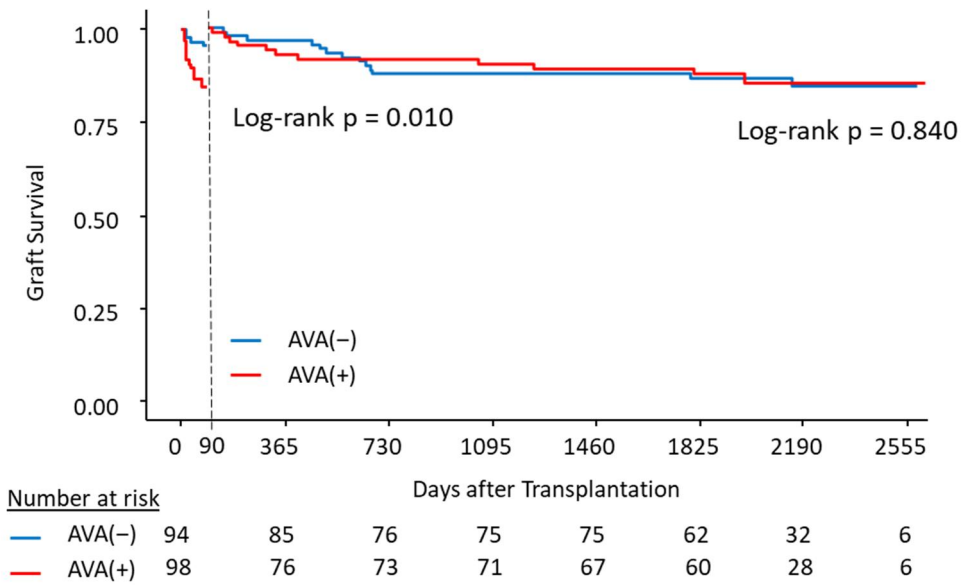

Figure 14 Landmark analysis at 90 days for graft survival according to AVA

Role of anti−collagen II autoantibodies

Among the total study population, ACA were analyzed in 171 patients.

High titers of ACA (ACA+) were present in 47.9% (92 of 171) before transplantation. Patients with pre-transplant ACA+ were older (52.4 ± 12.7 vs. 49.0 ± 13.6 years), but no significant differences were observed in other baseline demographics (Table 7).

Table 7 Baseline characteristics according to anti-collagen II antibody status

	Anti-Collagen II (-) (N=79)	Anti-Collagen II (+) (N=92)	p-value
Sex (male), n (%)	53 (67.1%)	57 (62.0%)	0.59
Age (years), mean ± SD	49.0 ± 13.6	52.4 ± 12.7	0.098
BMI, kg/m2	22.2 ± 3.1	22.6 ± 3.9	0.449
Etiology			
- ischemic heart disease	11 (13.9%)	17 (18.5%)	0.552
- valvular heart disease	1 (1.3%)	5 (5.4%)	0.289
- idiopathic	45 (57.0%)	51 (55.4%)	0.963
- Retransplantation	3 (3.8%)	4 (4.3%)	1
Comorbidities			
-Hypertension	23 (29.1%)	31 (33.7%)	0.633
-Diabetes	19 (24.1%)	25 (27.2%)	0.772
-Insulin use	5 (6.3%)	7 (7.6%)	0.619
-History of malignancy	9 (11.4%)	2 (2.2%)	0.033
-Chronic kidney disease	9 (11.4%)	11 (12.0%)	1
Pretransplant management			

-Mechanical ventilation	11 (13.9%)	20 (21.7%)	0.261
-Ventricular assist device	14 (17.7%)	23 (25.0%)	0.334
--ECMO	13 (16.5%)	20 (21.7%)	0.497
--LVAD	1 (1.3%)	3 (3.3%)	0.724
-Renal replacement therapy	7 (8.9%)	17 (18.5%)	0.113
--Hemodialysis	1 (1.3%)	3 (3.3%)	0.724
--CRRT	7 (8.9%)	14 (15.2%)	0.303
Serology			
-CMV	74 (96.1%)	81 (98.8%)	0.568
-EBV	73 (96.1%)	77 (97.5%)	0.965
-HIV	1 (1.3%)	0 -	0.947
-HBsAg (+)	2 (2.6%)	5 (-5.7%)	0.553
PRA >10%	28 (35.9%)	41 (46.1%)	0.24
Donor specific antigen*	11 (15.5%)	17 (19.5%)	0.65
Blood type			0.307
-A	22 (27.8%)	37 (40.2%)	
-B	26 (32.9%)	21 (22.8%)	
-O	18 (22.8%)	21 (22.8%)	
-AB	13 (16.5%)	13 (14.1%)	

Patients with pre-transplant ACA+ had a significantly increased risk of graft failure at 1-year (HR 2.94, 95% CI 1.26 - 6.88, p=0.013), as shown in Figure 15. ACA+ also significantly increased the risk of all-cause mortality (HR 3.14, 95% CI 1.26 - 7.82, p=0.014), but did not show any relationship with acute rejections (HR 0.99, 95% CI 0.66 - 1.48, p=0.964) (Figure 16).

Unlike AVA, the prognostic value of ACA did not differ according to DSA status (Figure 17).

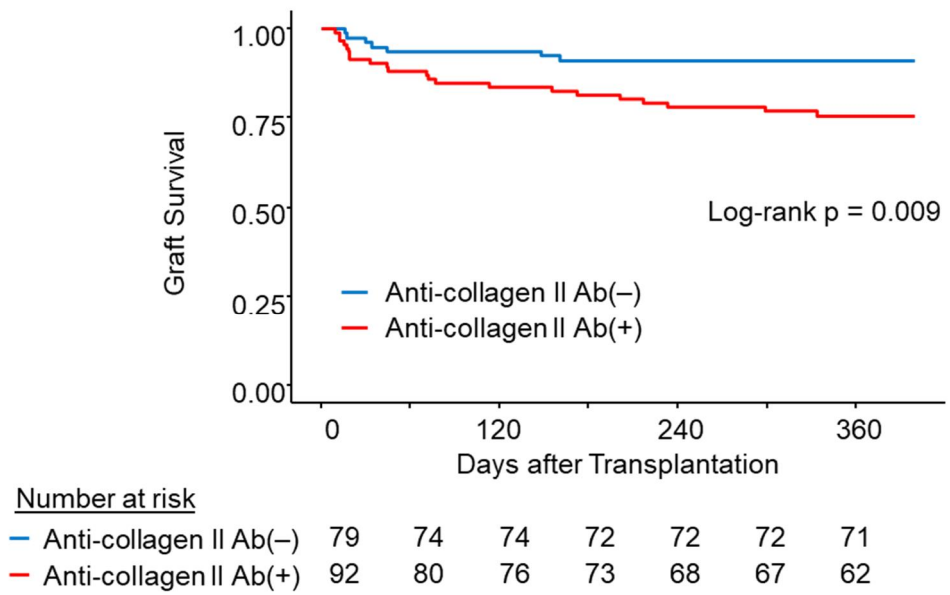

Figure 15 Kaplan–Meier curve for 1–year graft survival according to anti–collagen II Ab status

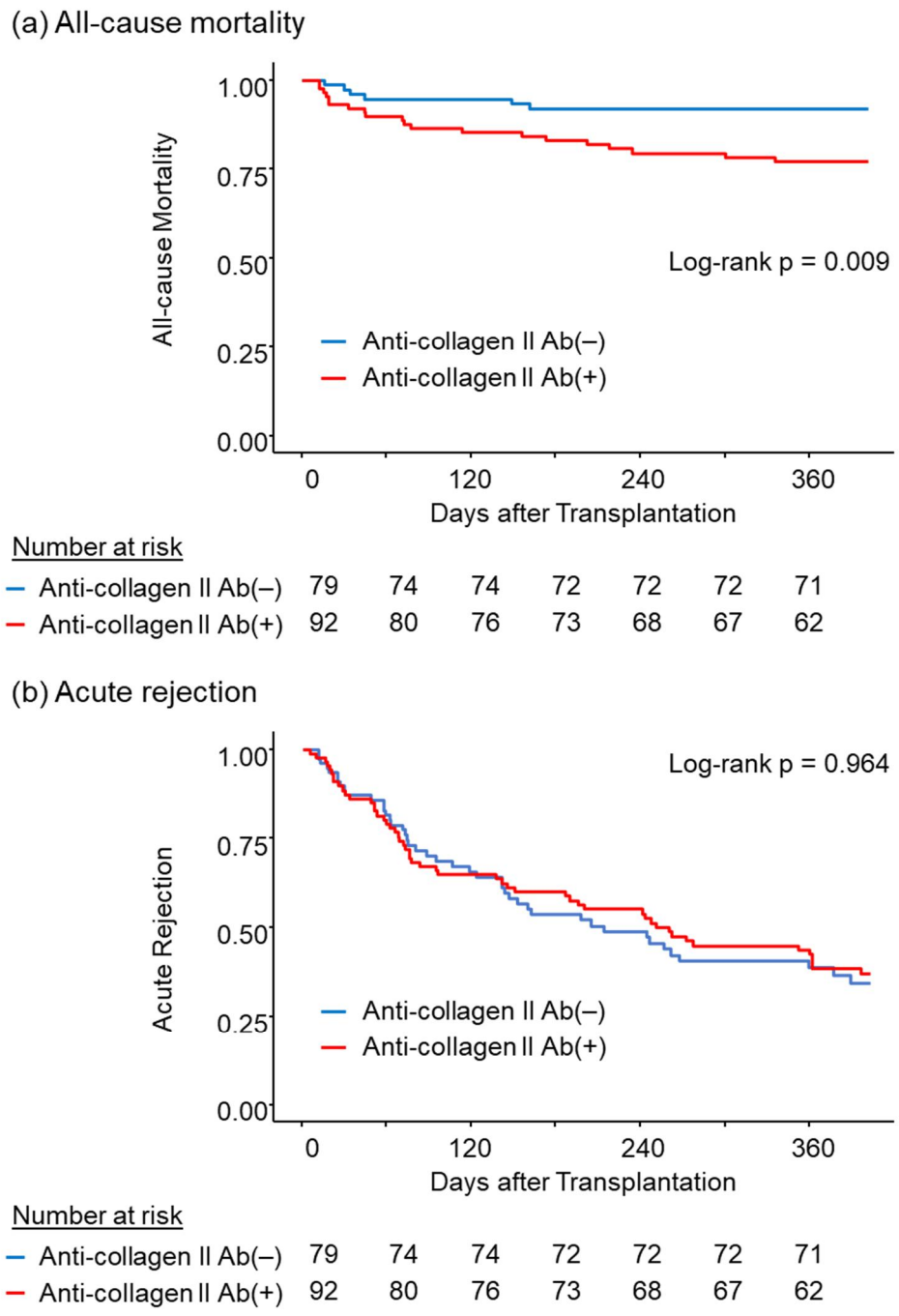

Figure 16 Kaplan–Meier curve according to anti–collagen II antibodies. (a) All–cause mortality. (b) Acute rejection.

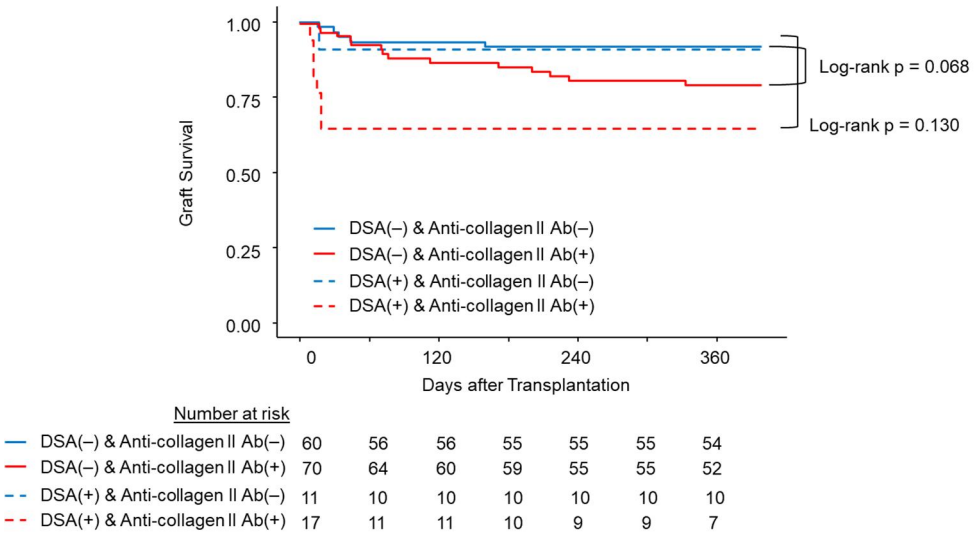

Figure 17 Kaplan–Meier curve for 1–year graft survival according to DSA x anti–collagen II Ab status

In analysis for long–term graft failure, patients with ACA+ showed significantly lower risk of events (log–rank p<0.001) compared with ACA– patients (Figure 18). Although landmark analysis performed at 90 days did not show a significantly difference in outcomes in the early period (log–rank p=0.066), persistent difference in outcomes were observed after 3 months (log–rank p=0.003) (Figure 19).

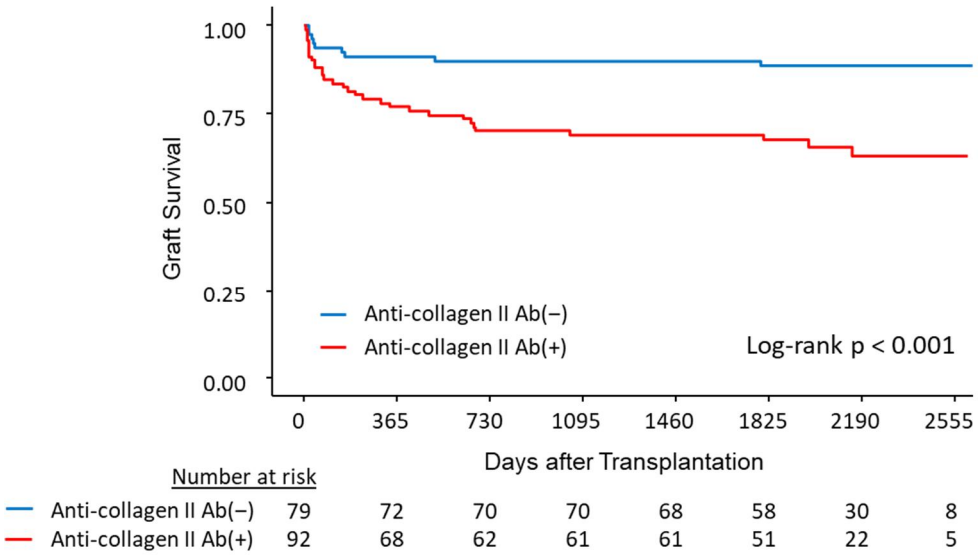

Figure 18 Kaplan−Meier curve for long−term graft survival according to anti−collagen II Ab status

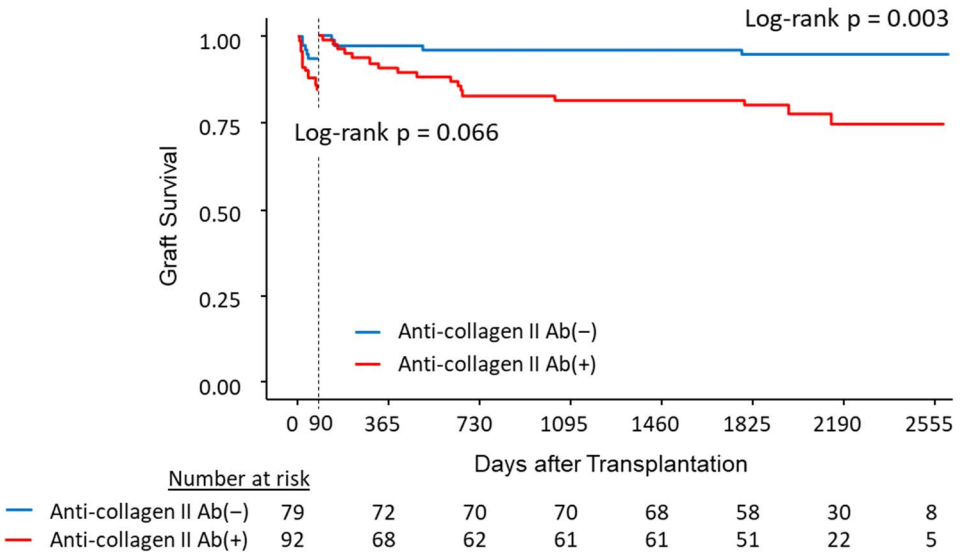

Figure 19 Landmark analysis at 90 days for graft survival according to anti−collagen II Ab status

Combined role of non−HLA antibodies

Outcomes of early and late graft failure were assessed using both

AVA and ACA. Among 171 patients with both antibody titer levels available, recipients with pre-transplant AVA+/ACA+ were associated with a significantly increased rate of graft failure at 1-year (log-rank p=0.001) and at long-term follow-up (log-rank p=0.001). For long-term graft failure, ACA+ further stratified outcomes in AVA+ patients (log-rank p=0.022) (Figure 20).

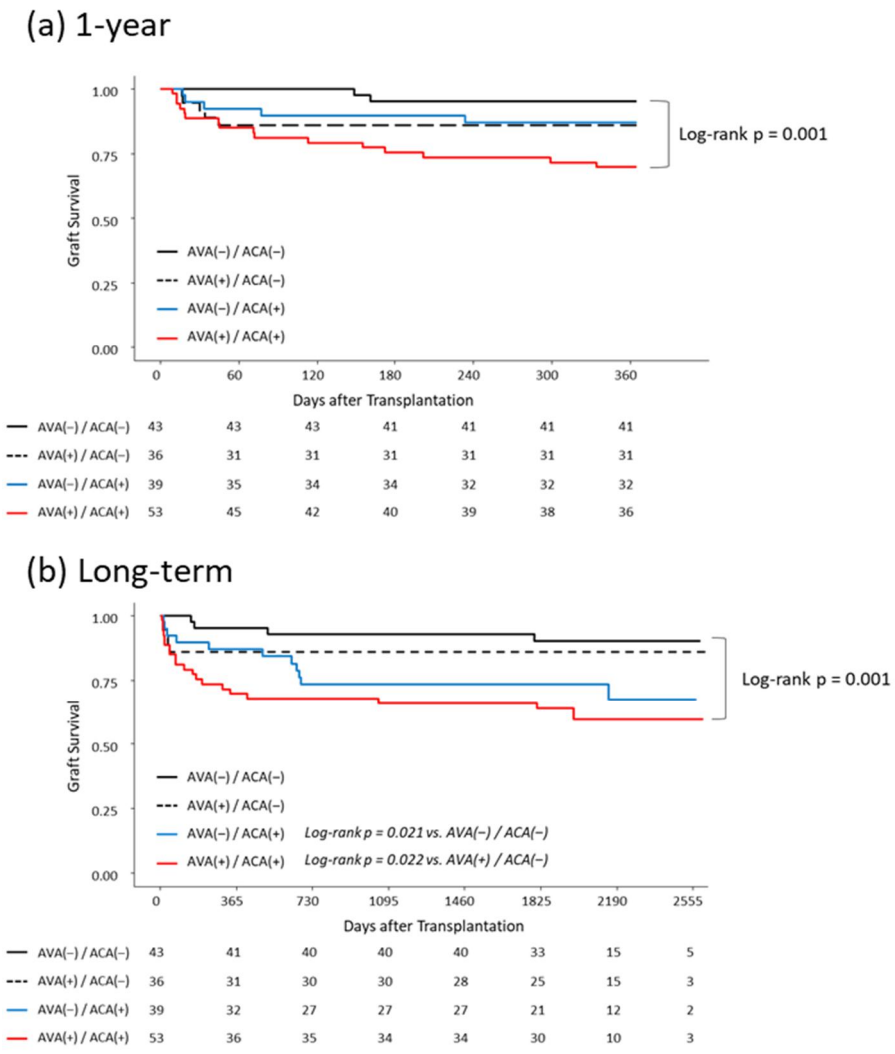

Figure 20 Early and late graft failure according to AVA / ACA status

Prognostic value of non−HLA autoantibodies

Different prediction models using 1) traditional risk factors derived from cox regression analysis (Model 1), 2) combination of Model 1 with AVA positivity (Model 2), and 3) combination of Model 2 with anti−collagen II positivity (Model 3) were constructed. Table 8

shows the likelihood ratio chi-square values, C-index, IDI, and NRI of each model. Addition of AVA enhanced the prediction power of model 2 (IDI=11%, p=0.002, NRI=23%, p=0.047). Further combination of anti-collagen II Ab did not affect model performance (p-value for IDI, NRI >0.05). The decision curve analysis in Figure 21 shows that addition of AVA increases the net benefit for predicting graft failure at 1-year.

Table 8 Performance of prediction models using non-HLA antibodies

	LR X^2	Δ from previous	IDI	NRI
Model 1	25.08			
p-value	<0.01			
Model 2	34.73	9.65	0.11 (0.02-0.22)	0.23 (0-0.40)
p-value	<0.01	<0.01	0.020	0.047
Model 3	37.54	2.81	0.03 (-0.01-0.11)	0.27 (-0.16-0.45)
p-value	<0.01	0.05	0.193	0.219

Model 1: DM, pre-transplant VAD, pre-transplant RRT, DSA
Model 2: Model 1 + AVA
Model 3: Model 2 + anti-collagen II antibody

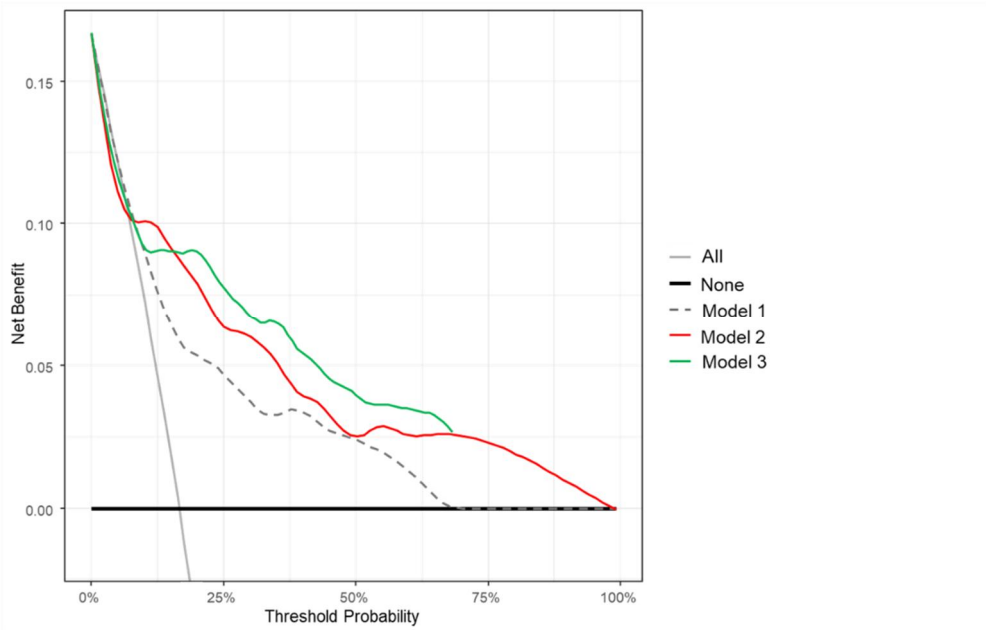

Figure 21 DCA analysis

Role of non-HLA autoantibodies using different positivity criteria

The positivity of non-HLA autoantibodies was also assessed by using the 75th percentile of the study population as cutoff thresholds for each non-HLA autoantibody. The proportion of patients having high AVA or ACA titers were significantly increased in patients experiencing graft failure, and mean MFI values were significantly higher for ACA in patients experiencing graft failure (Figure 22). Both AVA+ and ACA+ according to the 75th percentile was associated with significantly increased risk of graft failure at 1-year, whereas only ACA+ was associated with long-term graft failure. (Figures 23 and 24).

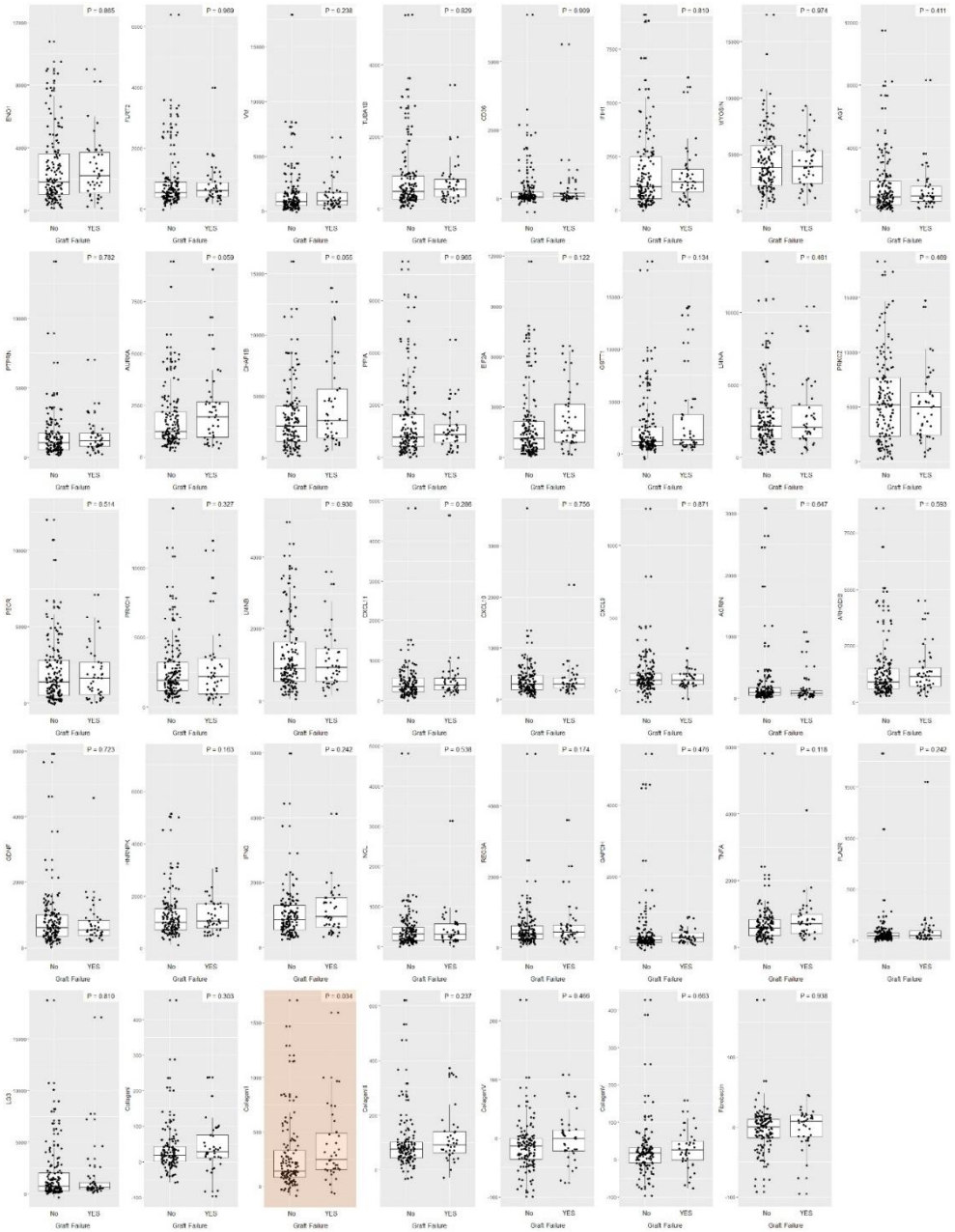

Figure 22 Non-HLA Ab MFI values according to presence of graft failure

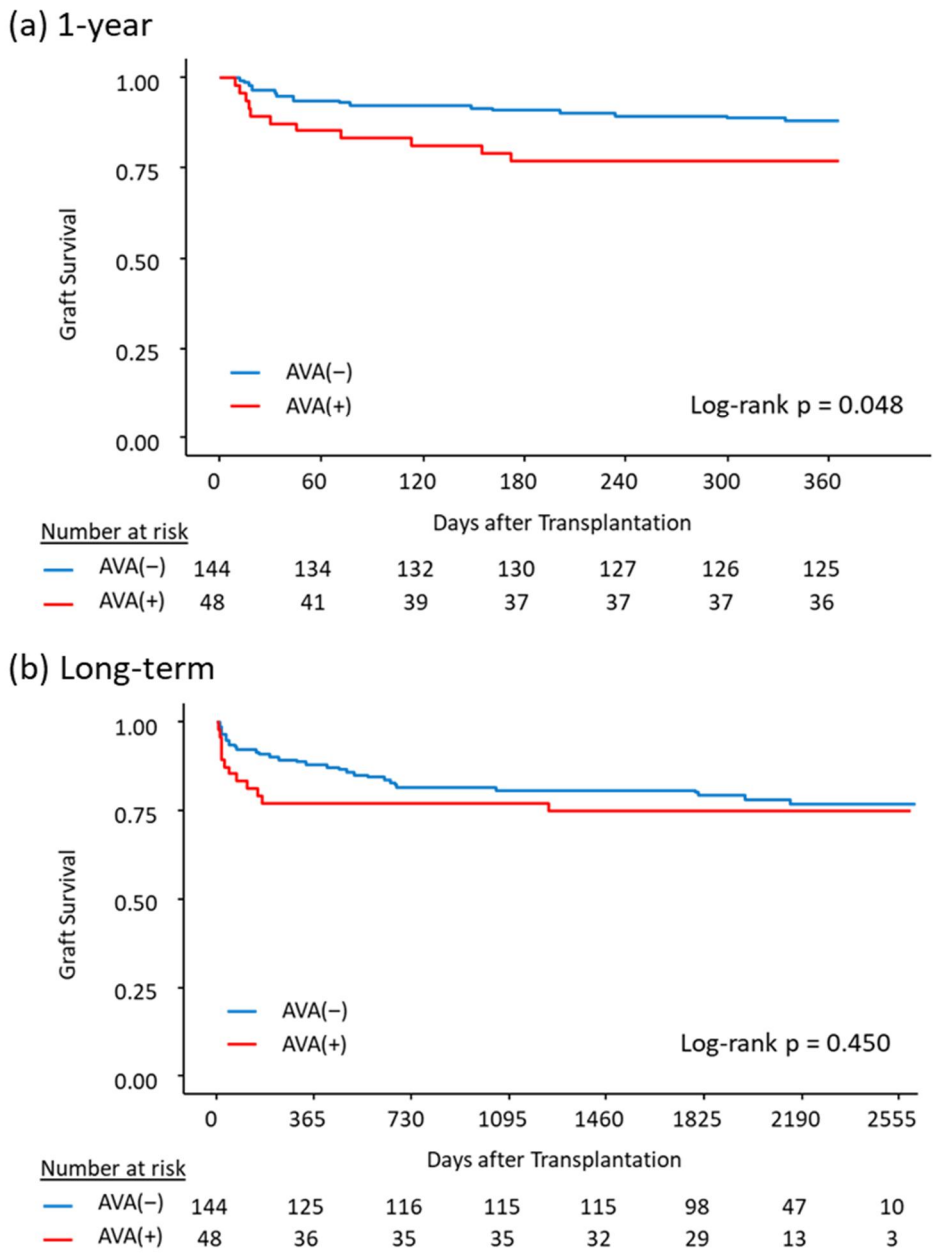

Figure 23 Kaplan-Meier curve for graft survival according to AVA status at (a) 1-year. (b) long term

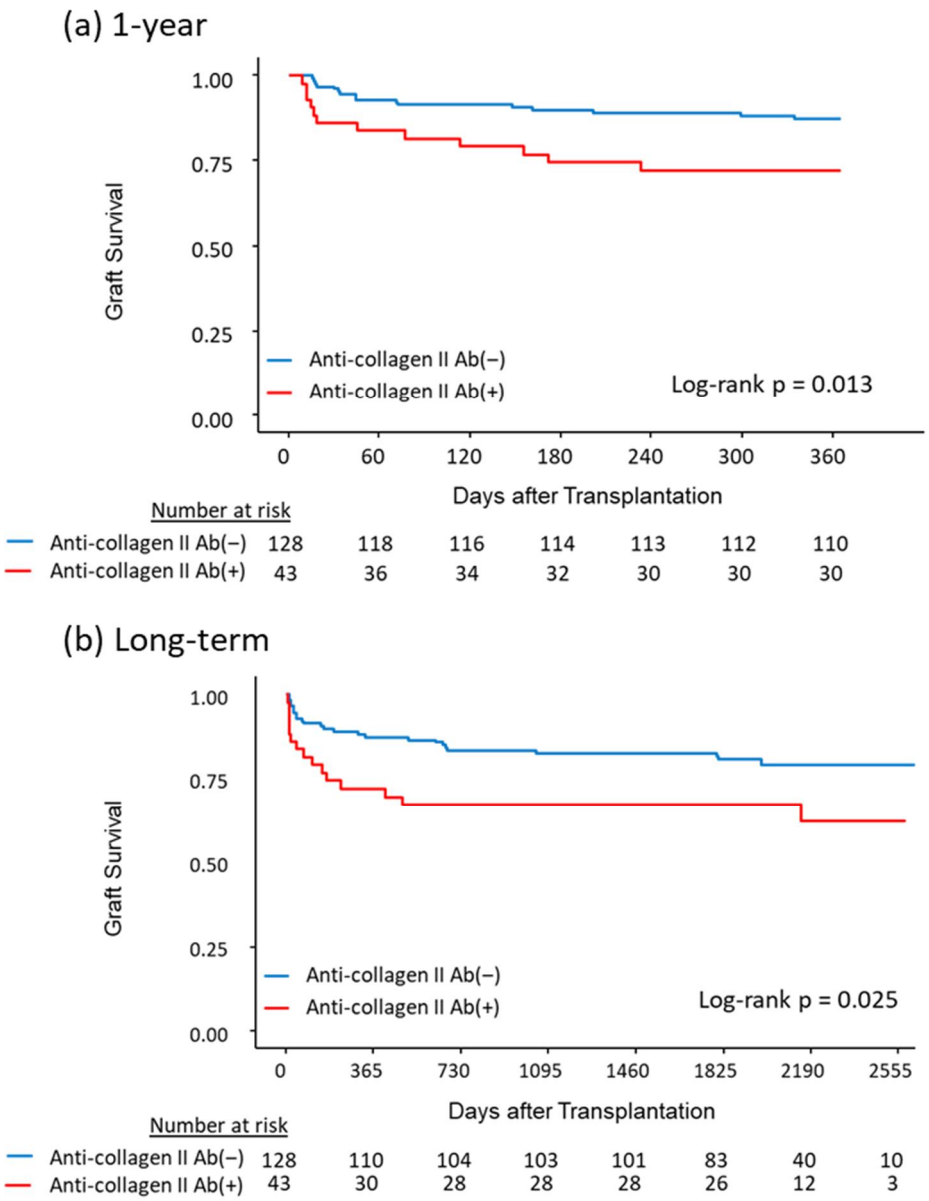

Figure 24 Kaplan-Meier curve for graft survival according to ACA status at (a) 1-year. (b) long term

Normal threshold values among Korean HTx patients

Using results of non-HLA Ab MFI values in 192 patients, 75%, 85%, and 95% percentile values in pre-transplant serum of Korean patients undergoing HTx were calculated (Table 9). These values

could be used as a threshold in guiding future research on Korean HTx patients.

Table 9 Normal threshold of non-HLA Ab according to 75%, 85%, and 95% percentile values

non-HLA Ab	75%	85%	95%	non-HLA Ab	75%	85%	95%
ENO1	3712.96	4876.73	8170.76	CXCL10	468.68	597.60	799.30
FLRT	886.69	1185.95	2710.88	CXCL9	112.84	158.76	268.49
VM	1654.48	2310.02	4543.48	AGRIN	156.40	272.62	845.17
TUBA1B	905.48	1170.95	2796.70	ARHGDIB	1490.44	1894.97	4149.47
CD36	230.20	508.80	1465.32	GDNF	993.64	1305.90	1681.85
IFIH1	2235.37	2925.86	5665.41	HNRNPK	1552.17	1739.90	2993.55
MYOSIN	5636.04	6711.59	8941.64	IFNG	1340.11	1532.13	2155.62
AGT	1845.69	2918.94	4996.52	NCL	522.41	663.31	989.63
PTPRN	1768.58	2429.95	3727.40	REG3A	625.31	809.26	1287.35
AURKA	2416.56	3155.60	4977.67	GAPDH	326.21	501.73	1051.90
CHAF1B	4355.63	5751.11	8585.67	TNFA	850.00	1045.15	1408.75
PPIA	2414.44	3376.38	6540.05	PLA2R	78.49	109.30	192.11
EIF2A	2345.60	3442.10	6392.95	LG3	1750.29	3156.58	7153.91
GSTT1	2816.44	4814.27	9361.30	Collagen I*	45.19	75.36	158.22
LMNA	3428.68	5038.87	7953.65	Collagen II*	378.49	580.13	978.09
PRKCZ	7620.69	9501.44	13566.52	Collagen III*	111.38	152.67	326.28
PECR	2783.85	4147.06	6015.07	Collagen IV*	3.12	14.45	58.00
PRKCH	3275.45	4382.91	7895.92	Collagen V*	34.82	59.55	115.89
LMNB	1603.40	2259.63	3445.33	Fibronectin*	12.96	17.31	28.90
CXCL11	562.14	712.49	960.04				

* Threshold values acquired from assessment of non-HLA Abs in 171 patients.

Discussion

The current analysis of Korean HTx patients using a prospective nationwide registry showed that antibodies to both HLA and non-HLA were significant prognostic markers for graft failure at 1-year. For patients with DSA+, there was a significantly higher rate of graft failure at 1-year. Non-HLA autoantibodies to vimentin also had a prognostic value in recognizing patients with poor early graft survival. Presence of AVA further discriminated outcomes in patients with DSA+. ACA+ also had a prognostic value in stratifying graft outcomes in both the early and late periods following HTx. Finally, compared with AVA-/ACA- patients, those with AVA+/ACA+ were associated with significantly increased risk of graft failure. Addition of non-HLA autoantibodies to vimentin and collagen II to traditional risk factors increased the performance of the prediction model for graft survival.

Sensitization and outcomes

Pre-sensitization occurs as a result of exposure to non-self HLA antigens, and well-known risk factors include pregnancy, prior organ transplantation, and blood transfusions. It has been reported to be

associated with higher rates of rejections,[9] increased prevalence of cardiac allograft vasculopathy (CAV), and lower graft survival after HTx.[25-28] Screening tests are performed to check whether the patient is pre-sensitized, and to perform desensitization treatment before transplantation if possible. However, unlike other solid organ transplantations, HTx are mostly performed as an emergency, and patients waiting for surgery are in critical, life-threatening conditions at the time of transplant. In most cases, desensitization treatment may not be possible, and hearts may need to be allocated to other recipients.

Among 104 pre-sensitized patients, 19 (18.3%) managed to undergo desensitization with IV rituximab, IVIG, or plasmapheresis as a single regimen or in combination therapy. When compared with pre-sensitized patients who had not received desensitization therapy, there was no significant difference in 1-year graft survival (log-rank p=0.933). However, the results need to be interpreted with caution, since there was a high proportion of DSA+ patients among those receiving desensitization treatment, and we also lacked data on whether specific treatments were given in the acute post-transplant periods. The number of patients who underwent desensitization was also too small to have statistical power.

The prevalence of pre-sensitization has been suggested to have racial variability and have different effect on outcome. A previous study conducted in a multi-ethnic population showed that Asians have a higher proportion of patients with high PRA values but were associated with better outcomes.[11] In the current cohort of Korean HTx patients, pre-sensitization rate was 36.1%, which is higher than the 21.1% reported for global average in the 2018 International Society of Heart and Lung Transplant (ISHLT) registry data, but comparable to other previous studies.[29] The overall 1-year survival of HTx patients was 88.6%, comparable to the ISHLT data of 85.4%, even with a higher proportion of pre-sensitized patients. This might be because the current registry was comprised with a mono-ethnic population. Other reasons for lower adverse events might be a higher prevalence of 'lean diabetic' HF patients in Asia, distinct from Western countries where patients are more obese.[30]

One thing to note is that DM and smoking history were associated with a lower incidence of pre-sensitization in univariate analysis. Although DM is a risk factor for HF development and progression, it has also been reported to alter the immune system,[31] which might explain the association with lower rate of pre-sensitization. Smoking also inhibits immune responses through

many components including nicotine.[32] Although smoking was not significantly associated with pre-sensitization in multivariate analysis, further evaluation on its effect on immune modulation is warranted.

Non-HLA antibodies and outcome

Traditionally, HLA antibodies have been known to be a critical factor in patient outcomes after solid organ transplantation. Extensive care is taken in management of pre-transplant patients to avoid being sensitized to possible donor antigens. Screening of PRA assess the probability of sensitization, and DSA are evaluated at the immediate pre-transplant to assess recipient eligibility. As waiting time for organ transplant increases, criteria for donor / recipient has become more lenient, but still is a key factor in determining outcomes. Measures may be taken to neutralize the effect of DSA before transplant, but most patients are in critical status and such measures cannot be always applied.

Some studies have raised the question of whether factors other than HLA antibodies could affect outcomes in organ transplantations. Antibodies to donor-specific major histocompatibility complex class I related chain A (MICA) have been associated with AMR in kidney transplantation and later in pancreas

and heart transplantations.[33, 34] In addition, antibodies to G Protein-coupled receptor AT_1 have shown detrimental effect on graft survival after kidney transplantation.[35] Presence of anti-AT_1R antibodies in HTx has also been linked with antibody- and cellular-mediated rejection and microvasculopathy.[17]

Vimentin is a type III intermediate filament which forms cytoskeleton in human cells including endothelial cells and contributes to cell structure, motility, signaling and proliferation.[36-38] Disruption cell integrity due to direct or indirect causes such as ischemia and inflammation could lead to production of epitopes for antibodies to vimentin.[39] In previous studies, anti-vimentin antibodies have been reported to have associations with rheumatoid arthritis[40] and early diagnosis of pancreas cancer.[41] More recently, they have been noticed for associations regarding antibody-mediated rejections in solid organ transplantations.

In 109 patients followed up for 5 years, the titers of anti-vimentin antibodies at 1- or 2-years post-transplant were independent predictors of coronary allograft vasculopathy.[42] Neth et al.[43] also demonstrated that antibodies to vimentin were increased in heart transplant patients with AMR or coronary allograft vasculopathy. More recently, See et al.[44] reported that DSA-

positive or those with AMR showed a higher reactivity to non-HLA antigens including vimentin. On the contrary, a retrospective study of 50 patients undergoing HTx, presence of pre-transplant AVA did not correlate with early outcomes of graft survival or rejections.[45] The Clinical Trials in Organ Transplantation (CTOT)-05 study, which was a prospective multicenter trial evaluating the risks of adverse outcomes after HTx, also summarized that reliable biomarkers for transplant outcomes remain elusive, with only possible correlations with serum alloantibodies.[46] Trials on the predictive values were heterogeneous in design and relatively small in number, cross-sectional, and single-center analyses. Unlike previous studies evaluating the prognostic value of post-transplant anti-vimentin antibodies, the current study assessed presence of pretransplant AVA and demonstrated a significant association with poor outcomes in the early period (<1 year) after HTx. Patients in DSA+ AVA- status had similar rates of graft survival compared to those with DSA-, showing that AVA status could have implications in organ allocation on top of DSA status.

Although role of AVA has not been fully investigated in human HTx, Mahesh et al.[47] demonstrated activation of vimentin-specific T and B cells, enhanced microvascular deposition of inflammatory cells after immunization with vimentin in a mice transplantation model.

Barber et al.[48] also showed that autoreactive CD8+ T-cells may be associated with adverse outcomes in human heart transplant patients. However, whether anti-vimentin antibodies were produced due to events proceeding or following HTx had not been determined. According to the current analysis, preformed antibodies to vimentin prior to transplantation were significant predictors of early outcomes. For patients surviving to 1-year and serum available for analysis, only 10.8% (8 out of 74) developed de novo AVA, while 46.6% (31 out of 68) showed negative conversion of AVA, suggesting that pre-transplant AVA may have a higher significance.

Although the current study could not provide a mechanism for increased graft failure in patients with AVA, increase in AMR could be a possible explanation. Previous studies have reported that AVA titers may differ according to post-transplant immunotherapy.[49] This may explain the finding that AVA+ was only associated with increased risk of graft failure in the early (< 1 year) period, but not in the late (> 1 year) period after transplantation. Further studies evaluating the changes in AVA status both before and after transplantation will be needed to better understand its association with outcomes in HTx. Nonetheless, patients with AVA may benefit from modified immune modulation and strict surveillance endomyocardial biopsy protocols. Positivity of AVA could be used to

tailor therapy during the early post transplantation period.

Transplant recipients with ACA+ were also at higher risk of graft-failure both in the early and late periods after transplantation. While pre-transplant AVA did not have a significant impact on outcomes in later periods, there seems to be an increased role of ACA after 1-year. However, type II collagen fibers are mostly distributed in the cartilage, and while antibodies to collagen types I and III have been suggested to be associated with AMR in kidney transplant recipients,[15] the role of collagen type II have not been well studied except for in pathogenesis of arthritis.[50] Therefore, the findings of the current analysis need to be interpreted with caution, as high titers of ACA may only be an incidental finding.

An interesting finding is that although AVA+ and ACA+ are both linked with adverse graft outcomes in the early period, the effect of pre-transplant ACA+ seems to persist in the long-term period. As mentioned before, the mechanism linking AVA+ and ACA+ with graft outcomes have not well been revealed, and the different time periods which these antibodies affect outcomes seem to suggest that the humoral immunity may be responsible for AVA, while cellular immunity might play a role for ACA. Another possible mechanism is that high titers of pre-transplant non-HLA antibodies may indicate a predisposition to a high autoimmune state, leading to adverse

outcomes after transplantation. More studies in pathways linking AVA, ACA, and graft outcomes in HTx will be needed to better understand the implication of non-HLA antibodies in HTx.

Finally, through the current study, the normal threshold values of non-HLA antibodies could be obtained. There have been no previous studies analyzing the presence and titer of non-HLA antibodies in the Korean HTx population. The results of the 75%, 85%, and 95% threshold values can be used in the future as a cut-off value for HTx research.

Limitations

The limitations of the current study are as follows. First, as with all analyses performed on registry data, missing values were frequent. Due to the emergency of the procedure and the recipient being in critical conditions, some patients needed to be rushed into surgery without a full pre-transplant work-up. Furthermore, as the analysis was performed using retrospective registry data, causal effect of each factor could not be fully assessed. Second, although data used in the analysis were acquired in a prospective nationwide registry, not all patients were enrolled in the study and blood samples were only available for a subset of patients. However, the registry collected data from the top 4 transplant centers in Korea which

represents more than 80% of total ongoing HTx during the study period.

Another limitation is lack of data on prior blood transfusions and pregnancies, which are well-known risk factors of pre-sensitization. Assessment of risk factors for pre-sensitization was limited due to lack of data on these major factors. Although females in general are known to be at a higher risk for pre-sensitization, detailed medical histories could have made further analyses possible. Additionally, it is highly probable that patients on ECMO support or on continuous renal replacement therapy (CRRT) received blood product transfusions. There was also no data on the specific uses of immunosuppressive therapy during the acute post-transplantation period. Patients with DSA+, or those pre-sensitized could have received additional immunosuppression therapy post-operatively, affecting outcomes.

Being on ECMO or renal replacement therapy also could have led to fluctuations in titers of non-HLA antibodies. To note, there were no differences in frequencies of ECMO or RRT according to AVA status. Matching serum samples at biopsy periods were also not available. Changes in AVA positivity or titer could have provided more information on prediction of acute rejections. The positivity of non-HLA antibodies to vimentin and collagen II were determined

using the 95% normal values provided by the manufacturer and characteristics of the normal population cannot be assessed. Ethnic differences were not assumed in the analysis. However, through the current analysis, the 75%, 85%, and 95% cutoff value of over 170 HTx recipients were derived, giving hope for future research.

In previous studies, antibodies specific to donor antigens have mostly been thought to be associated with hyper-acute AMR.[51] Unfortunately, detection of AMR was very limited in the current registry data. There was only 1 case of pathologically proven AMR among 224 biopsy-proven rejection episodes during 1-year of follow-up. Antibody-mediated rejections has been poorly defined and evaluated in the past. A survey conducted in 2010 states that over 50% of transplant centers diagnosed AMR based on cardiac dysfunction accompanied by a negative endomyocardial biopsy specimen.[52] Efforts have been taken to standardize diagnosis of AMR using histologic and immunopathologic evidence from endomyocardial biopsies. Unfortunately, these efforts were not taking place during the period. After interim analysis, measures have been taken to better assess AMR in recent years, and the KOTRY registry is currently acquiring specific data on AMR with hopes to conduct future studies to better understand the association between non-HLA antibodies and AMR.

Conclusion

In Korean HTx patients, risk factors associated with pre-sensitization were female sex and previous transplantation, while DM had a protective effect. Pre-sensitization was not associated with a significant difference in outcomes of graft failure at 1-year, but presence of DSA was associated with an increased risk of graft failure both in the early and late periods after HTx. Non-HLA autoantibodies to vimentin and type II collagen both increased the risk of early graft failure, but only anti-collagen II antibodies were associated with graft failure in the long-term period. There was also a synergistic effect of DSA and AVA in predicting early graft outcomes. Both HLA and non-HLA specific antibodies seem to impact graft outcome in HTx.

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국문 초록

서론

이식 전 감작(pre-sensitization)은 장기 이식 후 불량한 예후와 연관된 것으로 잘 알려진 위험 요소이다. 중증 심부전 환자 유병률이 증가하며 심장이식 대기시간도 증가하였고, 감작 위험 역시 높아졌다. 인간 백혈구 항원(HLA)에 대한 항체가 심장이식의 예후와 밀접한 연관이 있다는 것은 잘 알려진 사실이며, 최근에는 비HLA 항체의 중요도에 대한 연구도 보고된 바 있다. 본 연구를 통하여 한국인 심장이식 환자에서 HLA 항체인 공여자 특이 항체(DSA)와 비HLA 항체가 환자 예후에 미치는 복합적인 영향을 평가해보고자 한다.

방법

국내 4개 이식센터에서 2014년 1월부터 2016년 12월까지 중증 심부전으로 심장이식을 시행한 성인 환자의 정보를 전향적으로 수집하였다. 모든 분석은 2014년 설립된 한국장기이식연구단(KOTRY)의 데이터를 사용하여 수행되었다. 전체 대상자 중 혈청 공여에 동의한 환자를 대상으로 비HLA 항체의 존재를 실험실 분석하고, 감작 및 공여자 특이 HLA 항체, 비HLA 항체와 이식 후 1년 시점 및 장기 이식실패와 관계를 분석하였다.

결과

총 290명의 환자가 KOTRY에 등록되었다. 항체선별검사(PRA) 정보가 있는 288명의 환자 중 104명(36.1%)이 PRA 선별값이 10% 이상으로, 이식 전 감작으로 정의되었다. 감작 관련 위험인자는 여성(HR 3.73, 95% CI 2.17−6.42, p<0.001)과 재이식(HR 7.36, 95% CI 1.75−30.96, p=0.006)이었으며, 당뇨병이 있는 환자는 감작의 위험이 적은 것으로 나타났다(HR 0.44, 95% CI 0.22−0.88, p=0.020). 이식 전 감작은 이식 후 1년 시점 이식실패(HR 1.87, 95% CI 0.93−3.78, p=0.118)나 모든 원인에 의한 사망(HR 1.76, 95% CI 0.84−3.69, p=0.118)과 유의미한 연관성을 보이지 않았다. 이식 전 감작은 이식 후 급성 세포성 거부반응과도 유의미한 연관성이 없었다(log−rank p=0.700). 공여자 특이 항체(DSA)를 가진 환자의 경우 이식실패 위험이 유의하게 증가했으며, 대부분의 차이는 이식 후 1개월 이내에 발생하였다. 이식 전 감작 및 DSA+와 장기 이식실패 예후도 1년 시점 분석과 비슷한 결과가 관찰되었다(이식 전 감작 HR 1.42, 95% CI 0.65−3.10, p=0.378; DSA+ HR 2.47, 95% CI 1.16−5.27, p=0.019).

192명의 환자에서 실험실적 혈청분석을 통하여 비HLA 항체 유무를 조사하였다. 39개의 비HLA 항체 중 항비멘틴 항체(AVA) (HR 2.73, 95% CI 1.21−6.16, p=0.016)와 항 collagen II 항체 (ACA) (HR 2.76, 95% CI 1.12−6.81, p=0.027)가 1년 시점 이식실패와 연관성이 있었다. AVA는 98명(51.0%)의 환자에게서 확인되었으며, 남성에서 빈도가 더 높았다 (p=0.042). 이식 전 AVA의 존재는 1년 이식생존의 독

립적인 예측 변수였으나(log-rank p<0.001), 장기 이식편 예후와는 연관성이 없었다(log-rank p=0.120). 다만 DSA+ 환자에서 AVA의 존재는 이식실패의 위험을 추가적으로 높이는 것으로 나타났다(log-rank p = 0.002). 또한 AVA 항체 역가의 상승은 전통적인 위험인자 외에 1년 이식 생존에 대한 예측 모델을 증가시켰다(IDI=11%, p=0.002, NRI=23%, p=0.047). 이식 전 ACA 항체는 1년 이내(log-rank p=0.009) 및 장기(log-rank p<0.001) 이식실패와 연관성을 보였다. AVA-/ACA- 환자들과 비교하여, AVA+/ACA+ 환자들은 1년 이내, 그리고 장기적인 시점에서 이식실패 위험이 높은 것으로 밝혀졌다(log-rank p<0.001).

결론

심장이식을 받는 한국인 환자들을 분석한 결과, 이식 전 감작은 1년 시점 이식실패 및 사망 위험과 유의미한 연관성이 없었다. 그러나 비HLA 항체인 AVA를 활용할 경우 공여자 특이 항체가 존재하는 환자에서 추가로 예후를 분별할 수 있었으며, ACA는 1년 이내 및 장기 이식실패와 연관성이 밝혀졌다. 이식 전 공여자 특이항체와 함께 비HLA 항체 평가를 시행할 경우, 심장이식의 예후를 예측하고, 특정 환자의 면역요법을 조정하는 데 영향을 끼쳐 개인맞춤의학 실현에 도움이 될 것이다.