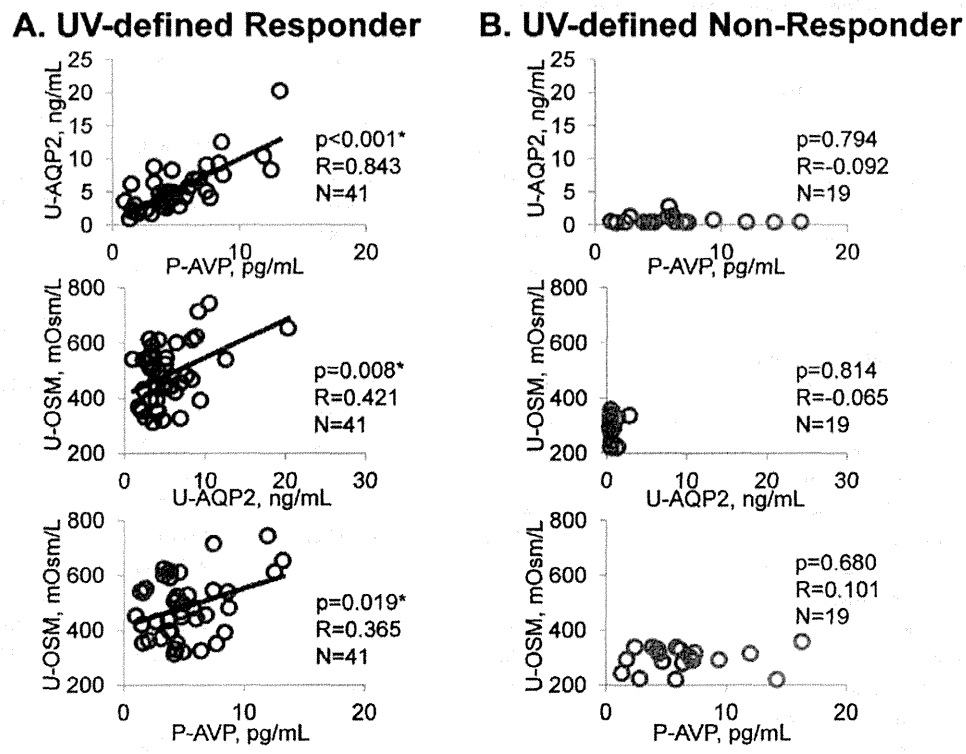

Figure 1. Relationship between urine aquaporin-2 (U-AQP2), urine concentration (U-OSM), and plasma arginine vasopressin (P-AVP) in the (**A**) urine volume (UV)-defined responders and (**B**) UV-defined non-responders to tolvaptan. *P<0.05 (Pearson's product-moment correlation coefficient).

P-AVP and Urine Parameters

In the UV-defined responders, whose UV increased after TLV initiation (from 1,348±360 to 2,082±883 ml/day), baseline P-AVP had a significant correlation with baseline U-AQP2 (average, 5.42±3.54 ng/ml; r=0.843, P<0.001). Higher baseline U-AQP2 was associated with highly concentrated urine (average, 485±110 mOsm/L; r=0.397, P=0.010) in the responders (**Figure 1A**). In the UV-defined non-responders, whose UV remained unchanged after TLV initiation (from 1,293±603 to 1,076±444 ml/day), baseline U-AQP2 remained extremely low (average, 0.76±0.59 ng/ml, P<0.001 vs. responders) regardless of baseline P-AVP level. Baseline U-OSM was low (average 298±43 mOsm/L, P<0.001 vs. responders) along with low U-AQP2 in the non-responders (**Figure 1B**). Consistently, significant correlation between P-AVP and U-OSM was observed only in the UV-defined responders but not in the non-responders. U-AQP2/P-AVP had a positive correlation with %change in UV after TLV initiation (r=0.300, P=0.020; **Figure 2A**), and all UV-defined responders satisfied U-AQP2/P-AVP ≥0.5×10^3, which was calculated on ROC analysis (area under the curve [AUC], 1.000; **Figure 2B**). U-AQP2 by itself had a high AUC for stratifying the UV-defined responders from the non-responders on ROC analysis (AUC, 0.875), but AUC of U-AQP2 was not as good as that of U-AQP2/P-AVP (1.000).

At 4–6 h after TLV initiation, U-AQP2 corrected by urinary concentration of creatinine significantly decreased compared with that of baseline in 5 UV-defined responders (15.0±2.1 vs. 5.6±4.1 μg/g creatinine, P<0.001), whereas the level remained low in 5 UV-defined non-responders (5.6±1.4 vs. 5.3±1.5 μg/g creatinine, P=0.625).

Baseline Characteristics vs. U-AQP2-Defined Response

Considering the aforementioned results, we redefined response to TLV according to U-AQP2/P-AVP level. Patients were then classified as AQP-defined responders with U-AQP2/P-AVP ≥0.5×10^3, or AQP-defined non-responders with U-AQP2/P-AVP <0.5×10^3, regardless of TLV use (**Figure 3**; **Table 2**).

Both the TLV(–) and TLV(+) groups had the same numbers of AQP-defined responders and of non-responders. In both groups, the AQP-defined non-responders were older, and had impaired renal function compared with the AQP-defined responders, but there were no significant differences in background data.

Among the AQP-defined responders, there were no significant differences in patient backgrounds between the TLV(–) and TLV(+) groups. Among the AQP-defined non-responders, the TLV(+) subjects had smaller physique and higher inotrope infusion rate compared with the TLV(–) group.

Baseline HF symptom score was similar in all 4 groups (responder with TLV, responder without TLV, non-responder with TLV, non-responder without TLV). At 1 month after enrollment, HF symptom score was most improved in the AQP-defined responder with TLV treatment.

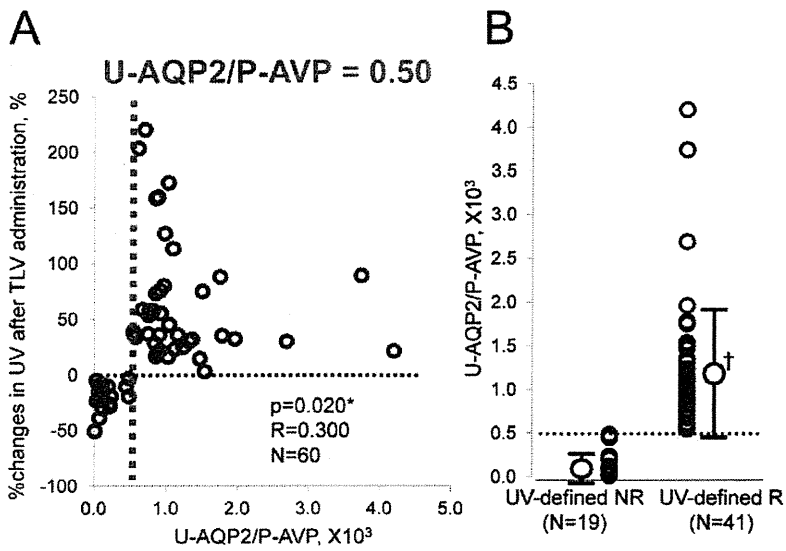

Figure 2. (**A**) Relationship between urine aquaporin-2/plasma arginine vasopressin (U-AQP2/P-AVP) and %change in urine volume (UV) after tolvaptan (TLV) and (**B**) comparison of U-AQP2/P-AVP between the UV-defined responders (R) and the UV-defined non-responders (NR). *P<0.05 (Pearson's product-moment correlation coefficient); †P<0.001 (Mann-Whitney test).

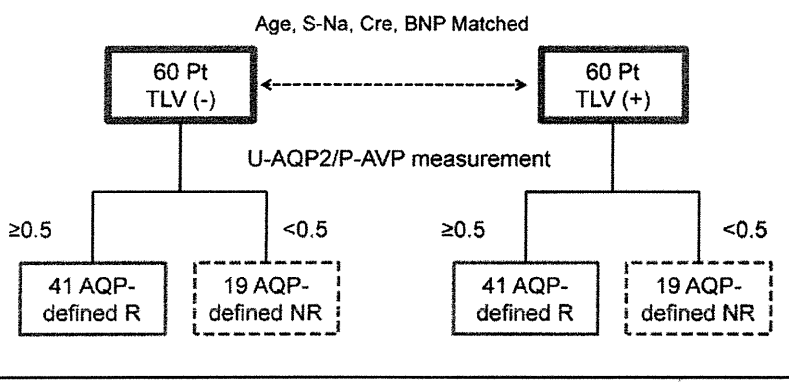

Figure 3. Study flow-chart. AQP, aquaporin; BNP, B-type natriuretic peptide; NR, non-responder; R, responder; TLV, tolvaptan; U-AQP2/P-AVP, urine aquaporin-2/plasma arginine vasopressin.

Risk Factors for AQP-Defined Non-Response at Baseline

On univariate logistic regression analysis higher age, lower dose of β-blocker, higher blood urea nitrogen and creatinine, and higher ejection fraction at baseline were significant predictors for AQP-defined non-response (n=38) in all patients (n=120; **Table 3**). Among them, higher age (≥60 years) and higher serum creatinine (≥1.3 mg/dl) were independent predictors for AQP-defined non-response on multivariate analysis.

Kaplan-Meier Analysis and TLV

TLV was given continuously to all TLV(+) patients until death (n=11) or ventricular assist device implantation (n=17). Survival in patients with TLV was as poor as that in patient without TLV over 2 years (P=0.479; **Figure 4A**), but rate of re-hospitalization due to worsening of HF was significantly reduced by TLV treatment (P=0.002; **Figure 4B**). Survival curves were stratified by U-AQP2/P-AVP level in the TLV(+) group and the propensity-matched TLV(−) group (**Figures 4C,D**). As shown in **Figure 4C**, the AQP-defined responders had a higher survival rate than those without TLV (95% vs. 74%, P=0.034). In contrast, there were no significant differences in 2-year survival among the AQP-defined non-responders regardless of TLV treatment (P=0.272). Readmission-free rate was markedly improved by TLV treatment in the AQP-defined responders (81% vs. 45%, P<0.001), but was not changed in the non-responders (18% vs. 15%, P=0.874). Combined events of death and/or HF re-hospitalization were significantly lower in the AQP-defined responders when they were treated with TLV (24% vs. 57%, P=0.014; **Figure S1**).

Discussion

We have found that all UV-defined responders whose UV increased after TLV initiation, had U-AQP2/P-AVP ≥0.5×10³. The AQP-defined responders who had U-AQP2/P-AVP ≥0.5 had better survival after 2-year TLV treatment over the propensity-matched AQP-defined responders without TLV treatment.

Table 2. Baseline Characteristics vs. AQP-Defined Response

	TLV(−)		P-value (R vs. NR)	TLV(+)				P-value (R vs. NR)
	R (n=41)	NR (n=19)		R (n=41)	P-value (vs. TLV(−))	NR (n=19)	P-value (vs. TLV(−))	
Demographic parameters								
Age (years)	49.7±18.4	66.9±13.6	0.001*	49.6±18.4	0.995	66.0±19.1	0.869	0.002*
Male	35 (85.4)	17 (89.5)	0.663	29 (70.7)	0.109	12 (63.2)	0.062	0.557
Body weight (kg)	57.8±11.4	58.7±9.1	0.756	58.0±12.7	0.942	52.7±9.7	0.059	0.118
BSA (m^2)	1.70±0.18	1.71±0.15	0.883	1.69±0.19	0.924	1.61±0.18	0.027*	0.074
Etiology of ischemia	7 (1.7)	2 (10.5)	0.509	6 (14.6)	0.762	3 (15.8)	0.500	0.907
SBP (mmHg)	101.7±13.7	104.7±10.5	0.350	98.6±13.0	0.165	96.7±11.3	0.058	0.585
DBP (mmHg)	65.4±9.1	65.0±5.5	0.796	64.2±8.9	0.541	62.6±6.4	0.237	0.489
HR (beats/min)	77.8±17.2	74.2±16.2	0.550	84.8±17.5	0.066	79.3±11.7	0.228	0.151
Concomitant medication								
TLV (mg daily)	–	–	–	4.8±3.0	–	4.9±3.5	–	0.842
Furosemide (mg daily)	45.7±17.8	51.7±18.9	0.082	54.4±26.5	0.059	53.8±46.7	0.993	0.950
Aldosterone antagonist (mg daily)	24.5±19.7	25.0±22.0	0.925	34.2±21.5	0.061	27.6±27.5	0.189	0.322
Trichlormethiazide (mg daily)	0.1±0.3	0.2±0.5	0.233	0.3±1.0	0.091	0.2±0.5	0.881	0.555
β-blocker (mg daily)	7.2±6.3	4.9±3.2	0.074	7.7±7.8	0.080	5.6±5.9	0.899	0.129
ACEI/ARB (mg daily)	3.5±3.6	3.6±2.7	0.930	2.6±1.8	0.141	3.1±2.6	0.550	0.463
Furosemide	38 (92.7)	19 (100)	0.312	41 (100)	0.120	19 (100)	–	–
Aldosterone antagonist	29 (70.7)	12 (63.2)	0.557	35 (85.4)	0.109	13 (68.4)	0.890	0.127
Trichlormethiazide	1 (2.4)	2 (10.5)	0.233	5 (12.2)	0.101	2 (10.5)	0.698	0.611
β-blocker	40 (97.6)	17 (89.5)	0.233	37 (90.2)	0.180	16 (84.2)	0.500	0.498
ACEI/ARB	36 (87.8)	17 (89.5)	0.611	35 (85.4)	0.746	14 (73.7)	0.202	0.277
I.v. inotropes	18 (43.9)	4 (21.0)	0.088	25 (61.0)	0.122	10 (52.6)	0.044*	0.542
Laboratory parameters								
Plasma AVP (pg/ml)	4.9±3.9	7.5±8.1	0.067	5.1±2.9	0.714	8.1±9.3	0.901	0.177
Hemoglobin (g/dl)	11.6±2.7	11.4±2.2	0.824	12.2±2.1	0.255	11.0±2.0	0.594	0.053
Platelets ($\times10^3/\mu l$)	19.8±9.1	19.2±8.8	0.822	18.8±5.8	0.562	18.5±7.8	0.789	0.866
Serum albumin (g/dl)	3.4±0.7	3.3±0.5	0.515	3.6±0.6	0.175	3.4±0.7	0.713	0.064
Serum sodium (mEq/L)	135.9±4.3	135.3±4.2	0.636	133.7±5.8	0.054	133.4±6.4	0.286	0.875
Serum potassium (mEq/L)	4.2±0.5	4.4±0.6	0.156	4.3±0.5	0.691	4.2±0.4	0.152	0.538
Serum BUN (mg/dl)	22.7±8.7	34.3±18.2	0.002*	27.4±12.7	0.068	38.5±16.5	0.692	0.006*
Serum creatinine (mg/dl)	1.1±0.4	2.0±0.9	<0.001*	1.2±0.5	0.066	1.7±0.7	0.188	0.009*
Serum total bilirubin (mg/dl)	1.2±0.7	0.9±0.5	0.121	1.6±1.2	0.094	1.3±1.2	0.309	0.363
Serum AST (IU/L)	38.3±44.0	24.2±7.5	0.076	29.0±13.7	0.074	31.2±22.3	0.067	0.641
Serum ALT (IU/L)	35.3±44.0	24.2±15.4	0.069	28.4±23.9	0.102	23.1±18.9	0.490	0.395
Plasma BNP ($\log_{10}$ pg/ml)	2.64±0.46	2.81±0.43	0.072	2.69±0.43	0.246	2.66±0.40	0.072	0.763
Urine AQP2 (ng/ml)	4.87±4.38	1.63±2.15	<0.001*	5.42±3.54	0.533	0.76±0.59	0.103	<0.001*
Urine AQP2/Plasma AVP ($\times10^3$)	1.77±2.46	0.22±0.13	<0.001*	1.21±0.76	0.165	0.17±0.15	0.205	<0.001*
Echocardiographic parameters								
LV diastolic diameter (mm)	60.6±14.0	60.4±11.4	0.970	63.9±14.4	0.294	58.3±18.6	0.669	0.206
LV systolic diameter (mm)	51.9±15.8	48.4±15.9	0.429	55.7±15.6	0.283	46.7±19.7	0.766	0.061
Ejection fraction (%)	31.2±16.6	39.5±22.7	0.164	30.2±17.1	0.285	36.8±20.6	0.847	0.064
Ejection fraction ≥50%	6 (14.6)	5 (26.3)	0.277	5 (12.2)	0.723	6 (31.6)	0.512	0.098
Cardiac index ($L\cdot min^{-1}\cdot m^{-2}$)	2.2±0.5	2.2±0.5	0.740	2.1±0.4	0.766	2.0±0.3	0.111	0.142
HF symptom score (before)	6.2±1.0	6.3±1.1	0.740	6.1±1.1	0.512	6.0±1.1	0.213	0.814
HF symptom score (after 1 month)	5.6±1.1	5.7±1.2	0.784	4.8±0.8	0.036*	5.8±1.3	0.618	0.018*

Data given as mean±SD or n (%). *P<0.05 (Tukey test when ANOVA was significant).
NR, AQP-defined non-responder; R, AQP-defined responder. Other abbreviations as in Table 1.

Measurement of U-AQP2

AQP2 is the recently characterized AVP-regulated water channel protein, and its shuttle trafficking in principal cells determines the water permeability of the apical membrane and then dominates urine-concentrating ability.[22,23] Secreted AVP binds to the V_2 receptor, which is located at the basolateral membrane of principal cells in the collecting duct. Activation of the V_2 receptor triggers trafficking of AQP2 from intracellular storage vesicles to the apical membrane by way of cAMP-dependent phosphorylation of the AQP2 protein.[24] Approxi-

Table 3. Predictors of U-AQP2/P-AVP <0.5 at Baseline

	AQP-defined R (n=82)	AQP-defined NR (n=38)	Univariate analysis		Multivariate analysis	
			P-value	OR	P-value	OR
Demographic parameters						
Age (years)	49.7±18.3	66.5±16.4	<0.001*	1.056		
Age ≥60 years	24 (29.3)	28 (73.7)	<0.001*	6.757	0.004*	4.425
Male	64 (78.0)	29 (76.3)	0.833	0.907		
Body weight (kg)	57.9±12.0	55.7±9.7	0.335	0.983		
BSA (m^2)	1.70±0.19	1.65±0.18	0.140	0.194		
Etiology of ischemia	13 (15.9)	5 (13.2)	0.701	0.805		
SBP (mmHg)	99.7±13.4	100.2±11.7	0.822	1.003		
DBP (mmHg)	64.9±9.0	63.8±6.0	0.513	0.984		
HR (beats/min)	81.3±17.4	76.8±12.9	0.157	0.982		
Concomitant medication						
Furosemide (mg daily)	48.5±23.2	53.7±35.2	0.339	1.007		
Spironolactone (mg daily)	29.3±21.0	26.3±24.6	0.491	0.994		
Trichlormethiazide (mg daily)	0.2±0.7	0.2±0.5	0.938	1.022		
β-blocker (mg daily)	7.5±7.1	4.5±4.7	0.026*	0.912		
β-blocker ≤8.0 mg	46 (56.1)	30 (78.9)	0.018*	2.933	0.367	1.645
ACEI/ARB (mg daily)	3.1±2.9	3.4±2.7	0.598	1.036		
Furosemide	79 (96.3)	38 (100)	–	–		
Spironolactone	64 (78.0)	25 (65.8)	0.156	0.541		
Trichlormethiazide	6 (7.3)	4 (10.5)	0.556	1.490		
β-blocker	77 (93.9)	33 (86.8)	0.203	0.429		
ACEI/ARB	71 (86.6)	31 (81.6)	0.477	0.686		
Catecholamine infusion	43 (52.4)	14 (36.8)	0.114	0.529		
Laboratory parameters						
Hemoglobin (g/dl)	11.9±2.4	11.2±2.1	0.159	0.881		
Platelets ($\times 10^3/\mu l$)	19.3±7.6	18.9±8.2	0.774	0.993		
Serum albumin (g/dl)	3.5±0.7	3.3±0.6	0.067	0.565		
Serum sodium (mEq/L)	134.8±5.2	134.4±5.4	0.687	0.985		
Serum potassium (mEq/L)	4.3±0.5	4.3±0.5	0.487	1.326		
Serum BUN (mg/dl)	24.1±11.3	37.4±17.2	<0.001*	1.070		
Serum creatinine (mg/dl)	1.1±0.4	1.8±0.8	<0.001*	6.897		
Serum creatinine ≥1.3 mg/dl	17 (20.7)	27 (71.1)	<0.001*	9.346	<0.001*	7.634
Serum total bilirubin (mg/dl)	1.4±1.0	1.1±0.9	0.393	1.026		
Serum AST (IU/L)	35.9±29.1	25.3±17.4	0.070	0.970		
Serum ALT (IU/L)	34.9±35.8	21.1±17.1	0.074	0.975		
Plasma BNP ($\log_{10}$ pg/ml)	2.63±0.46	2.78±0.43	0.096	2.212		
Echocardiographic parameters						
LV diastolic diameter (mm)	62.2±14.3	59.3±15.3	0.315	0.986		
LV systolic diameter (mm)	53.8±15.8	47.6±17.7	0.058	0.976		
Ejection fraction (%)	29.2±16.9	40.2±21.4	0.008*	1.030		
Ejection fraction ≥26%	42 (51.2)	28 (73.7)	0.022*	2.667	0.452	1.511
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.2±0.4	2.1±0.4	0.580	0.771		

Data given as mean±SD or n (%). *P<0.05 (logistic regression).
AQP, aquaporin; NR, non-responder; OR, odds ratio; P-AVP, plasma arginine vasopressin; R, responder; U-AQP2, urine aquaporin-2. Other abbreviations as in Table 1.

mately 3% of AQP2 in the kidney is excreted daily in urine,[15] but only phosphorylated and translocated AQP2 has a chance for excretion into urine because unphosphorylated AQP2 remains in the cytoplasm. Therefore, U-AQP2 is considered as an index of AVP and V_2 receptor activation in the collecting duct.[25]

U-AQP2 level is increased in the situation of elevated P-AVP, including dehydration, HF, and syndrome of inappropriate secretion of anti-diuretic hormone.[26–28] Lower U-AQP2 is associated with decreased AVP action, including hydration or central/nephrogenic diabetes insipidus (NDI).[26] There have been no studies, however, discussing the relationship between U-AQP2 and responsiveness to TLV before the present study.

U-AQP2 in UV-Defined Responders/Non-Responders to TLV

We previously reported that the preserved potential of the collecting duct is essential for responsiveness to TLV other than hemodynamics or electrolyte, and most of the UV-defined

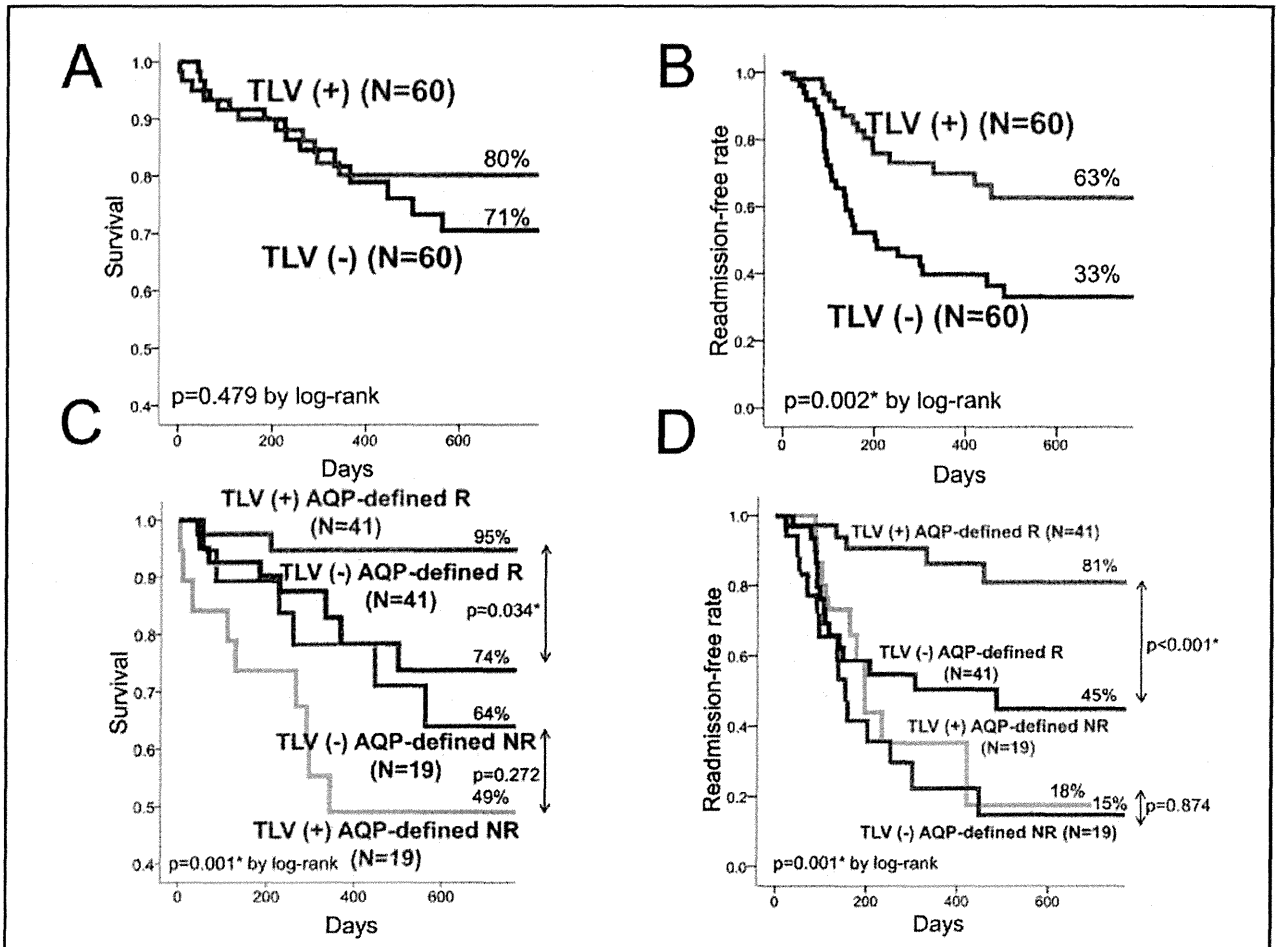

Figure 4. (**A**) Two-year survival and (**B**) readmission-free rate between tolvaptan (TLV)-treated patients and the propensity-matched patients without TLV treatment. (**C**) Two-year survival and (**D**) readmission-free rate stratified by urine aquaporin-2/plasma arginine vasopressin level. AQP, aquaporin; NR, non-responder; R, responder. *P<0.05 (log-rank test).

responders had baseline U-OSM >350 mOsm/L, indicating preserved urine-concentrating ability.[13] Baseline P-AVP had a significant correlation with baseline U-AQP2, and higher U-AQP2 was associated with highly concentrated urine in the UV-defined responders (**Figure 1A**). In normal subjects, Kanno et al found an increase in U-AQP2 by AVP stimulation, and Rai et al noted a positive correlation between U-AQP2 and U-OSM.[15,26]

In contrast, baseline U-AQP2 was low regardless of baseline P-AVP level, and U-OSM remained low along with low baseline U-AQP2 in the UV-defined non-responders (**Figure 1B**), which may result from the deterioration of collecting duct function. The results were consistent with our previous hypothesis that the unresponsiveness to TLV was attributable to a similar pathogenesis to NDI.[13] Elder patients with chronic kidney disease (CKD) have a similar physiology to that of acquired NDI in general, given that they lose urine-concentrating ability.[29] We observed that the UV-defined non-responders had higher age and worse renal dysfunction compared with the UV-defined responders. AQP2 and V_2 receptor expression were found to be downregulated in CKD, although detailed mechanisms leading to attenuated expression are yet to be determined.[30,31]

U-AQP2 decreased after TLV in the UV-defined responders, which had been previously reported by Martin et al, and decreases of U-AQP2 excretion indicated V_2 receptor antagonistic effects by TLV.[32] We also observed unchanged U-AQP2 after TLV initiation in the UV-defined non-responders, which could translate into clinical unresponsiveness to TLV.

U-AQP2/P-AVP as a Novel Predictor of Responsiveness to TLV

U-AQP2 level is basically dependent on P-AVP level when downstream pathways from V_2 receptors are intact. Therefore, we used U-AQP2/P-AVP to assess the viability of the collecting duct, and found excellent predictability for the responsiveness to TLV at a cut-off of 0.5×10^3 (**Figure 2**). U-Cre was also significantly correlated with U-APQ2 in UV-defined responders (P=0.001 and r=0.462) because U-Cre is one of the major osmotic components in urine. We selected P-AVP2 for the correction of U-AQP2, however, given its stronger correlation (P<0.001 and r=0.843). In contrast, U-Cre was used for the normalization of U-AQP2 before and after TLV treatment, because P-AVP was no longer a good parameter after TLV

treatment.

We previously reported the criteria to predict responsiveness to TLV, which consisted of (1) higher baseline U-OSM; and (2) sufficient decrease of U-OSM at 4–6 h after TLV initiation,[13] but responsiveness could not be determined until 4 h after TLV initiation using these criteria. In contrast, use of U-AQP2/P-AVP enabled the stratification of HF patients into AQP-defined responders and AQP-defined non-responders before TLV treatment (**Figure 3**). Moreover, U-AQP2 was a more straightforward marker than U-OSM, reflecting the biological activity of the collecting duct. Logistic regression analysis showed that higher age and higher serum creatinine were independent predictors of AQP-defined non-response. The result was again consistent with our hypothesis that unresponsiveness to TLV was attributable to the impaired activity of the collecting duct, which is usually accompanied by advanced CKD and/or aging.

Improved Survival in AQP-Defined Responders After TLV

Previous authors including us reported that TLV could improve clinical parameters without apparent adverse events during the short-term study period.[5–11] The EVEREST study, however, did not find a survival advantage of long-term TLV treatment.[33] As shown in **Figure 4A**, TLV treatment was not associated with better survival rate in the overall group, and the results appeared to be consistent with the EVEREST study.

Is it true that TLV does not improve survival in patients with HF? A sub-analysis of the EVEREST study showed that patients with severe hyponatremia (<130 mEq/L) were associated with reduced cardiovascular morbidity and mortality on long-term TLV treatment.[34] The ACTIVE in CHF trial showed that 60-day mortality was lower in TLV-treated patients with renal dysfunction or severe systemic congestion compared to placebo.[35] These results have indicated that TLV may have a potential to improve patient prognosis when participants are adequately selected.

Among the AQP-defined responders, TLV treatment was accompanied by better survival after 2-year follow-up compared with the propensity-matched TLV(–) patients (**Figure 4C**). In contrast, TLV did not improve patient survival in the AQP-defined non-responders. Our novel definition for optimal patient selection may uncover sufficient effects of TLV for the improvement of prognosis. Although the potential mechanism for improvement of prognosis by TLV treatment is unknown, the resolution of congestion by TLV may translate into better survival with significant recovery of HF symptoms in the AQP-defined responders. More aggressive control of congestion by TLV may result in reduced re-hospitalization due to worsening of HF, as was observed in the TLV-treated responders.

We acknowledge that our study has several limitations. First, the present study was conducted retrospectively in a single center, and consequently included a limited number of patients. Although the propensity-matching score analysis was performed to recruit background-matched patients as a control group, a prospective randomized trial should be performed among the AQP-defined responders. Second, the initial dose of TLV was determined by attending physicians considering the stability of patient hemodynamics and degree of congestion, although the initial dose of TLV did not affect responsiveness to TLV in this study. Third, the present study had patient selection bias because TLV treatment was determined by attending physicians. Although insignificant, patients in the TLV(+) group took a relatively higher dose of diuretics than the TLV(–) group, probably because TLV was preferentially indicated in those who were refractory to conventional diuretics. And fourth, U-AQP2 at 4–6 h after TLV was measured among only 10 patients in the present study. A future study with a larger number of subjects would clarify the time course of U-AQP2 after TLV treatment.

Conclusions

Response to TLV can be predicted beforehand by the novel index, U-AQP2/P-AVP. AQP-defined responders with U-AQP2/P-AVP $\geq 0.5\times10^3$ had a better prognosis during 2-year TLV treatment.

Acknowledgments

Grant-in-Aid from Fukuda Foundation for medical Technology to K.K. Also, K.K. has a potential conflict of interest with Otsuka Pharmaceutical, which consists of lecture fees and endowment of the Department belonged to.

References

1. Uretsky BF, Verbalis JG, Generalovich T, Valdes A, Reddy PS. Plasma vasopressin response to osmotic and hemodynamic stimuli in heart failure. *Am J Physiol* 1985; **248**(3 Pt 2): H396–H402.
2. Lanfear DE, Sabbah HN, Goldsmith SR, Greene SJ, Ambrosy AP, Fought AJ, et al. Association of arginine vasopressin levels with outcomes and the effect of V2 blockade in patients hospitalized for heart failure with reduced ejection fraction: Insights from the EVEREST trial. *Circ Heart Fail* 2013; **6:** 47–52.
3. Goldsmith SR. Vasopressin as vasopressor. *Am J Med* 1987; **82:** 1213–1219.
4. Nielsen S, Kwon TH, Christensen BM, Promeneur D, Frokiaer J, Marples D. Physiology and pathophysiology of renal aquaporins. *J Am Soc Nephrol* 1999; **10:** 647–663.
5. Imamura T, Kinugawa K, Minatsuki S, Muraoka H, Kato N, Inaba T, et al. Tolvaptan can improve clinical course in responders. *Int Heart J* 2013; **54:** 377–381.
6. Kinugawa K, Imamura T, Komuro I. Experience of a vasopressin receptor antagonist, tolvaptan, under the unique indication in Japanese heart failure patients. *Clin Pharmacol Ther* 2013; **94:** 449–451.
7. Costello-Boerrigter LC, Smith WB, Boerrigter G, Ouyang J, Zimmer CA, Orlandi C, et al. Vasopressin-2-receptor antagonism augments water excretion without changes in renal hemodynamics or sodium and potassium excretion in human heart failure. *Am J Physiol Renal Physiol* 2006; **290:** F273–F278.
8. Schrier RW, Gross P, Gheorghiade M, Berl T, Verbalis JG, Czerwiec FS, et al. Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. *N Engl J Med* 2006; **355:** 2099–2112.
9. Udelson JE, Orlandi C, Ouyang J, Krasa H, Zimmer CA, Frivold G, et al. Acute hemodynamic effects of tolvaptan, a vasopressin V2 receptor blocker, in patients with symptomatic heart failure and systolic dysfunction: An international, multicenter, randomized, placebo-controlled trial. *J Am Coll Cardiol* 2008; **52:** 1540–1545.
10. Berl T, Quittnat-Pelletier F, Verbalis JG, Schrier RW, Bichet DG, Ouyang J, et al. Oral tolvaptan is safe and effective in chronic hyponatremia. *J Am Soc Nephrol* 2010; **21:** 705–712.
11. Matsuzaki M, Hori M, Izumi T, Fukunami M. Efficacy and safety of tolvaptan in heart failure patients with volume overload despite the standard treatment with conventional diuretics: A phase III, randomized, double-blind, placebo-controlled study (QUEST study). *Cardiovasc Drugs Ther* 2011; **25** (Suppl 1): S33–S45.
12. Kinugawa K, Sato N, Inomata T, Shimakawa T, Iwatake N, Mizuguchi K. Efficacy and safety of tolvaptan in heart failure patients with volume overload. *Circ J* 2014; **78:** 844–852.
13. Imamura T, Kinugawa K, Shiga T, Kato N, Muraoka H, Minatsuki S, et al. Novel criteria of urine osmolality effectively predict response to tolvaptan in decompensated heart failure patients: Association between non-responders and chronic kidney disease. *Circ J* 2013; **77:** 397–404.
14. Imamura T, Kinugawa K, Minatsuki S, Muraoka H, Kato N, Inaba T, et al. Urine osmolality estimated using urine urea nitrogen, sodium and creatinine can effectively predict response to tolvaptan in decompensated heart failure patients. *Circ J* 2013; **77:** 1208–1213.
15. Rai T, Sekine K, Kanno K, Hata K, Miura M, Mizushima A, et al. Urinary excretion of aquaporin-2 water channel protein in human and rat. *J Am Soc Nephrol* 1997; **8:** 1357–1362.
16. Kinugawa K. How to treat stage D heart failure? When to implant

left ventricular assist devices in the era of continuous flow pumps? *Circ J* 2011; **75:** 2038–2045.
17. Umenishi F, Summer SN, Cadnapaphornchai M, Schrier RW. Comparison of three methods to quantify urinary aquaporin-2 protein. *Kidney Int* 2002; **62:** 2288–2293.
18. Sasaki S, Ohmoto Y, Mori T, Iwata F, Muraguchi M. Daily variance of urinary excretion of AQP2 determined by sandwich ELISA method. *Clin Exp Nephrol* 2012; **16:** 406–410.
19. Garin O, Ferrer M, Pont A, Rue M, Kotzeva A, Wiklund I, et al. Disease-specific health-related quality of life questionnaires for heart failure: A systematic review with meta-analyses. *Qual Life Res* 2009; **18:** 71–85.
20. Kato N, Kinugawa K, Seki S, Shiga T, Hatano M, Yao A, et al. Quality of life as an independent predictor for cardiac events and death in patients with heart failure. *Circ J* 2011; **75:** 1661–1669.
21. Joffe MM, Rosenbaum PR. Invited commentary: Propensity scores. *Am J Epidemiol* 1999; **150:** 327–333.
22. Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S. Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* 1993; **361:** 549–552.
23. Sasaki S, Fushimi K, Saito H, Saito F, Uchida S, Ishibashi K, et al. Cloning, characterization, and chromosomal mapping of human aquaporin of collecting duct. *J Clin Invest* 1994; **93:** 1250–1256. Erratum in: *J Clin Invest* 1994; **94:** following 216.
24. Radin MJ, Yu MJ, Stoedkilde L, Miller RL, Hoffert JD, Frokiaer J, et al. Aquaporin-2 regulation in health and disease. *Vet Clin Pathol* 2012; **41:** 455–470.
25. Elliot S, Goldsmith P, Knepper M, Haughey M, Olson B. Urinary excretion of aquaporin-2 in humans: A potential marker of collecting duct responsiveness to vasopressin. *J Am Soc Nephrol* 1996; **7:** 403–409.
26. Kanno K, Sasaki S, Hirata Y, Ishikawa S, Fushimi K, Nakanishi S, et al. Urinary excretion of aquaporin-2 in patients with diabetes insipidus. *N Engl J Med* 1995; **332:** 1540–1545.
27. Ishikawa Se, Saito T, Fukagawa A, Higashiyama M, Nakamura T, Kusaka I, et al. Close association of urinary excretion of aquaporin-2 with appropriate and inappropriate arginine vasopressin-dependent antidiuresis in hyponatremia in elderly subjects. *J Clin Endocrinol Metab* 2001; **86:** 1665–1671.
28. Funayama H, Nakamura T, Saito T, Yoshimura A, Saito M, Kawakami M, et al. Urinary excretion of aquaporin-2 water channel exaggerated dependent upon vasopressin in congestive heart failure. *Kidney Int* 2004; **66:** 1387–1392.
29. Kleeman CR, Adams DA, Maxwell MH. An evaluation of maximal water diuresis in chronic renal disease. I. Normal solute intake. *J Lab Clin Med* 1961; **58:** 169–184.
30. Teitelbaum I, Mcguinness S. Vasopressin resistance in chronic renal failure: Evidence for the role of decreased V-2 receptor messenger-RNA. *J Clin Invest* 1995; **96:** 378–385.
31. Bedford JJ, Leader JP, Walker RJ. Aquaporin expression in normal human kidney and in renal disease. *J Am Soc Nephrol* 2003; **14:** 2581–2587.
32. Martin PY, Abraham WT, Lieming X, Olson BR, Oren RM, Ohara M, et al. Selective V2-receptor vasopressin antagonism decreases urinary aquaporin-2 excretion in patients with chronic heart failure. *J Am Soc Nephrol* 1999; **10:** 2165–2170.
33. Konstam MA, Gheorghiade M, Burnett JC Jr, Grinfeld L, Maggioni AP, Swedberg K, et al. Effects of oral tolvaptan in patients hospitalized for worsening heart failure: The EVEREST Outcome Trial. *JAMA* 2007; **297:** 1319–1331.
34. Hauptman PJ, Burnett J, Gheorghiade M, Grinfeld L, Konstam MA, Kostic D, et al. Clinical course of patients with hyponatremia and decompensated systolic heart failure and the effect of vasopressin receptor antagonism with tolvaptan. *J Card Fail* 2013; **19:** 390–397.
35. Gheorghiade M, Gattis WA, O'Connor CM, Adams KF Jr, Elkayam U, Barbagelata A, et al. Effects of tolvaptan, a vasopressin antagonist, in patients hospitalized with worsening heart failure: A randomized controlled trial. *JAMA* 2004; **291:** 1963–1971.

Supplementary Files

Supplementary File 1

Figure S1. Composite endpoints of death and/or heart failure rehospitalization during 2 years vs. (A) use of tolvaptan (TLV) and (B) urine aquaporin-2/plasma arginine vasopressin.

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-14-0244

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Heart Failure

Status 2 Patients Had Poor Prognosis Without Mechanical Circulatory Support

– Indications for Device Implantation –

Teruhiko Imamura, MD; Koichiro Kinugawa, MD, PhD; Masaru Hatano, MD; Takeo Fujino, MD, PhD; Toshiro Inaba, MD; Hisataka Maki, MD, PhD; Osamu Kinoshita, MD, PhD; Eisuke Amiya, MD, PhD; Kan Nawata, MD, PhD; Atsushi Yao, MD, PhD; Shunei Kyo, MD, PhD; Minoru Ono, MD, PhD; Issei Komuro, MD, PhD

Background: Indication for mechanical circulatory support (MCS) has been a matter of debate in less sick status 2 patients.

Methods and Results: Data were obtained from 183 consecutive patients assigned to stage D heart failure (HF) who were evaluated by the institutional review board of the University of Tokyo Hospital and then listed for heart transplantation as status 1 or 2 of the Japan Organ Transplant Network. Patients with status 2 (n=38) had a prognosis as poor as those dependent on inotropes (n=54) or MCS (n=91; P=0.615, log-rank test), and only 4 of them had eventual ventricular assist device (VAD) implantation (10.5%). Patients who eventually received VAD (n=92) had better 4-year survival than those without MCS among status 1 and 2 (P=0.030, log-rank test). On Cox regression analysis plasma B-type natriuretic peptide (BNP) >740 pg/ml was the only significant predictor for 4-year survival among the status 2 group (P=0.014; hazard ratio, 8.267). Ten patients with status 2 died: 6 due to acute hemodynamic compromise and 4 due to ventricular fibrillation.

Conclusions: Prognosis in status 2 patients was as poor as that of those dependent on inotrope infusion or VAD, mostly because of out-of-hospital sudden death without MCS. Status 2 patients with considerably high plasma BNP may be good candidates for continuous flow VAD therapy. (*Circ J* 2014; **78:** 1396–1404)

Key Words: Heart failure; Heart transplantation; INTERMACS

Survival in patients with stage D heart failure (HF) has remained unsatisfactory in the era of guideline-directed optimal medical therapy consisting of β-blockers, angiotensin-converting enzyme inhibitors (ACEI), aldosterone antagonists, and cardiac resynchronization therapy with or without defibrillators (CRT-D).[1] Although heart transplantation (HTx) is the ultimate solution for such refractory patients, approximately 90% of Japanese recipients eventually require implantation of ventricular assist device (VAD) for bridge to HTx (BTT) because of the long waiting period due to severe donor shortage.[2]

The current Japanese reimbursement system requires the approval of the institutional review board for the eligibility of HTx and successive HTx listings on the Japan Organ Transplant (JOT) Network prior to continuous flow (CF) VAD implantation.[3] In Japan, extracorporeal (EC) VAD had been widely used as the only durable device until CF LVAD became available in 2011, and because of its EC nature, EC VAD was usually implanted under unstable hemodynamics. Nowadays EC VAD is still implanted in patients with cardiogenic shock as bridge to decision, and such patients may be listed for HTx after confirming eligibility later.[4] Currently, EC VAD is also indicated for patients with small body surface area. HTx recipients listed on JOT Network are classified into 2 groups according to patient condition, that is, (1) 'status 1' for patients dependent on mechanical support including VAD or i.v. infusion of inotropes, equivalent to INTERMACS profile 1–3 or the United Network for Organ Sharing (UNOS)

Received January 20, 2014; revised manuscript received February 22, 2014; accepted February 26, 2014; released online April 3, 2014
Time for primary review: 28 days

Department of Cardiovascular Medicine (T. Imamura, M.H., T.F., T. Inaba, H.M., E.A., A.Y., I.K.), Department of Therapeutic Strategy for Heart Failure (K.K., S.K.), and Department of Thoracic Surgery (O.K., K.N., M.O.), Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Mailing address: Koichiro Kinugawa, MD, PhD, Department of Therapeutic Strategy for Heart Failure, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: kinugawa-tky@umin.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-14-0077

Table 1. Patient Characteristics

	Total (n=183)	Status 2 (n=38)	Status 1 VAD (–) (n=54)	P-value vs. Status 2	Status 1 VAD (+) (n=91)	P-value vs. Status 2
Demographic parameters						
Age (years)	40.0±14.4	38.8±17.7	40.7±14.1	0.728	40.1±13.3	0.895
Male	135 (74.2)	26 (68.4)	46 (85.2)	0.075	63 (69.2)	0.075
Body surface area (m^2)	1.56±0.27	1.53±0.38	1.58±0.25	0.132	1.57±0.22	0.098
Etiology of ischemia	27 (14.8)	4 (10.5)	5 (9.3)	0.412	18 (19.8)	0.024†
SBP (mmHg)	90.5±11.1	87.6±9.7	86.0±8.2	0.760	94.4±11.8	0.003*
Heart rate (beats/min)	83.4±14.8	82.7±14.5	84.2±17.1	0.885	83.1±13.6	0.990
History of NSVT	65 (35.7)	13 (34.2)	27 (50.0)	0.026†	25 (27.4)	0.423
Etiology						
DCM	114 (62.3)	22 (57.9)	39 (72.2)	–	53 (58.2)	–
ICM	29 (15.8)	4 (10.6)	5 (9.3)	–	20 (22.0)	–
ACHD	3 (1.6)	2 (5.3)	0 (0)	–	1 (1.1)	–
dHCM	14 (7.7)	4 (10.6)	6 (11.4)	–	4 (4.4)	–
Secondary cardiomyopathy	5 (2.7)	2 (5.3)	1 (1.9)	–	2 (2.2)	–
Cardiac sarcoidosis	3 (1.6)	2 (5.3)	1 (1.9)	–	0 (0)	–
RCM	3 (1.6)	2 (5.3)	0 (0)	–	1 (1.1)	–
Myocarditis	12 (6.6)	0 (0)	2 (3.8)	–	10 (11.0)	–
Concomitant treatment						
Furosemide (mg daily)	39.1±33.7	47.4±32.0	59.6±36.7	0.128	23.3±23.3	<0.001*
Furosemide	150 (82.0)	37 (97.4)	52 (96.3)	0.945	61 (67.0)	<0.001†
Aldosterone antagonist (mg daily)	33.3±21.7	37.2±23.3	31.3±20.9	0.405	32.9±21.6	0.571
Aldosterone antagonist	157 (85.8)	36 (94.7)	46 (85.2)	0.734	75 (82.4)	0.834
β-blocker (mg daily)	11.9±11.8	10.7±7.1	7.4±5.8	0.354	15.1±14.8	0.111
β-blocker	172 (94.0)	36 (94.7)	51 (94.4)	0.998	85 (93.4)	0.984
ACEI/ARB (mg daily)	3.0±2.8	3.5±3.1	3.4±3.0	0.986	2.6±2.6	0.231
ACEI/ARB	153 (83.6)	35 (92.1)	50 (92.6)	0.945	68 (74.7)	0.142
CRT-D	77 (42.3)	22 (57.9)	34 (63.0)	0.634	21 (23.1)	<0.001†
Laboratory parameters						
White blood cells ($\times10^3/\mu l$)	6.3±1.7	5.9±1.5	6.3±1.5	0.564	6.4±1.9	0.280
Hemoglobin (g/dl)	11.8±2.0	12.9±1.9	12.3±2.1	0.281	11.0±1.6	<0.001*
Platelets ($\times10^3/\mu l$)	22.4±9.1	21.5±7.1	22.0±8.7	0.954	23.1±10.0	0.641
Serum sodium (mEq/L)	136.2±4.5	136.0±5.3	134.4±4.1	0.187	137.4±4.0	0.209
Serum potassium (mEq/L)	4.2±0.4	4.2±0.4	4.3±0.4	0.837	4.2±0.3	1.000
Serum BUN (mg/dl)	23.1±4.3	23.1±4.3	25.1±5.8	0.096	22.2±4.0	0.001*
Serum creatinine (mg/dl)	0.9±0.4	0.9±0.4	1.0±0.4	0.275	0.8±0.3	0.060
Serum albumin (g/dl)	3.8±0.5	4.0±0.6	3.8±0.5	0.150	3.6±0.5	<0.001*
Serum GOT (IU/L)	28.7±12.6	29.1±12.3	27.1±12.3	0.727	29.4±12.9	0.990
Serum GPT (IU/L)	26.3±17.2	27.3±18.1	27.1±18.1	0.964	24.8±15.1	0.746
Serum LDH (IU/L)	335.8±186.3	260.9±165.1	252.0±83.2	0.966	417.6±204.2	<0.001*
Serum total bilirubin (mg/dl)	1.1±0.8	1.1±0.6	1.3±0.7	0.464	1.1±0.9	0.985
Plasma BNP ($\log_{10}$pg/ml)	2.83±3.03	2.90±2.81	3.04±3.20	0.353	2.58±2.77	0.099
Echocardiographic parameters						
LV diastolic diameter (mm)	62.2±17.0	65.3±15.5	71.4±15.5	0.164	55.5±16.7	0.003*
LV systolic diameter (mm)	55.3±16.8	57.6±15.9	64.5±16.7	0.087	48.8±16.1	0.011*
IVSD (mm)	7.8±1.7	7.6±1.7	7.8±1.9	0.933	7.9±1.5	0.610
PWD (mm)	8.0±1.7	7.9±1.7	7.9±1.9	0.989	8.2±1.5	0.684
LVMI (g/m^2)	150.5±77.8	171.6±68.6	177.2±71.0	0.932	126.7±76.1	<0.001*
Ejection fraction (%)	23.7±11.0	24.7±13.9	21.1±8.7	0.058	25.0±10.4	0.970
AR (grade)	0.2±0.4	0.2±0.6	0.2±0.4	0.977	0.2±0.4	0.998
MR (grade)	1.2±0.9	1.3±0.7	1.6±0.9	0.257	0.8±1.0	0.003*
TR (grade)	1.1±0.7	1.1±0.6	1.3±0.7	0.491	1.0±0.7	0.678
Hemodynamic parameters						
mRAP (mmHg)	8.1±4.4	6.9±8.6	6.3±4.3	0.321	8.4±4.6	0.218
mPAP (mmHg)	23.8±8.9	24.9±8.6	28.0±10.5	0.068	21.7±6.3	0.108
PCWP (mmHg)	16.8±8.1	18.4±7.9	21.9±8.6	0.070	13.2±5.8	0.001*
CI ($L\cdot min^{-1}\cdot m^{-2}$)	2.3±0.7	2.2±0.5	2.0±0.4	0.427	2.5±0.7	0.025*
PVR (WU)	2.2±1.2	2.1±1.4	2.5±1.1	0.169	2.1±1.1	0.987
RVSWI (g/m^2)	6.2±3.2	6.4±2.6	7.3±3.6	0.374	5.4±3.0	0.250
CVP/PCWP	0.5±0.3	0.4±0.2	0.4±0.2	0.971	0.7±0.4	<0.001*

(**Table 1**'s footnote is on the next page.)

Data given as mean±SD or n (%). *P<0.05 (unpaired t-test or Mann-Whitney test as appropriate); †P<0.05 (Chi-squared test or Fisher's exact test as appropriate).
ACEI, angiotensin-converting enzyme inhibitor; ACHD, adult congenital heart disease; AR, aortic regurgitation; ARB angiotensin receptor blocker; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CI, cardiac index; CRT-D, cardiac resynchronization therapy with defibrillator; CVP, central venous pressure; DCM, dilated cardiomyopathy; dHCM, dilated phase of hypertrophic cardiomyopathy; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ICM, ischemic cardiomyopathy; IVSD, interventricular septum diameter; LDH, lactate dehydrogenase; LV left ventricle; LVMI, left ventricular mass index; mPAP, mean pulmonary artery pressure; MR, mitral regurgitation; mRAP, mean right atrial pressure; NSVT, non-sustained ventricular tachycardia; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; PWD, posterior wall diameter; RCM, restrictive cardiomyopathy; RVSWI, right ventricular stroke work index; SBP, systolic blood pressure; TR, tricuspid regurgitation; VAD, ventricular assist device.

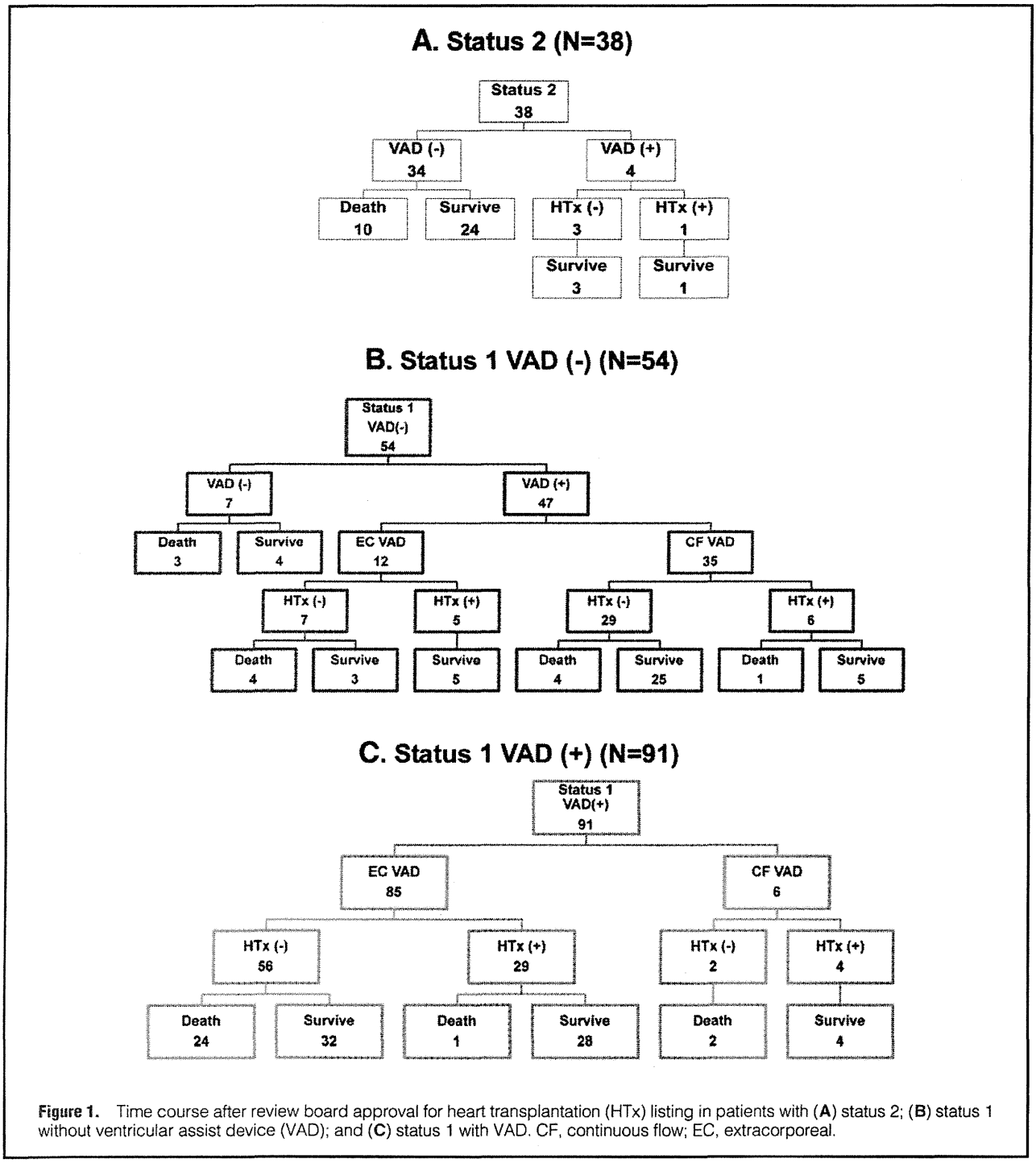

Figure 1. Time course after review board approval for heart transplantation (HTx) listing in patients with (**A**) status 2; (**B**) status 1 without ventricular assist device (VAD); and (**C**) status 1 with VAD. CF, continuous flow; EC, extracorporeal.

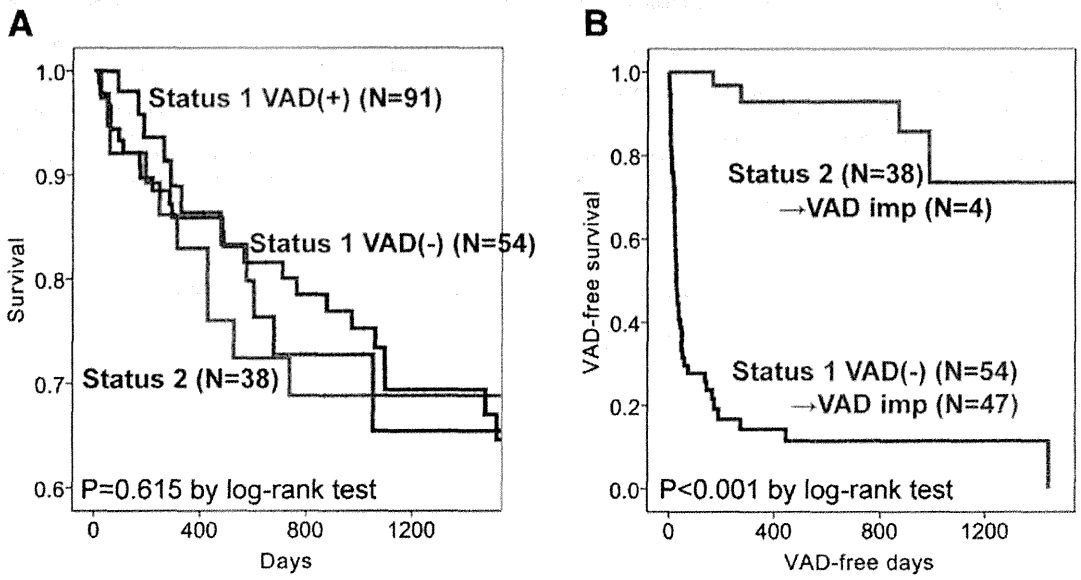

Figure 2. (**A**) Four-year survival among the 3 groups; and (**B**) ventricular assist device (VAD)-free survival in status 2 patients and in status 1 without VAD over 4 years. imp, implantation.

status code 1A and 1B; or (2) "status 2" for patients with New York Heart Association (NYHA) IV symptom but free from continuous inotrope infusion or mechanical circulatory support (MCS), equivalent to INTERMACS profile 4–6 or UNOS status code status 2.[5–7]

Considering its cost, adverse events, and quality of life during VAD treatment,[8] all listed patients are not necessarily considered as candidates for VAD therapy. Thus far, MCS as a tool for BTT has been indicated mostly for patients with status 1, and a number of authors including us have reported preoperative survival risk factors for optimal selection among them.[3,9–16] The indication for MCS in patients with status 2, however, has not been well described. Patients with status 2 are basically outpatients, and it may be difficult for health-care providers to respond to acute hemodynamic deterioration. On the one hand, many people consider that MCS indication is too early for status 2 patients, but, on the other hand, we should not be too late. Therefore, we compared patient prognosis between status 1 and 2 with or without MCS, and identified a therapeutic borderline between medical and MCS therapy in status 2 patients.

Methods

Patient Selection

We retrospectively analyzed 183 consecutive patients with stage D HF who were evaluated by the review board for HTx listing in the University of Tokyo Hospital and then listed for HTx on JOT Network between January 2003 and August 2013. All patients had been treated with guideline-directed medical therapy consisting of β-blockers, ACEI or angiotensin II receptor blockers (ARB), and aldosterone antagonists unless contraindicated. CRT-D was introduced if indicated. Before evaluation by the board, all patients received full examination to confirm eligibility for HTx.[6] In patients assigned to status 2, peak oxygen consumption (peak $\dot{V}O_2$) $\leq 14\,ml \cdot kg^{-1} \cdot min^{-1}$ on cardiopulmonary exercise test was an indispensable eligibility for HTx listing.

EC VAD was implanted as BTT or bridge to decision in patients with acute decline of hemodynamics with or without the approval of the review board. A small number of CF VAD was also implanted in patients under the clinical trials before review board approval for HTx. After confirming eligibility for HTx, they were listed as status 1.

Written informed consent was obtained at admission from the patients and/or their family members in all cases. The study protocol was approved by the Ethics Committee of Graduate School of Medicine, University of Tokyo (application number 779 [1]).

Variables Evaluated

Patients were enrolled at the time of approval by the institutional review board for HTx. All patients enrolled were listed on JOT Network later. Patient demographic, laboratory, echocardiographic, and hemodynamic parameters obtained <1 month before review board approval were analyzed in this study. In patients with VAD, postoperative data were used. History of non-sustained ventricular tachycardia (NSVT) was confirmed on 24-h Holter electrocardiogram. To evaluate effects of different types of β-blocker, the dose of bisoprolol was normalized to the approximately equivalent dose of carvedilol according to efficacy. For example, we regarded 5 mg of bisoprolol as 20 mg of carvedilol.[17] In the same manner, the doses of ACEI/ARB were normalized to the approximately equivalent dose of enalapril. For example, 4 mg of candesartan was regarded as 5 mg of enalapril.[18]

Statistical Analysis

All statistical analysis was done using PASW Statistics 18 (SPSS, Chicago, IL, USA) or JMP9 (SAS Institute, Cary, NC, USA). Categorical variables were summarized as frequencies and percentages, and compared using Chi-squared test or

Table 2. Predictors of Survival in Patients Without VAD at the Time of Review Board Approval

	Death (n=22)	Survival (n=70)	P-value	Hazard ratio	95% confidence interval
Demographic parameters					
Age (years)	39.6±18.5	40.0±14.7	0.631	0.993	0.966–1.021
Male	17 (77.3)	55 (78.6)	0.952	1.030	0.384–2.763
Body surface area (m^2)	1.57±0.26	1.47±0.46	0.147	0.434	0.141–1.340
Etiology of ischemia	2 (9.1)	7 (10.0)	0.815	0.840	0.195–3.613
Systolic blood pressure (mmHg)	88.2±10.5	86.2±8.3	0.391	1.018	0.977–1.061
Heart rate (beats/min)	83.0±14.6	85.4±20.2	0.681	1.005	0.980–1.031
History of NSVT	9 (40.9)	31 (44.3)	0.907	0.952	0.418–2.167
Concomitant treatment					
Furosemide (mg daily)	62.7±46.7	52.0±30.7	0.508	1.004	0.993–1.015
Spironolactone (mg daily)	33.5±25.4	33.8±20.1	0.906	1.001	0.983–1.020
β-blocker (mg daily)	8.0±7.2	9.0±6.4	0.732	0.989	0.927–1.054
ACEI/ARB (mg daily)	3.7±4.7	3.3±2.2	0.301	1.066	0.944–1.203
CRT-D	11 (50.0)	45 (64.3)	0.397	0.699	0.305–1.602
Laboratory parameters					
White blood cells ($\times10^3/\mu l$)	6.1±1.8	6.2±1.4	0.639	0.964	0.934–1.074
Hemoglobin (g/dl)	12.5±2.0	12.5±2.1	0.287	1.108	0.917–1.338
Platelets ($\times10^3/\mu l$)	23.7±10.7	21.2±7.0	0.336	1.023	0.977–1.071
Serum sodium (mEq/L)	134.0±6.4	135.4±4.0	0.694	0.984	0.906–1.068
Serum potassium (mEq/L)	4.3±0.4	4.2±0.4	0.394	1.514	0.583–3.935
Serum BUN (mg/dl)	25.0±7.4	24.1±4.5	0.406	1.032	0.958–1.112
Serum creatinine (mg/dl)	1.0±0.5	1.0±0.4	0.987	0.991	0.348–2.823
Serum albumin (g/dl)	3.7±0.4	4.0±0.6	0.124	0.604	0.318–1.148
Serum GOT (IU/L)	33.3±13.1	26.2±11.6	0.076	1.033	0.974–1.063
Serum GPT (IU/L)	31.0±23.1	26.8±17.6	0.475	1.008	0.987–1.029
Serum LDH (IU/L)	301.3±200.2	255.4±77.6	0.243	1.006	0.988–1.034
Serum total bilirubin (mg/dl)	1.2±0.7	1.2±0.8	0.821	1.072	0.587–1.959
Plasma BNP (log_{10}pg/ml)	3.01±2.88	2.98±3.18	0.324	0.976	0.923–1.084
Echocardiographic parameters					
LV diastolic diameter (mm)	67.1±19.4	69.4±14.5	0.749	0.996	0.971–1.022
LV systolic diameter (mm)	58.4±20.0	62.7±14.7	0.575	0.993	0.969–1.017
IVS (mm)	7.9±2.5	7.7±1.7	0.867	1.018	0.824–1.258
PW (mm)	8.1±2.1	7.9±1.7	0.976	1.003	0.809–1.244
LVMI (g/m^2)	182.2±76.5	172.5±67.8	0.664	1.001	0.996–1.006
Ejection fraction (%)	26.2±15.0	21.1±9.8	0.268	1.017	0.987–1.048
AR (grade)	0.2±0.7	0.2±0.4	0.465	1.354	0.601–3.052
MR (grade)	1.4±0.9	1.5±0.8	0.628	0.997	0.546–1.441
TR (grade)	1.2±0.8	1.2±0.7	0.945	1.022	0.552–1.893
Hemodynamic parameters					
mRAP (mmHg)	9.2±3.3	7.4±4.4	0.087	1.112	0.978–1.228
mPAP (mmHg)	29.9±7.5	27.4±10.6	0.219	1.028	0.983–1.075
PCWP (mmHg)	22.2±6.4	20.0±8.9	0.179	1.037	0.984–1.093
CI ($L\cdot min^{-1}\cdot m^{-2}$)	2.0±0.5	2.1±0.5	0.403	0.64	0.225–1.819
PVR (WU)	2.3±0.9	2.4±1.3	0.889	0.974	0.668–1.419
RVSWI (g/m^2)	7.2±2.8	6.9±3.3	0.730	1.025	0.890–1.180
CVP/PCWP	0.4±0.2	0.4±0.3	0.272	3.531	0.371–33.58

Data given as mean±SD or n (%). *P<0.05 (Cox regression analysis).
Abbreviations as in Table 1.

Fisher's exact test as appropriate. Continuous variables are represented as mean±SD unless otherwise specified, and compared using unpaired t-test or Mann-Whitney test as appropriate. Variables of status 1 and 2 with/without VAD were compared using ad-hoc Tukey test when analysis of variance confirmed significance. Kaplan-Meier analysis was done with log-rank test for survival over 4 years. Cox regression analysis was used to examine significant factors for survival. Receiver operating characteristic (ROC) analysis was used to calculate a cut-off value of plasma B-type natriuretic peptide (BNP) for survival in the status 2 group. All hypothesis tests reported are 2-tailed, and P<0.05 was set as significant.

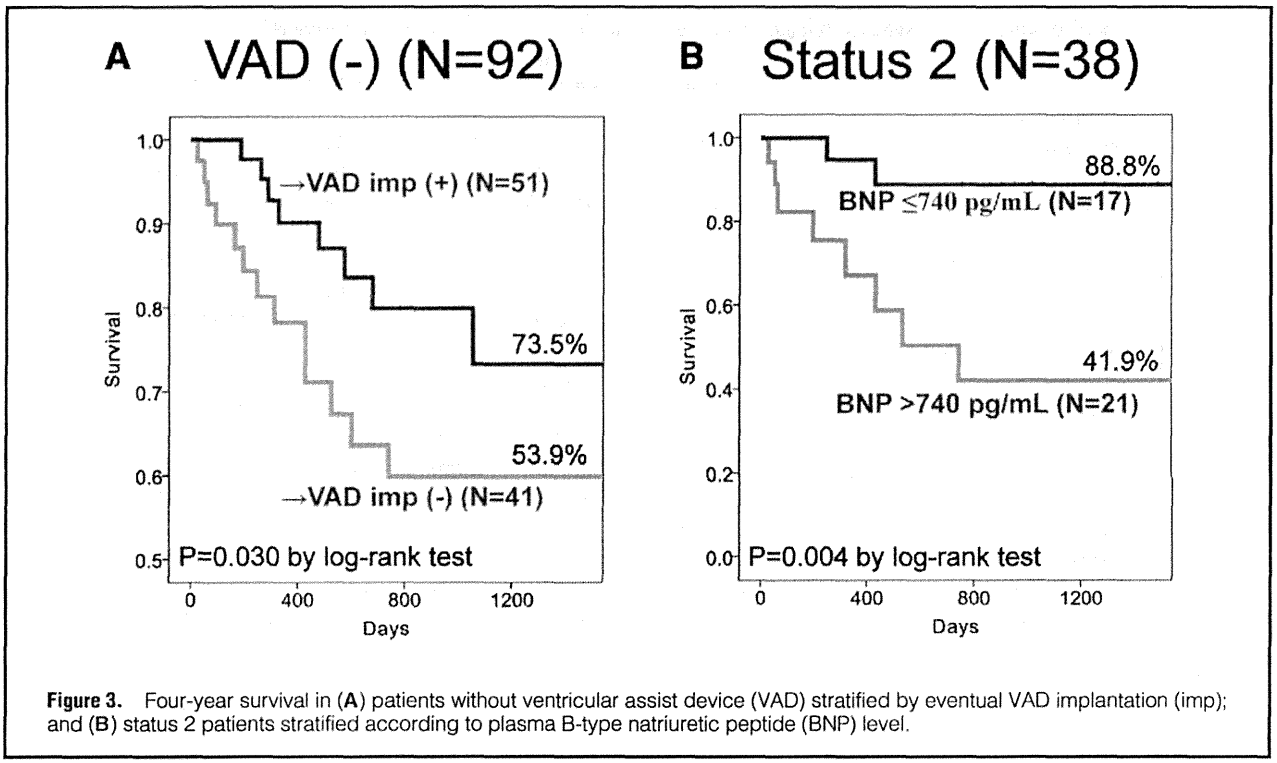

Figure 3. Four-year survival in (**A**) patients without ventricular assist device (VAD) stratified by eventual VAD implantation (imp); and (**B**) status 2 patients stratified according to plasma B-type natriuretic peptide (BNP) level.

Results

Patient Baseline Characteristics

Dilated cardiomyopathy and ischemic cardiomyopathy were the dominant etiologies of HF in all 3 groups (**Table 1**). Dilated phase of hypertrophic cardiomyopathy was another major etiology of HF in the status 2 and the status 1 without VAD groups. Fulminant myocarditis was the third major etiology of HF in the status 1 with VAD group.

There were no statistical differences in demographic, laboratory, echocardiographic, and hemodynamic parameters between the status 2 and the status 1 without VAD groups except for higher prevalence of previous NSVT in the latter group (34.2% vs. 50.0%, P=0.026). The status 1 with VAD group had a higher prevalence of improved hemodynamics along with unloaded left ventricle regardless of lower dose of diuretics (all P<0.05 compared with status 2).

Clinical Course Over 4 Years at Time of Review Board Approval

In the status 2 group (n=38), only 4 patients (10.5%) received VAD treatment eventually, and all of them survived during the study period (**Figure 1A**). Peak $\dot{V}O_2$ averaged 12.0±3.6 ml·kg^{-1}·min^{-1}, and all patients were assigned INTERMACS profile 4–6 at the time of enrollment (profile 4, 12 patients, 31.6%; profile 5, 21 patients, 55.3%; profile 6, 5 patients, 13.2%). Of the patients without VAD treatment, 10 patients (29.4%) died (6 due to acute hemodynamic compromise and 4 due to ventricular fibrillation [Vf]). Of the 4 patients who died due to Vf, 2 patients had received CRT-D previously.

In the status 1 without VAD group (n=54), 47 patients (87.0%) received VAD treatment eventually (CF, 35 patients, 74.5%; EC, 12 patients, 25.5%; **Figure 1B**). All patients were assigned INTERMACS profile 2 or 3 at the time of enrollment (profile 2, 23 patients, 42.6%; profile 3, 31 patients, 57.4%). Eleven patients (20.3%) received HTx eventually and, of them, only 1 patient died. Five patients (14.3%) died during CF VAD support, whereas 4 patients (33.3%) died during EC VAD support. Cause of death was multiple organ failure or stroke after VAD implantation.

In the VAD group (n=91), 85 patients (93.4%) had already received EC VAD, and 6 patients (6.6%) had CF VAD under the clinical trials (**Figure 1C**). All patients were assigned INTERMACS profile 1 or 2 before VAD implantation (profile 1, 50 patients, 54.9%; profile 2, 41 patients, 45.1%). Thirty-three patients (36.3%) received HTx eventually and, of them, only 1 patient died. While waiting for HTx, 26 patients (44.8%) died under VAD treatment.

There were no significant differences in overall survival among the 3 groups over 4 years (status 2, 68.8%; status 1 without VAD, 65.5%; and status 1 with VAD, 64.6%, P=0.615; **Figure 2A**). Patients assigned status 1 without VAD had markedly lower VAD-free survival than those of status 2 over 4 years (11.5% vs. 73.5%, P<0.001; **Figure 2B**).

Risk Analysis for Survival in Patients Without VAD at Time of Review Board Approval

On Cox regression analysis there were no significant predictors for 4-year survival in patients without VAD treatment (including both status 1 and 2) at the time of review board approval (n=92; **Table 2**), whereas more than half of the patients (n=51, 55%) eventually received VAD therapy as shown in **Figure 2B**, and clinical course was affected by MCS. Consistently, eventual VAD implantation significantly stratified 4-year survival in patients without VAD treatment according to Kaplan-Meier analysis (P=0.030, log-rank test; **Figure 3A**).

In contrast, Cox regression analysis showed that higher plasma BNP was the only significant predictor of 4-year mortality in status 2 patients (P=0.024, hazard ratio [HR], 8.267; **Table 3**). On ROC analysis the cut-off level of plasma BNP was 740 pg/ml (area under the curve, 0.704; sensitivity, 0.800;

Table 3. Predictors of Survival in Status 2 Patients at the Time of Review Board Approval

Total n=38	Death (n=10)	Survive (n=28)	P-value	Hazard ratio	95% confidence interval
Demographic parameters					
Age (years)	32.9±17.1	41.0±17.6	0.163	0.977	0.945–1.010
Male	6 (60.0)	20 (71.4)	0.363	0.556	0.156–1.973
Body surface area (m^2)	1.31±0.51	1.48±0.32	0.217	0.420	0.106–1.662
Etiology of ischemia	0 (0)	4 (14.3)	0.495	0.042	0.001–372.6
Systolic blood pressure (mmHg)	86.2±12.0	88.1±9.0	0.596	0.983	0.921–1.048
Heart rate (beats/min)	90.0±16.8	80.1±13.0	0.068	1.043	0.998–1.090
History of NSVT	5 (50.0)	9 (32.1)	0.972	1.022	0.297–3.515
Peak $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	10.9±3.3	11.9±3.8	0.708	1.033	0.873–1.221
Concomitant treatment					
Furosemide (mg daily)	46.0±40.9	47.9±29.1	0.718	0.996	0.975–1.018
Spironolactone (mg daily)	37.5±27.0	37.1±22.4	0.850	1.002	0.977–1.028
β-blocker (mg daily)	9.1±8.2	11.3±6.7	0.451	0.962	0.870–1.064
ACEI/ARB (mg daily)	3.1±4.3	3.6±2.6	0.802	1.032	0.806–1.322
CRT-D	4 (40.0)	18 (64.3)	0.181	0.420	0.118–1.496
Laboratory parameters					
White blood cells ($\times 10^3/\mu l$)	5.4±1.4	6.1±1.5	0.087	0.596	0.37–1.023
Hemoglobin (g/dl)	13.3±1.6	12.8±2.0	0.273	1.203	0.865–1.672
Platelets ($\times 10^3/\mu l$)	19.3±5.0	22.2±7.6	0.361	0.951	0.854–1.059
Serum sodium (mEq/L)	135.4±8.0	136.2±4.1	0.868	1.010	0.902–1.130
Serum potassium (mEq/L)	4.2±0.4	4.2±0.3	0.573	0.652	0.147–2.885
Serum BUN (mg/dl)	22.5±6.3	23.3±3.4	0.672	0.972	0.851–1.109
Serum creatinine (mg/dl)	0.9±0.4	0.9±0.5	0.432	0.521	0.102–2.653
Serum albumin (g/dl)	3.8±0.5	4.1±0.6	0.355	0.669	0.285–1.568
Serum GOT (IU/L)	33.3±14.6	27.6±11.3	0.151	1.031	0.989–1.076
Serum GPT (IU/L)	28.6±18.9	26.8±18.1	0.664	1.008	0.974–1.042
Serum LDH (IU/L)	336.4±282.5	269.6±63.8	0.223	1.005	0.997–1.014
Serum total bilirubin (mg/dl)	1.2±0.7	1.0±0.6	0.504	1.391	0.529–3.62
Plasma BNP (log_{10}pg/ml)	2.96±0.35	2.70±0.34	0.024*	8.267	1.041–65.66
Plasma BNP >740pg/ml	8 (80.0)	9 (32.1)	0.014*	7.037	1.487–33.29
Echocardiographic parameters					
LV diastolic diameter (mm)	64.6±21.4	65.6±13.3	0.829	0.995	0.954–1.038
LV systolic diameter (mm)	54.7±21.3	58.6±13.8	0.545	0.988	0.952–1.026
IVS (mm)	7.4±2.5	7.7±1.6	0.636	0.916	0.636–1.318
PW (mm)	7.8±2.5	7.9±1.3	0.800	0.947	0.621–1.444
LVMI (g/m^2)	177.1±82.9	169.6±64.3	0.813	1.001	0.992–1.010
Ejection fraction (%)	30.8±18.3	23.6±11.8	0.314	1.018	0.983–1.055
AR (grade)	0.3±0.9	0.1±0.4	0.394	1.461	0.611–3.497
MR (grade)	1.5±0.9	1.3±0.6	0.253	1.774	0.665–4.734
TR (grade)	1.1±0.9	1.1±0.6	0.681	0.824	0.328–2.072
Hemodynamic parameters					
mRAP (mmHg)	7.8±3.8	6.7±4.2	0.513	1.051	0.905–1.221
mPAP (mmHg)	27.8±8.7	24.1±8.6	0.263	1.043	0.969–1.123
PCWP (mmHg)	21.0±6.1	17.7±8.3	0.331	1.039	0.961–1.124
CI ($L \cdot min^{-1} \cdot m^{-2}$)	2.2±0.6	2.2±0.5	0.991	0.993	0.304–3.246
PVR (WU)	1.8±0.8	2.2±1.4	0.918	0.974	0.594–1.597
RVSWI (g/m^2)	6.7±2.7	6.3±2.6	0.574	1.069	0.848–1.347
CVP/PCWP	0.4±0.2	0.4±0.2	0.892	0.786	0.024–25.88

Data given as mean±SD or n (%). *P<0.05 (Cox regression analysis).
Abbreviations as in Table 1.

specificity, 0.607), and plasma BNP concentration >740pg/ml had HR 7.037 on Cox regression analysis (P=0.014). Kaplan-Meier analysis significantly stratified 4-year survival in patients with status 2 according to plasma BNP level (P=0.004, log-rank test; **Figure 3B**).

Discussion

Among 183 consecutive patients with stage D HF who were evaluated by the review board of the University of Tokyo Hospital for HTx listing, the prognosis of status 2 patients was

as poor as those of status 1 with or without VAD treatment over 4 years. Eventual VAD implantation provided significantly better 4-year survival in patients without VAD treatment regardless of any baseline characteristics. On Cox regression analysis plasma BNP >740 pg/ml was the only significant predictor for 4-year mortality among status 2 patients.

Patient Prognosis

The VAD group had the sickest preoperative background because a large proportion of them had had INTERMACS profile 1, and received MCS due to cardiogenic shock. The group of status 1 without VAD, which was equivalent to INTERMACS profile 2 or 3, also had a sicker background, because i.v. inotropes could not be discontinued due to refractory HF. Surprisingly, less sick patients with status 2, equivalent to INTERMCS profile 4–6, had a prognosis as poor as other 2 groups. Nader et al consistently reported that UNOS status 2 patients, equivalent to those with JOT status 2, had a poor prognosis without transplantation (3-year survival with/without HTx, 87% vs. 57%, P<0.01).[19] Patient baseline characteristics including end-organ function and plasma BNP level of the status 1 group were as good as those of status 2 at the time of review board approval. Accordingly, not only MCS but intensive inotropic support could successfully maintain hemodynamics for a certain period. Approximately 90% of inotrope-dependent patients, however, needed VAD therapy within 1 year (cf. Figure 2B), and we should remember that inotropes are not as powerful as MCS for support on a month-to-month basis.

VAD Indication in Status 1 and 2

Previously established predictors of survival in HF patients, such as hyponatremia, chronic kidney disease, and high plasma BNP,[20–22] were not risk factors for survival among patients without VAD. Instead, VAD implantation provided significantly better survival. Among patients who are destined to receive MCS, such biomarkers may not simply become good predictors for survival. Consistent with this, Kelsey et al recently reported that previously developed preoperative health status had a limited association with outcome in patients who received HeartMate II VAD.[23]

CF VAD is indicated in patients with status 1, whereas those with status 2 have rarely received VAD treatment thus far in Japan.[9,10,24] INTERMACS similarly reported that not many (18.3%) of less sick patients (ie, profile 4–7) received VAD treatment in the past.[8] Among the status 2 group, 4 patients (10.5%) eventually received VAD treatment via status 1 due to worsening of HF, and all patients remained alive. The other 34 patients (89.5%) did not receive VAD implantation simply because they were assigned the less sick status 2. Boyle et al found that patients assigned INTERMACS profile 4–7, that is, almost equivalent to status 2 in Japan, had better 3-year survival after CF VAD implantation than the group who was more acutely ill.[25] Then, the next question is whether all patients with status 2 should receive VAD implantation.

Selection for VAD in Status 2

Plasma BNP >740 pg/ml was the only significant risk factor for mortality in the status 2 group, and 20 patients (52.6%) had plasma BNP >740 pg/ml. In other words, approximately half of the status 2 patients had been followed as outpatients without MCS, albeit with considerably high plasma BNP. Consistent with this, Kato et al reported that patients with peak $\dot{V}O_2$ 10–14 $ml \cdot min^{-1} \cdot kg^{-1}$ had a worse prognosis when they had plasma BNP ≥506 pg/ml.[26] All 10 deceased patients died due to acute deterioration of hemodynamics or fatal ventricular tachyarrhythmia. Their plasma BNP was already high (1197±943 pg/ml at the time of review board approval. Status 2 patients with higher plasma BNP appear to have high risk for sudden death, and may be good candidates for MCS in terms of survival benefit. Moreover, most of the deceased patients were followed in other hospitals, where VAD treatment could not be carried out. It might be better for such high-risk patients to be followed at an institution where VAD is available. In contrast, 3 of 4 patients with status 2 who eventually received VAD implantation had plasma BNP <740 pg/ml. The exacerbation of HF was relatively gradual among them and LVAD could be successfully implanted after they were inotrope dependent for a certain time period. It is of note that BNP level can stratify different time frames of HF progression.

Among 10 deceased patients, 4 out-hospital patients died suddenly due to Vf. Two patients had already received CRT-D, and they had been assigned "modifier A", the significance of which we previously proposed.[27] Patents with modifier A cannot be rescued only by CRT-D because of the deterioration of hemodynamics during electrical storm. Such patients may also be good candidates for MCS considering its advantage in avoidance of sudden death due to hemodynamic deterioration following fatal ventricular tachyarrhythmia, although sometimes these patients appear too well to receive MCS.

Guidelines state that CRT may be considered for ambulatory NYHA IV patients but not indicated for those who are inotrope dependent.[28] Nevertheless, CRT-D is often indicated for patients with advanced HF before VAD treatment partly because of hesitation in VAD implantation. In agreement with this, 56 patients (60.9%) had received CRT-D before review board approval among those who had not had VAD treatment. CRT-D, however, was not significantly associated with better 4-year survival according to Cox regression analysis. Furthermore, CRT-D was not sufficient to rescue patients with modifier A. Therefore, the evidence-based indication for CRT-D should be more strictly observed in order to minimize the percentage of non-responders as well as to save medical expenses.

Recently, the Medical Arm of INTERMACS (MEDAMACS) and the randomized evaluation of VAD intervention before inotropic therapy (REVIVE-IT) have been conducted in the USA to assess medically treated patients with profile 4–6 who might become candidates for VAD treatment.[8,29] The results would provide a reasonable indication for MCS in less sick patients.

Study Limitations

This study was conducted retrospectively at a single center, and consequently included a limited number of patients. The present institutional review board, however, is one of the major committees in Japan and a number of patients (62 patients; 33.9%) were referred from other institutes. Nevertheless, data from all HTx centers in Japan would definitely strengthen statistical power.

Only 4 VAD implants in the status 2 group were carried out, and we could not analyze the effects of VAD implantation on prognosis. Although the impact of VAD implantation on mortality appears to be clear in the combined group of status 1 and status 2, the survival benefit of VAD implantation in status 2 patients was not able to be demonstrated in this study.

Conclusions

Prognosis in status 2 patients was as poor as those dependent on inotrope infusion or VAD treatment because of out-of-hospital sudden death without MCS. Status 2 patients with high

plasma BNP may be good candidates for CF VAD therapy.

Acknowledgments

Grant-in-Aid from Fukuda Foundation for medical Technology to K.K. The authors have no conflict of interest.

References

1. Lindenfeld J, Feldman AM, Saxon L, Boehmer J, Carson P, Ghali JK, et al. Effects of cardiac resynchronization therapy with or without a defibrillator on survival and hospitalizations in patients with New York Heart Association class IV heart failure. *Circulation* 2007; **115:** 204–212.
2. Kitamura S. Heart transplantation in Japan: A critical appraisal for the results and future prospects. *Gen Thorac Cardiovasc Surg* 2012; **60:** 639–644.
3. Imamura T, Kinugawa K, Shiga T, Endo M, Kato N, Inaba T, et al. Preoperative levels of bilirubin or creatinine adjusted by age can predict their reversibility after implantation of left ventricular assist device. *Circ J* 2013; **77:** 96–104.
4. Suwa H, Seguchi O, Fujita T, Murata Y, Hieda M, Watanabe T, et al. Paracorporeal ventricular assist device as a bridge to transplant candidacy in the era of implantable continuous-flow ventricular assist device. *J Artif Organs* 2014; **17:** 16–22.
5. Kinugawa K. How to treat stage D heart failure: When to implant left ventricular assist devices in the era of continuous flow pumps. *Circ J* 2011; **75:** 2038–2045.
6. Nakatani T. Heart transplantation. *Circ J* 2009; **73**(Suppl A)**:** A55–A60.
7. Slaughter MS. UNOS status of heart transplant patients supported with a left ventricular assist device: Is it time to reconsider the status criteria? *Tex Heart Inst J* 2011; **38:** 549–551.
8. Kirklin JK, Naftel DC, Kormos RL, Stevenson LW, Pagani FD, Miller MA, et al. Fifth INTERMACS annual report: Risk factor analysis from more than 6,000 mechanical circulatory support patients. *J Heart Lung Transplant* 2013; **32:** 141–156.
9. Imamura T, Kinugawa K, Shiga T, Endo M, Kato N, Inaba T, et al. Novel risk scoring system with preoperative objective parameters gives a good prediction of 1-year mortality in patients with a left ventricular assist device. *Circ J* 2012; **76:** 1895–1903.
10. Yoshioka D, Sakaguchi T, Saito S, Miyagawa S, Nishi H, Yoshikawa Y, et al. Predictor of early mortality for severe heart failure patients with left ventricular assist device implantation: Significance of INTERMACS level and renal function. *Circ J* 2012; **76:** 1631–1638.
11. Rao V, Oz MC, Flannery MA, Catanese KA, Argenziano M, Naka Y. Revised screening scale to predict survival after insertion of a left ventricular assist device. *J Thorac Cardiovasc Surg* 2003; **125:** 855–862.
12. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. *Crit Care Med* 1985; **13:** 818–829.
13. Lietz K, Long JW, Kfoury AG, Slaughter MS, Silver MA, Milano CA, et al. Outcomes of left ventricular assist device implantation as destination therapy in the post-REMATCH era: Implications for patient selection. *Circulation* 2007; **116:** 497–505.
14. Levy WC, Mozaffarian D, Linker DT, Sutradhar SC, Anker SD, Cropp AB, et al. The Seattle Heart Failure Model: Prediction of survival in heart failure. *Circulation* 2006; **113:** 1424–1433.
15. Holman WL, Kormos RL, Naftel DC, Miller MA, Pagani FD, Blume E, et al. Predictors of death and transplant in patients with a mechanical circulatory support device: A multi-institutional study. *J Heart Lung Transplant* 2009; **28:** 44–50.
16. Toda K, Fujita T, Kobayashi J, Shimahara Y, Kitamura S, Seguchi O, et al. Impact of preoperative percutaneous cardiopulmonary support on outcome following left ventricular assist device implantation. *Circ J* 2012; **76:** 88–95.
17. Hori M, Nagai R, Izumi T, Matsuzaki M. Efficacy and safety of bisoprolol fumarate compared with carvedilol in Japanese patients with chronic heart failure: Results of the randomized, controlled, double-blind, Multistep Administration of bisoprolol IN Chronic Heart Failure II (MAIN-CHF II) study. *Heart Vessels* 2014; **29:** 238–247.
18. McKelvie RS, Yusuf S, Pericak D, Avezum A, Burns RJ, Probstfield J, et al. Comparison of candesartan, enalapril, and their combination in congestive heart failure: Randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study. The RESOLVD Pilot Study Investigators. *Circulation* 1999; **100:** 1056–1064.
19. Moazami N, Shah NR, Ewald GA, Geltman EM, Moorhead SL, Pasque MK. Should UNOS Status 2 patients undergo transplantation? *Heart Surg Forum* 2006; **9:** E823–E827.
20. Gheorghiade M, Rossi JS, Cotts W, Shin DD, Hellkamp AS, Pina IL, et al. Characterization and prognostic value of persistent hyponatremia in patients with severe heart failure in the ESCAPE Trial. *Arch Intern Med* 2007; **167:** 1998–2005.
21. Damman K, Valente MA, Voors AA, O'Connor CM, van Veldhuisen DJ, Hillege HL. Renal impairment, worsening renal function, and outcome in patients with heart failure: An updated meta-analysis. *Eur Heart J* 2014; **35:** 455–469.
22. Aspromonte N, Valle R, Peacock WF, Vanderheyden M, Maisel A. Inpatient monitoring and prognostic importance of B-type natriuretic peptide. *Congest Heart Fail* 2008; **14**(4 Suppl 1)**:** 30–34.
23. Flint KM, Matlock DD, Sundareswaran KS, Lindenfeld J, Spertus JA, Farrar DJ, et al. Pre-operative health status and outcomes after continuous-flow left ventricular assist device implantation. *J Heart Lung Transplant* 2013; **32:** 1249–1254.
24. Iwashima Y, Yanase M, Horio T, Seguchi O, Murata Y, Fujita T, et al. Serial changes in renal function as a prognostic indicator in advanced heart failure patients with left ventricular assist system. *Ann Thorac Surg* 2012; **93:** 816–823.
25. Boyle AJ, Ascheim DD, Russo MJ, Kormos RL, John R, Naka Y, et al. Clinical outcomes for continuous-flow left ventricular assist device patients stratified by pre-operative INTERMACS classification. *J Heart Lung Transplant* 2011; **30:** 402–407.
26. Kato TS, Collado E, Khawaja T, Kawano Y, Kim M, Farr M, et al. Value of peak exercise oxygen consumption combined with B-type natriuretic peptide levels for optimal timing of cardiac transplantation. *Circ Heart Fail* 2013; **6:** 6–14.
27. Imamura T, Kinugawa K, Shiga T, Endo M, Inaba T, Maki H, et al. Early decision for a left ventricular assist device implantation is necessary for patients with modifier A. *J Artif Organs* 2012; **15:** 301–304.
28. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol* 2009; **53**(15)**:** e1–e90, doi: 10.1016/j.jacc.2008.11.013.
29. Baldwin JT, Mann DL. NHLBI's program for VAD therapy for moderately advanced heart failure: The REVIVE-IT pilot trial. *J Card Fail* 2010; **16:** 855–858.

Cardiac Allograft Vasculopathy Can Be Distinguished From Donor-Transmitted Coronary Atherosclerosis by Optical Coherence Tomography Imaging in a Heart Transplantation Recipient

Double Layered Intimal Thickness

Teruhiko IMAMURA,[1] MD, Koichiro KINUGAWA,[2] MD, Takahide MURASAWA,[3] ME, Yukie KAGAMI,[4] RN, Miyoko ENDO,[4] RN, Hironori MURAOKA,[1] MD, Takeo FUJINO,[1] MD, Toshiro INABA,[1] MD, Hisataka MAKI,[1] MD, Masaru HATANO,[1] MD, Osamu KINOSHITA,[5] MD, Kan NAWATA,[5] MD, Shunei KYO,[2] MD, Issei KOMURO,[1] MD, *and* Minoru ONO,[5] MD

SUMMARY

Although survival after heart transplantation (HTx) has improved in recent years, cardiac allograft vasculopathy (CAV) is still the leading cause of remote morbidity and mortality in HTx recipients, partly because of difficulty with its diagnosis. In general, routine surveillance for CAV is advocated with coronary angiography accompanied by intravascular ultrasound (IVUS) if necessary. However, these modalities have limitations with respect to low spatial resolution, and sufficient qualitative/quantitative assessment of coronary intima has not been accomplished. Recently, optical coherence tomography (OCT) has emerged as a novel intracoronary imaging technique using an optical analogue of ultrasound with a spatial resolution of 10-20 μm, which is 10 times greater than IVUS. We here experienced a 49-year-old male who received a HTx 3 years ago, and OCT was executed during low molecular weight dextran injection. OCT demonstrated distinct double intimal layers probably consisting of a donor-transmitted atherosclerotic layer and an inner intimal proliferation due to CAV, which was indistinguishable by IVUS and virtual histological analyses. We believe that OCT imaging is not only a new loadstar during treatment of CAV but also a new generation modality for screening for early CAV in HTx recipients. (Int Heart J 2014; 55: 178-180)

Key words: Intima, Everolimus, Intravascular ultrasound

Although major improvements have been made in surgical techniques and treatments for acute rejection, accelerated cardiac allograft vasculopathy (CAV) still limits the remote survival in heart transplantation (HTx) recipients.[1] CAV is a pathologically multifaceted disorder that affects epicardial coronary arteries with different types of lesions including intimal fibromuscular hyperplasia, atherosclerosis, and inflammation.[2] In distinction from general coronary atherosclerosis, which is marked by focal and eccentric fibrofatty atheroma, CAV involves the entire coronary vasculature diffusely with marked intimal proliferation and concentric vascular thickening and fibrosis.[3] Typically, HTx recipients do not experience angina because of perioperative denervation, but eventually present with left ventricular dysfunction as a consequence of progressed myocardial ischemia.[4] Therefore, an International Society for Heart and Lung Transplantation (ISHLT) working group recommended regular surveys with coronary angiography regardless of the recipient's symptoms for early detection of CAV, accompanied by subsequent IVUS when CAV is suspected.[5]

Recently, optical coherence tomography (OCT) has emerged as a new generation catheter-based modality that acquires images at a spatial resolution of 10-20 μm, enabling visualization of blood vessel wall microstructure in vivo at an unprecedented level of detail.[6] However, little is known about adaptation of OCT for analyses of CAV. Hence, we experienced a chance to conduct OCT along with coronary angiography and IVUS in a heart transplantation (HTx) recipient, and discuss the utility of OCT.

CASE REPORT

In 2010, a 46-year-old male with dilated cardiomyopathy received a HTx from a male adult donor after undergoing 2 years of left ventricular assist device support. His postoperative course was uneventful under prescription of tacrolimus, mycophenolate mophetil, prednisolone, and 2.5 mg/day of rosuvas-

From the Departments of [1] Cardiovascular Medicine, [2] Therapeutic Strategy for Heart Failure, [3] Medical Engineering, [4] Organ Transplantation, and [5] Cardiothoracic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

Address for correspondence: Koichiro Kinugawa, MD, Department of Therapeutic Strategy for Heart Failure, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, Japan. E-mail: kinugawa-tky@umin.ac.jp

Received for publication September 17, 2013. Revised and accepted September 26, 2013.

Released advance online J-STAGE March 14, 2014.

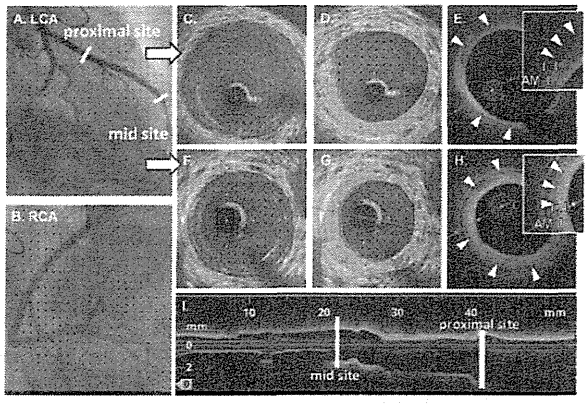

Figure. Coronary angiography images of left coronary artery (**A**) and right coronary artery (**B**); intravascular ultrasound images of the proximal (**C**) and the mid (**F**) sites of LAD; iMAP images of the proximal (**D**) and mid (**G**) sites of LAD; optimal coherence tomography images of the proximal (**E**) and mid (**H**) sites of LAD. Figure 1I shows longitudinal OCT image of LAD. A indicates adventitia; M, media; I, intima; I_d, donor-transmitted atherosclerosis; and I_c, intimal proliferation due to CAV. Arrowheads represent borderline between donor-transmitted atherosclerosis layer and intimal proliferation layer due to CAV.

tatin, except for 1 instance of cellular rejection with ISHLT grade 3A at 3 weeks after HTx, which was treated by steroid pulse therapy. At 3 months after HTx, diffuse slight plaque at a mid to proximal site of the left anterior descending coronary artery (LAD) was observed by intravascular ultrasound (IVUS). After detection of diffuse mild plaque at a mid to proximal site of the LAD by IVUS at 6 months after HTx, mycophenolate mophetil was switched to everolimus. Prednisolone was tapered off in August 2012.

In August 2013, he was admitted to our hospital for regular follow-up. His height and weight were 180 cm and 66 kg. His plasma B-type natriuretic peptide concentration was 56.4 pg/mL, and his serum creatinine concentration was 1.12 mg/dL on admission. Trough concentrations of tacrolimus and everolimus were 6.7 and 3.9 ng/mL, respectively. His ejection fraction on transthoracic echocardiography by Simpson's biplane method was 67% with a left ventricular end-diastolic diameter of 46 mm. Cytomegalovirus antigenemia and %panel reactive activity were assayed but both were negative.

According to a hemodynamic study and endomyocardial biopsy, his intracardiac pressure was normal together with no cellular rejection (ISHLT grade 0) and no complement deposition. Coronary angiography indicated no significant stenosis in any coronary artery (Figure 1A and B). IVUS images were recorded (iLab™, Boston Scientific, Corporation, Natick MA) from the mid portion to the left main coronary artery with an automated pullback system at a speed of 0.5 mm/s, using a 2.5F, 40-MHz IVUS catheter (Atlantis™ SR Pro, Boston Scientific Corporation, Natick, MA, USA). Analyzed IVUS images showed diffuse concentric plaque at a mid to proximal site of the LAD (maximal %plaque area was 38.4% at the mid site) (Figures 1C and F). iMAP images (Boston Scientific Corporation) revealed histological tissue characterization of the LAD, and over 70% of the intimal area was occupied with fibrotic component (Figures 1D and G). Subsequently, the IVUS catheter was replaced with a 2.7F OCT catheter (C8 Dragon-Fly™ JP, St Jude Medical, St Paul, MN, USA). During low molecular weight dextran injection for the clearance of blood (30 mL at 4 mL/s by power injection), OCT images (C8-XR™ system, ILUMIEN™ OPTIS™ Imaging system, St Jude Medical) were recorded from the mid to proximal portion of the LAD at an automatic pull-back speed of 20 mm/s and a frame rate of 100/s. Three layers of components consisting of intima, media, and adventitia were observed separately. Moreover, double homogenous intimal layers that were separated by a thin threshold line were observed (Figures 1E, H, and I).

Discussion

Considering the future of coronary angiography that visualizes only the coronary lumen and CAV that facilitates diffuse and concentric proliferation of intima, an early diagnosis of CAV only by coronary angiography is sometimes difficult. In contrast, IVUS can quantify coronary plaque, and some recent investigators recommend IVUS for routine surveillance of CAV.[7] Consistently, although there appeared to be no angiographic stenosis in the LAD in the present case (Figure 1A and B), mild plaque was detected at a mid to proximal site of the LAD by IVUS analyses (Figure 1D and E). However, intimal thickening is only indirectly evaluated as the intima-media thickening by IVUS because the boundary of intima and media cannot be distinguished by this method as shown also in the present case.[8] OCT is a new imaging procedure with a spatial resolution of approximately 10-20 μm, which is 10-fold greater than that of IVUS.[6] As shown in Figures 1E and H, OCT could obviously identify the layer of media as a lower-echoic line, which could not be identified by IVUS. When assessing the quality of an intracoronary structure accurately, OCT seems to have more potential than IVUS.[9]

Although few reports have performed OCT analyses for CAV,[10,11] Cassar, *et al* introduced "layered complex plaque" as one of the advanced types of CAV. They speculated that such multi-layer patterns consisting of multi-components within intimal thickening may be a pathological hallmark of repeatedly healed intimal erosions through progression of CAV.[12] We could detect a clear and pronounced boundary line within the intimal layer at the mid to proximal site in the LAD, but both layers seemed homogeneous. Consistently, iMAP images, which are obtained by using a pattern recognition algorithm on the spectra obtained from a fast Fourier transformation and histology-derived database,[13] could not distinguish between the 2 intimal layers histologically. Both layers mainly consisted of a fibrotic component. Soon after HTx, we observed only slight diffuse plaque at the mid to proximal site in the LAD by IVUS imaging, which must have been donor-transmitted atherosclerosis. Considering these results, there may be 2 mono-component intimal layers consisting of donor-transmitted atherosclerosis (shown as "I_d" in Figure 1E and H) and successive inner intimal proliferation due to CAV after HTx (shown as "I_c" in Figure 1E and H), which could not be distinguished by conventional modalities other than OCT. Repeated observation of the inner layer by OCT imaging would strengthen this double layer hypothesis.

Statins,[14] vasodilators,[15] and immunosuppressive agents such as mycophenolate mofetil[16] and everolimus[17] are used to treat CAV. However, there have been no standardized loadstars

for the treatment of CAV thus far. Quantitational assay of intimal thickening purely due to CAV separated from donor-transmitted atherosclerosis by OCT imaging would become a key loadstar for assessing the effectiveness of a specific treatment against CAV. Moreover, by distinguishing newly developed CAV from donor-transmitted atherosclerosis, an early and accurate diagnosis of CAV may be feasible.

Should OCT be recommended for all HTx recipients as a screening procedure? Different from IVUS, OCT imaging requires displacement of red blood cells from the vessel lumen during the procedure, and this is generally accomplished by using radiographic contrast.[18] However, there is concern that injection of radiographic contrast would worsen renal dysfunction because HTx recipients often have higher levels of serum creatinine due to daily administration of immunosuppressive agents, like in the present patient.[19] We adopted here low molecular weight dextran instead of radiographic contrast, as recommended by Frick, *et al.*[20] The quality of OCT images was equally high and sufficient to be analyzed compared with those with conventional radiographic contrast. We would like to emphasize that OCT imaging during low molecular weight dextran injection may be a novel new generation procedure for routine surveillance of CAV in HTx recipients.

References

1. Stehlik J, Edwards LB, Kucheryavaya AY, *et al.* The Registry of the International Society for Heart and Lung Transplantation: Twenty-eighth Adult Heart Transplant Report--2011. J Heart Lung Transplant 2011; 30: 1078-94.
2. Lu WH, Palatnik K, Fishbein GA, *et al.* Diverse morphologic manifestations of cardiac allograft vasculopathy: a pathologic study of 64 allograft hearts. J Heart Lung Transplant 2011; 30: 1044-50.
3. Rahmani M, Cruz RP, Granville DJ, McManus BM. Allograft vasculopathy versus atherosclerosis. Circ Res 2006; 99: 801-15. (Review)
4. Willman VL, Cooper T, Hanlon CR. Return of neural responses after autotransplantation of the heart. Am J Physiol 1964; 207: 187-9.
5. Mehra MR, Crespo-Leiro MG, Dipchand A, *et al.* International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. J Heart Lung Transplant 2010; 29: 717-27.
6. Tearney GJ, Regar E, Akasaka T, *et al.* Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. J Am Coll Cardiol 2012; 59: 1058-72.
7. Torres HJ, Merello L, Ramos SA, *et al.* Prevalence of cardiac allograft vasculopathy assessed with coronary angiography versus coronary vascular ultrasound and virtual histology. Transplant Proc 2011; 43: 2318-21.
8. Kume T, Akasaka T, Kawamoto T, *et al.* Assessment of coronary intima--media thickness by optical coherence tomography: comparison with intravascular ultrasound. Circ J 2005; 69: 903-7.
9. McCabe JM, Croce KJ. Optical coherence tomography. Circulation 2012; 126: 2140-3. (Review)
10. Khandhar SJ, Yamamoto H, Teuteberg JJ, *et al.* Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTCAV study). J Heart Lung Transplant 2013; 32: 596-602.
11. Ichibori Y, Nakatani D, Sakata Y, *et al.* Cardiac allograft vasculopathy progression associated with intraplaque neovascularization. J Am Coll Cardiol 2013; 61: e149.
12. Cassar A, Matsuo Y, Herrmann J, *et al.* Coronary atherosclerosis with vulnerable plaque and complicated lesions in transplant recipients: new insight into cardiac allograft vasculopathy by optical coherence tomography. Eur Heart J 2013; 34: 2610-7.
13. Heo JH, Brugaletta S, Garcia-Garcia HM, *et al.* Reproducibility of intravascular ultrasound iMAP for radiofrequency data analysis: Implications for design of longitudinal studies. Catheter Cardiovasc Interv (in press)
14. Wenke K, Meiser B, Thiery J, *et al.* Simvastatin initiated early after heart transplantation: 8-year prospective experience. Circulation 2003; 107: 93-7.
15. Erinc K, Yamani MH, Starling RC, *et al.* The effect of combined angiotensin-converting enzyme inhibition and calcium antagonism on allograft coronary vasculopathy validated by intravascular ultrasound. J Heart Lung Transplant 2005; 24: 1033-8.
16. Eisen HJ, Kobashigawa J, Keogh A, *et al.* Three-year results of a randomized, double-blind, controlled trial of mycophenolate mofetil versus azathioprine in cardiac transplant recipients. J Heart Lung Transplant 2005; 24: 517-25.
17. Eisen HJ, Tuzcu EM, Dorent R, *et al.* Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. N Engl J Med 28 2003; 349: 847-58.
18. Kataiwa H, Tanaka A, Kitabata H, Imanishi T, Akasaka T. Safety and usefulness of non-occlusion image acquisition technique for optical coherence tomography. Circ J 2008; 72: 1536-7.
19. Lindenfeld J, Miller GG, Shakar SF, *et al.* Drug therapy in the heart transplant recipient: part II: immunosuppressive drugs. Circulation 2004; 110: 3858-65. (Review)
20. Frick K, Michael TT, Alomar M, *et al.* Low molecular weight dextran provides similar optical coherence tomography coronary imaging compared to radiographic contrast media. Catheter Cardiovasc Interv (in press)

Urine Sodium Excretion After Tolvaptan Administration Is Dependent Upon Baseline Serum Sodium Levels

A Possible Explanation for the Improvement of Hyponatremia With Scarce Chance of Hypernatremia by a Vasopressin Receptor Antagonist

Teruhiko IMAMURA,[1] MD, Koichiro KINUGAWA,[2] MD, Shun MINATSUKI,[1] MD, Hironori MURAOKA,[1] MD, Naoko KATO,[2] PhD, Toshiro INABA,[1] MD, Hisataka MAKI,[1] MD, Masaru HATANO,[1] MD, Atsushi YAO,[1] MD, *and* Issei KOMURO,[1] MD

SUMMARY

Several studies have demonstrated that tolvaptan (TLV) can improve hyponatremia in advanced heart failure (HF) patients with rare chance of hypernatremia. However, changes in serum sodium concentrations (S-Na) in patients with or without hyponatremia during TLV treatment have not been analyzed.

Ninety-seven in-hospital patients with decompensated HF who had received TLV at 3.75-15 mg/day for 1 week were enrolled. Among 68 "responders", who had achieved any increases in urine volume (UV) during the first day, urinary sodium excretion during 24 hours ($U\text{-}NaEx_{24}$) increased significantly during one week of TLV treatment along with higher baseline S-Na ($P < 0.05$ and $r = 0.325$). Considering a cut-off value (S-Na, 132 mEq/L; AUC, 0.711) for any increases in $U\text{-}NaEx_{24}$, we defined "hyponatremia" as S-Na < 132 mEq/L. In hyponatremic responders ($n = 25$), S-Na increased significantly, although 1 week was not sufficient for normalization (125.8 ± 5.0 versus 128.9 ± 4.3 mEq/L, $P < 0.05$), along with unchanged $U\text{-}NaEx_{24}$ (2767 ± 2703 versus 2972 ± 2950 mg/day, NS). In contrast, in normonatremic responders ($n = 43$), S-Na remained unchanged (136.6 ± 3.1 versus 137.4 ± 2.9 mEq/L, NS) along with increased $U\text{-}NaEx_{24}$ (2201 ± 1644 versus 4198 ± 3550 mg/day, $P < 0.05$).

TLV increased S-Na only in hyponatremic responders by way of pure aquaresis, but increased $U\text{-}NaEx_{24}$ only in normonatremic responders, which explains the scarcity of hypernatremia. Epithelial Na-channels in the distal nephrons, whose repression by TLV increases urinary sodium excretion, may be attenuated by reduced ATP-supply in worse hemodynamics under hyponatremia. (Int Heart J 2014; 55: 131-137)

Key words: Heart failure, Vasopressin, Urine osmolality

The orally active vasopressin antagonists vaptans provide potential effects to treat chronic water-retaining disorders.[1] Among them, the vasopressin type 2 (V2) receptor antagonist tolvaptan (TLV) has been available for patients with heart failure (HF) with symptomatic congestion or hyponatremia.[2] TLV has been demonstrated to ameliorate congestion, stabilize hemodynamics, and improve renal function without any significant adverse effects.[3-7] We also reported the efficacy and safety of TLV in (1) amelioration of congestion even in stage D HF patients and (2) improvement of renal function by converting ongoing diuretics to TLV.[8,9]

With respect to serum sodium concentration (S-Na), various studies in Europe and the United States have demonstrated the efficacy of TLV to improve hyponatremia with little chance of hypernatremia, ie, S-Na > 145 mEq/L (eg, 1.7% of hypernatremia in the EVEREST study and 0% in the QUEST study).[2,6,10-12] In Japan, we can administer TLV to HF patients to treat their congestion regardless of baseline S-Na as long as hypernatremia or rapid increases in S-Na do not develop. However, no studies have examined the efficacy and safety of TLV in patients with normonatremia thus far. Therefore, we have analyzed and compared the effect of TLV on S-Na between patients with and without hyponatremia.

METHODS

Study design and patients: Of the patients who were hospitalized for decompensated HF at the University of Tokyo Hospital between February 2011 and May 2013, consecutive 97 pa-

From the Departments of [1] Cardiovascular Medicine, [2] Therapeutic Strategy for Heart Failure, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

This work was supported in part by a Grant-in-Aid for a JSPS Postdoctoral Research Fellow from the Japan Society for the Promotion of Science (no. 224943) to N.K., a grant for clinical epidemiology research from the Japanese Heart Foundation, the Japanese Association for Cerebro-Cardiovascular Disease Control and AstraZeneca to N.K. and K.K., and a domestic collaborative research grant from the Pfizer Health Research Foundation to N.K. and K.K.

Address for correspondence: Koichiro Kinugawa, MD, Department of Therapeutic Strategy for Heart Failure, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: kinugawa-tky@umin.ac.jp

Received for publication August 1, 2013. Revised and accepted October 10, 2013.

Released advance online J-STAGE March 14, 2014.