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ORIGINAL RESEARCH

Circulating Progenitor Cells Are Associated With Bioprosthetic Aortic Valve Deterioration: A Preliminary Study

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BACKGROUND: Mechanisms underlying bioprosthetic valve deterioration are multifactorial and incompletely elucidated. Reparative circulating progenitor cells, and conversely calcification-associated osteocalcin expressing circulating progenitor cells, have been linked to native aortic valve deterioration. However, their role in bioprosthetic valve deterioration remains elusive. This study sought to evaluate the contribution of different subpopulations of circulating progenitor cells in bioprosthetic valve deterioration.

METHODS AND RESULTS: This single-center prospective study enrolled 121 patients who had peripheral blood mononuclear cells isolated before bioprosthetic aortic valve replacement and had an echocardiographic follow-up ≥2 years after the procedure. Using flow cytometry, fresh peripheral blood mononuclear cells were analyzed for the surface markers CD34, CD133, and osteocalcin. Bioprosthetic valve deterioration was evaluated by hemodynamic valve deterioration (HVD) using echocardiography, which was defined as an elevated mean transprosthetic gradient ≥30 mm Hg or at least moderate intraprosthetic regurgitation. Sixteen patients (13.2%) developed HVD during follow-up for a median of 5.9 years. Patients with HVD showed significantly lower levels of reparative $CD34^+CD133^+$ cells and higher levels of osteocalcin-positive cells than those without HVD ($CD34^+CD133^+$ cells: 125 [80, 210] versus 270 [130, 420], P=0.002; osteocalcin-positive cells: 3060 [523, 5528] versus 670 [180, 1930], P=0.005 respectively). Decreased level of $CD34^+CD133^+$ cells was a significant predictor of HVD (hazard ratio, 0.995 [95% CI, 0.990%–0.999%]).

CONCLUSIONS: Circulating levels of $CD34^+CD133^+$ cells and osteocalcin-positive cells were significantly associated with the subsequent occurrence of HVD in patients undergoing bioprosthetic aortic valve replacement. Circulating progenitor cells might play a vital role in the mechanism, risk stratification, and a potential therapeutic target for patients with bioprosthetic valve deterioration.

Key Words: aortic valve stenosis ■ bioprosthetic valve deterioration ■ circulating progenitor cells ■ structural valve degeneration ■ valve replacement

The implantation of bioprosthetic valves (BVs) in patients requiring aortic valve replacement continues to increase since recently it has been reported that there was no survival difference between mechanical valves and BVs in patients aged 50 to 69 years who underwent aortic valve replacement.[1] Furthermore, transcatheter aortic valve implantation, which uses BVs, is widely gaining popularity as an interventional treatment option for patients with aortic valve stenosis. Also, the approach to the case of valve dysfunction by transcatheter valve-in-valve implantations for valve dysfunction has further stimulated the increased use of aortic BV. However, all BVs are susceptible to deterioration as structural valve degeneration is inevitable. Because

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CLINICAL PERSPECTIVE

What Is New?

- This study sought to investigate the contribution of different subpopulations of circulating progenitor cells in bioprosthetic aortic valve deterioration and the predictive value of circulating progenitor cells measured before aortic valve replacement in patients who underwent bioprosthetic valve replacement.
- Lower levels of circulating $CD34^+CD133^+$ cells and higher osteocalcin-positive cells were significantly associated with the incidence of hemodynamic valve deterioration.
- Decreased levels of $CD34^+CD133^+$ cells emerged as a significant predictor of hemodynamic valve deterioration.

What Are the Clinical Implications?

- Circulating progenitor cells might play a vital role in hemodynamic valve deterioration progression and provide additional potential insight for the risk stratification and therapeutic target of patients undergoing aortic valve replacement.
- Future therapeutic strategies directed toward reduced circulating progenitor cells that represent the vascular dysfunction may potentially provide a novel management option for improving prognosis in patients with bioprosthetic valve replacement. Further large prospective studies that test circulating progenitor cells in patients undergoing aortic valve replacement as therapeutic targets are warranted.

Nonstandard Abbreviations and Acronyms

AVR	aortic valve replacement
BV	bioprosthetic valve
BVD	bioprosthetic valve deterioration
CPCs	circulating progenitor cells
CPC-OCN	osteocalcin expressing CPCs
HVD	hemodynamic valve deterioration
PPM	patient-prosthesis mismatch

BV deterioration (BVD) is a life-threatening and growing problem that eventually requires a reoperation valve replacement and might result in adverse outcomes, there is an increasing need to predict BVD development in the decision of the type of prosthetic valves.

BVD is mediated by progressive leaflet tissue stiffening and calcification, resulting in stenosis and leakage of the prosthetic valve.[2] However, mechanisms underlying BVD are multifactorial and remain to be elucidated. We have previously reported that reduced numbers of reparative circulating progenitor cells (CPCs) and increased number of CPC expressing the osteoblastic cell surface marker osteocalcin (CPC-OCN) were associated with native calcific aortic valve stenosis.[3,4] Although BVs lack valve interstitial cells and are not capable of regeneration unlike native aortic valves, native aortic valve stenosis and BVD share mechanisms such as leaflet tissue remodeling and inflammation contributing to leaflet calcification.[5] Hence, similar to the native valve, low levels of CPCs and high levels of CPC-OCN might potentially contribute to early and accelerated valvular deterioration. This study, therefore, sought to investigate the contribution of different subpopulations of CPCs in bioprosthetic aortic valve deterioration and the predictive value of CPCs measured before aortic valve replacement (AVR) in patients who underwent bioprosthetic valve replacement.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

This prospective observational study enrolled consecutive patients clinically and echocardiographically diagnosed with aortic valve stenosis and collected blood samples for CPC measurement at Mayo Clinic. Patients who underwent invasive AVR (surgical AVR or transcatheter aortic valve implantation) and were implanted with BVs were included in the present study. In the final analysis, patients who had a complete Doppler echocardiographic follow-up for ≥2 years after AVR at our institution were studied. AVR was performed within 60 days of CPC measurement. The indications for invasive therapies, valve type, and procedure strategy selected were based on the consensus of the institutional heart team.

The study protocol was approved by the Mayo Clinic Institutional Review Board (# 06–002204). The present study complied with the Declaration of Helsinki for the investigation in human beings, and all patients provided written informed consent before enrollment in this study.

Flow Cytometry

Flow cytometry was performed as described previously.[3,4,6–8] Briefly, peripheral blood mononuclear cells were isolated from fresh blood samples obtained in EDTA tubes using a Ficoll density gradient. Immunofluorescent cell staining was undertaken with the following fluorescent conjugated antibodies: CD34-PerCP Cy 5.5 (Becton Dickinson), CD133/2-phycoerythrin

(Miltenyi Biotec GmbH), and the appropriate isotype controls. Osteocalcin-expressing cells were recognized using an anti-human osteocalcin antibody (Santa Cruz Biotechnology) with a fluorescein isothiocyanate secondary antibody (Jackson ImmunoResearch).

Cell fluorescence was measured immediately after staining (FACSCalibur, Becton Dickinson) and was analyzed using CellQuest software (Becton Dickinson). A total of 150 000 events were counted, and data were acquired within the lymphocyte gate (determined using forward and side scatter characteristics). Threshold settings of backgrounds for isotype controls were <0.3% for CD34 and CD133, and 1% for osteocalcin. The results are expressed as counts per 100 000 events within the lymphocyte gate.

Echocardiographic Assessment

Transthoracic echocardiography examinations were performed preprocedure and before discharge from the hospital and repeated during follow-up. Two-dimensional echocardiography was performed as was previously described.[9] M-mode and Doppler echocardiography was performed by experienced sonographers according to the American Society of Echocardiography guidelines and interpreted offline by experienced investigators in a blinded fashion.[9] Cardiac morphology was assessed using diameters in standard 4- and 2-chamber views. Left ventricular ejection fraction was calculated using the biplane Simpson method. Valvular stenosis and regurgitation were quantified using an integrated approach and graded as none, mild, mild to moderate, moderate, moderate to severe, or severe according to the respective guidelines.[9] Transprosthetic flow velocity was determined by continuous-wave Doppler imaging, and the transprosthetic mean gradient was calculated using the modified Bernoulli formula. The absolute change in mean gradient was calculated as the difference between the follow-up and postoperative transthoracic echocardiography, and annualized change in mean gradient was calculated by dividing the change from postoperative to the latest echocardiogram by the time interval between the 2 assessments. Prosthetic regurgitation was evaluated by color Doppler, and the origin of the jet was visualized in several views to differentiate it from paraprosthetic regurgitation. Prosthetic regurgitation severity was assessed as recommended by the American Society of Echocardiography and classified as mild, moderate, or severe.[9] Patient-prosthesis mismatch (PPM) was defined as no PPM if the effective orifice area indexed to body surface area >0.85 cm^2/m^2, moderate if it was >0.65 cm^2/m^2 and ≤0.85 cm^2/m^2, and severe if it was ≤0.65 cm^2/m^2.

Clinical Measures and Follow-Up

Clinical evaluation, medical history, blood sample data, and transthoracic echocardiography results were collected prospectively by a detailed review of medical records in a blinded fashion by investigators. Traditional cardiovascular risk factors were evaluated according to the respective guidelines. In the present study, patients were followed up for the occurrence of BVD. BVD was detected by clinical hemodynamic valve deterioration (HVD) using a transthoracic echocardiogram, which was defined as an elevated mean transprosthetic gradient (≥30 mm Hg) or at least moderate intraprosthetic regurgitation.[10,11] Morphological valve leaflet abnormalities, which include thickening and calcification, were also evaluated. To focus on structural valve deterioration related to BVD, worsening prosthetic regurgitation, increased mean gradient attributable to thrombosis or pannus on a leaflet, or infective endocarditis or periprosthetic regurgitation were not classified as HVD. The date of the first detection of HVD was included in the final analysis to determine the occurrence of HVD development.

Statistical Analysis

The patients were divided into 2 groups, with and without the occurrence of HVD. Clinical characteristics, echocardiogram variables, and CPCs data were compared between these 2 groups. Statistical analysis was performed using SPSS version 25.0 (SPSS, Inc., Chicago, IL) and R version 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria) software. Categorical data are expressed as absolute frequencies and percentages and were compared by the χ^2 or Fisher exact tests. Continuous variables are expressed as mean±SD for normally distributed variables or as median (25th–75th percentile) for non-normally distributed variables and were compared using Student *t*-tests and the Mann–Whitney *U*-test, respectively. Correlation between 2 parameters were evaluated using linear regression analysis. Receiver operating characteristic (ROC) curves were analyzed to assess the best cut-off values of the level of $CD34^+CD133^+$ cells for predicting HVD. The optimal cut-off value was calculated using the Youden index. Survival curves were estimated using Kaplan–Meier estimates and were compared using log-rank tests. A Cox proportional hazards regression model was used to estimate hazard ratios for the occurrence of HVD and to identify independent predictors of HVD. The covariates with $P<0.10$ in the univariate analysis were included in the multivariate analysis. A collinearity index was used for checking linear combinations among covariates and Akaike information criterion for avoiding overfitting. A 2-sided $P<0.05$ was considered statistically significant.

RESULTS

Baseline Patient Characteristics and Echocardiogram Data

A total of 319 patients were diagnosed with aortic valve stenosis and had blood samples collected for CPCs analyses. Two hundred thirty-four of these patients underwent invasive AVR. Of those, 168 were implanted with BVs. Ten patients who died within 2 years after discharge, 14 patients who died >2 years after surgery without echocardiogram follow-up, and 9 patients who were lost to follow-up were also excluded. No patients died related to valvular failure or heart failure within 2 years after an early postoperative period. Among the remaining 135 patients, 3 patients had inadequate flow cytometry results because of poor fluorescence staining, and 11 patients had incomplete echocardiogram follow-up data. Thus, the final analysis was performed on 121 patients (Figure 1), of which 104 patients underwent surgical AVR and 17 underwent transcatheter aortic valve implantation.

Detailed preoperative clinical characteristics and procedural data are shown in Table 1. The mean age of the study population at the time of AVR was 73±8 years, and 34 of 121 patients (28.1%) were women. There were no significant differences in cardiovascular risk factors between the 2 groups. The prevalence of diabetes tended to be higher in the patients with HVD than in those without. Thirty-three patients (27.3%) concomitantly underwent coronary artery bypass surgery. There was no significant difference in implanted valve type between the patients with and without HVD. No significant differences in preoperative echocardiographic data were found between groups. However, transprosthetic peak velocity and mean gradient of postoperative echocardiogram were significantly higher in the patients with HVD than those without (median 2.65, interquartile range [2.38–2.95] versus 2.35 [2.00–2.60] m/s, P=0.029; 17 [12–19] versus 12 [9–15] mm Hg, P=0.030, respectively) (Table 2). Moderate PPM was detected in 20 patients (16.5%) and severe PPM in 8 patients (6.6%). Annualized changes in mean transprosthetic valve gradient were 3.51 [1.53–5.19] and 0.00 [–0.85–1.00] in patients with and without HVD. Patients who were excluded from this study because of postoperative death without echocardiogram follow-up were older and showed higher NT-proBNP (N-terminal pro-B-type natriuretic peptide) than patients included in the present study. There was no significant difference in patient characteristics and pre/postoperative echocardiogram data between the study population and those who were excluded because of incomplete echocardiographic follow-up (Table S1, Table S2).

CPCs Levels Before AVR

The median time between the blood sample test for CPC measurement to AVR was 42 days. Patients with HVD had significantly lower levels of $CD34^+CD133^+$ cells. These patients had higher levels of osteocalcin-positive (OCN^+) cells ($CD34^+CD133^+$ cells: 120 [80, 150] versus 270 [120, 420], P=0.001; OCN^+ cells: 3235 [675, 7120] versus 700 [200, 3020], P=0.026 respectively) (Figure 2). Also, there were significant differences in the level of $CD34^+CD133^+$ cells and OCN^+ cells between the patients with and without morphological valve abnormalities ($CD34^+CD133^+$ cells: 125 [80, 210] versus 270 [130, 420], P=0.002; OCN^+ cells: 3060 [523, 5528] versus 670 [180, 1930], P=0.005 respectively), whereas the level of $CD34^+$ cells did not show significant differences between the patients with and without HVD or morphological valve abnormalities

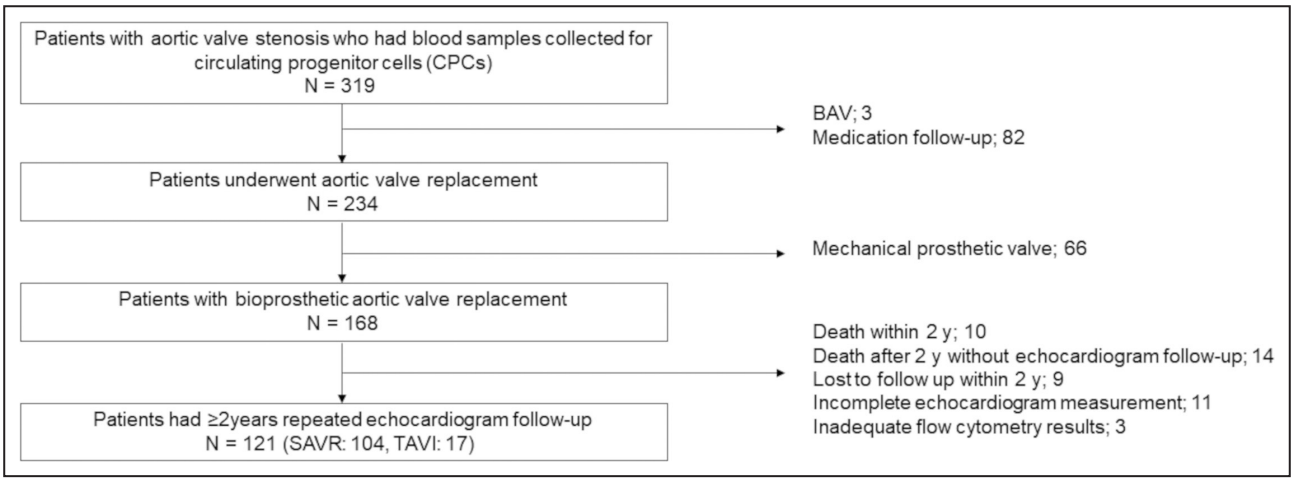

Figure 1. Study flowchart.
A flowchart showing the enrollment process with a total of 121 patients in the final analysis. BAV indicates balloon aortic valvuloplasty; CPC, circulating progenitor cells; SAVR, surgical aortic valve replacement; and TAVI, transcatheter aortic valve implantation.

Table 1. Baseline Characteristics at the Procedure and Procedural Data With and Without HVD

	Total N=121	HVD (+) n=16	HVD (–) n=105	*P* value
Demographics				
Age, y	73±8	69±9	73±8	0.079
Women, n (%)	34 (28.1)	5 (31.2)	29 (27.6)	0.998
Body surface area, m^2	2.03 [1.87–2.16]	2.04 [1.87–2.21]	2.03 [1.87–2.16]	0.584
Medical history, n (%)				
Hypertension, n (%)	93 (76.9)	14 (87.5)	79 (75.2)	0.444
Hyperlipidemia, n (%)	91 (75.2)	9 (56.2)	82 (78.1)	0.115
Diabetes, n (%)	27 (22.3)	7 (43.8)	20 (19.0)	0.059
Smoker, n (%)	44 (36.4)	6 (37.5)	38 (36.2)	1
History of CAD, n (%)	69 (57.0)	9 (56.2)	60 (57.1)	1
Laboratory data				
T-chol, mg dL^{-1}	167 [140–193]	167 [143–199]	167 [140–193]	0.708
HDL-chol, mg dL^{-1}	49 [40–61]	45 [38–58]	49 [40–61]	0.447
LDL-chol, mg dL^{-1}	85 [69–108]	88 [73–109]	84 [67–107]	0.508
Triglycerides, mg dL^{-1}	113 [79–157]	104 [79–195]	116 [82–156]	0.731
Creatinine, mg dL^{-1}	1.00 [0.80–1.20]	1.00 [0.80–1.10]	1.00 [0.80–1.20]	0.626
eGFR, mL min^{-1} 1.73 m^{-2}	62.0 [51.0–74.0]	58.5 [55.3–68.8]	62.0 [51.0–74.0]	0.903
NT-proBNP, ng L^{-1}	417 [161, 1159]	704 [247, 1722]	396 [153, 1025]	0.137
Procedural data				
Concomitant CABG, n (%)	33 (27.3)	2 (12.5)	31 (29.5)	0.261
Valve size, mm	23 [23–26]	23 [21–27]	23 [23–26]	0.351
Type of valve, n (%)				0.239
Stent less	3 (2.5)	1 (6.2)	2 (1.9)	
Stented porcine	26 (21.5)	3 (18.8)	23 (21.9)	
Stented pericardial	75 (62.0)	12 (75.0)	63 (60.0)	
TAVI	17 (14.0)	0 (0.0)	17 (16.2)	

CAD indicates coronary artery disease; CABG, coronary artery bypass grafting; eGFR, estimated glomerular filtration rate; HDL-chol, high-density lipoprotein cholesterol; HVD, hemodynamic valve deterioration; LDL-chol, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TAVI, transcatheter aortic valve implantation; and T-chol, total cholesterol.

(840 [445, 1353] versus 840 [470, 1310], *P*=0.890; 785 [395, 1378] versus 910 [485, 1310], *P*=0.625). There were no significant correlations between preoperative and annualized change in mean gradient evaluated echocardiogram and CPCs levels (Table S3).

Factors Associated With HVD

During the follow-up period of 5.9 (2.9–8.2) years, morphological valve leaflet abnormalities were detected in 30 (24.8%), and HVD was in 16 of 121 patients (13.2%) in the present study. The clinical presentation of HVD was stenosis in 9, regurgitation in 3, and both in 4 patients. The median duration from replacement to the occurrence of HVD was 5.3 years. Morphological valve leaflet abnormalities were detected in all patients with HVD. In univariable Cox regression analysis, the levels of $CD34^+CD133^+$ cells were a significant factor (HR, 0.995 [95% CI, 0.990%–0.999%]; *P*=0.020). The level of $CD34^+CD133^+$ cells remained significant after adjustment for age and diabetes (Table 3). The optimal cut-off value of the level of $CD34^+CD133^+$ cells and the level of OCN^+ cells by receiver-operating characteristic curve analysis for predicting HVD were 245 counts/100 000 (area under the curve 0.749 [95% CI, 0.662%–0.837%], sensitivity 53.3%, specificity 100%) and 2550 counts/100 000 (area under the curve, 0.673 [95% CI, 0.526%–0.819%], sensitivity 74.3%, specificity 62.5%). Based on these thresholds, 65 of 121 (53.7%) patients showed lower levels of $CD34^+CD133^+$ cells, and 37 of 121 (30.6%) patients showed higher levels of OCN^+ cells, respectively. Kaplan–Meier analysis showed a significantly increased risk of HVD in patients with lower levels of $CD34^+CD133^+$ cells (*P*=0.005), whereas those with higher levels of $CD34^+CD133^+$ cells had no events during follow-up (Figure 3A). The level of $CD34^+CD133^+$ cells tended to be higher in the patients with late valve degeneration than those with early HVD when divided into 2 groups by the median follow-up duration (*P*=0.051; Figure S1). When we stratified the patients with lower levels of $CD34^+CD133^+$ cells by the level of OCN^+ cells, patients with lower levels of $CD34^+CD133^+$ cells and higher levels of OCN^+ cells

Table 2. Preoperative and Postoperative Echocardiogram Data

	Total N=121	HVD (+) n=16	HVD (–) n=105	*P* value
Preoperative data				
LVDd, mm	50 [47–53]	50 [47–53]	51 [47–53]	0.554
LVDs, mm	31 [29–34]	30 [28–35]	31 [29–34]	0.324
EF, %	64 [60–68]	67 [58–70]	64 [60–67]	0.289
LV mass index, g/m^2	111 [100–124]	118 [111–123]	108 [99–123]	0.334
Peak velocity, m/s	4.20 [3.90–4.60]	4.45 [4.18–4.85]	4.20 [3.80–4.60]	0.077
Mean gradient, mm Hg	44 [37–52]	46 [41–59]	43 [36–52]	0.204
Aortic valve area, cm^2	0.94 [0.82–1.14]	0.90 [0.83–1.02]	0.94 [0.82–1.15]	0.482
Postoperative data				
Peak velocity, m/s	2.40 [2.00–2.70]	2.65 [2.38–2.95]	2.35 [2.00–2.60]	0.029
Mean gradient, mm Hg	12 [9–16]	17 [12–19]	12 [9–15]	0.030
Effective orifice area, cm^2	2.12 [1.70–2.49]	2.06 [1.70–2.34]	2.17 [1.70–2.58]	0.477
Effective orifice area index, cm^2/m^2	1.05 [0.87–1.29]	0.95 [0.89–1.08]	1.06 [0.87, 1.32]	0.330
Patient-prosthesis mismatch, n (%)				0.891
None	93 (76.9)	13 (81.2)	80 (76.2)	
Moderate	20 (16.5)	2 (12.5)	18 (17.1)	
Severe	8 (6.6)	1 (6.2)	7 (6.7)	
Prosthetic regurgitation				0.111
None	110 (90.9)	14 (87.5)	96 (91.4)	
Mild	11 (9.1)	2 (12.5)	9 (8.6)	
Annualized change in mean gradient, mm Hg/year	0.18 [–0.70 to 1.61]	3.51 [1.53–5.19]	0.00 [–0.85 to 1.00]	<0.001

EF indicates ejection fraction; HVD, hemodynamic valve deterioration; LV, left ventricle; LVDd, left ventricular end-diastolic diameter; and LVDs, left ventricular end-systolic diameter.

(26/121, 21.5%) showed significantly worse outcomes (Figure 3B and 4).

DISCUSSION

The current study demonstrated for the first time the different CPCs subtypes and their predictive value for HVD before AVR in patients who underwent BV replacement. The major findings of this study are as follows: (1) During the median follow-up of 5.9 years, morphological valve leaflet abnormalities were detected in 24.8%, and HVD was in 13.2% of patients who underwent bioprosthetic valve replacement; (2) the levels of CD34$^+$CD133$^+$ cells were significantly lower, whereas the level of OCN$^+$ cells was significantly higher in patients with future morphological valve abnormalities as well as those with HVD compared with those without; (3) reduced level of CD34$^+$CD133$^+$ cells tested before AVR was a significant predictor of BVD defined by echocardiogram as HVD. The current study supports a potential role for CPC in the deterioration of valve in patients undergoing aortic tissue valve replacement.

Potential Role of CPCs in HVD

The current study extends our previous observations on the interaction between circulating CPC and valve disease. Moreover, the current study supports a potential role for CPCs in the mechanisms for BVD in humans. BVD has been reported to be caused by multiple activated processes characterized by leaflet mineralization, fibrosis caused by mechanical stress, lipid-mediated inflammation, and immune rejection processes.[2] In the absence of valve interstitial cells, which produce and remodel the extracellular matrix and provide a compensatory adaptive response to hydrodynamic and biochemical changes in the body,[12] the durability of BV directly depends on the chemically cross-linked extracellular matrix and recipient-related bioactive factors. Circulating progenitor cells are recognized for their role in the maintenance of vascular and tissue function and their capacity to contribute to re-endothelialization and neoangiogenesis. These cells can be identified with the surface expression of CD34 and CD133. CD34$^+$CD133$^+$ cells have been reported to contribute to re-endothelialization, neovascularization, and repair of endothelial function as well as reflecting

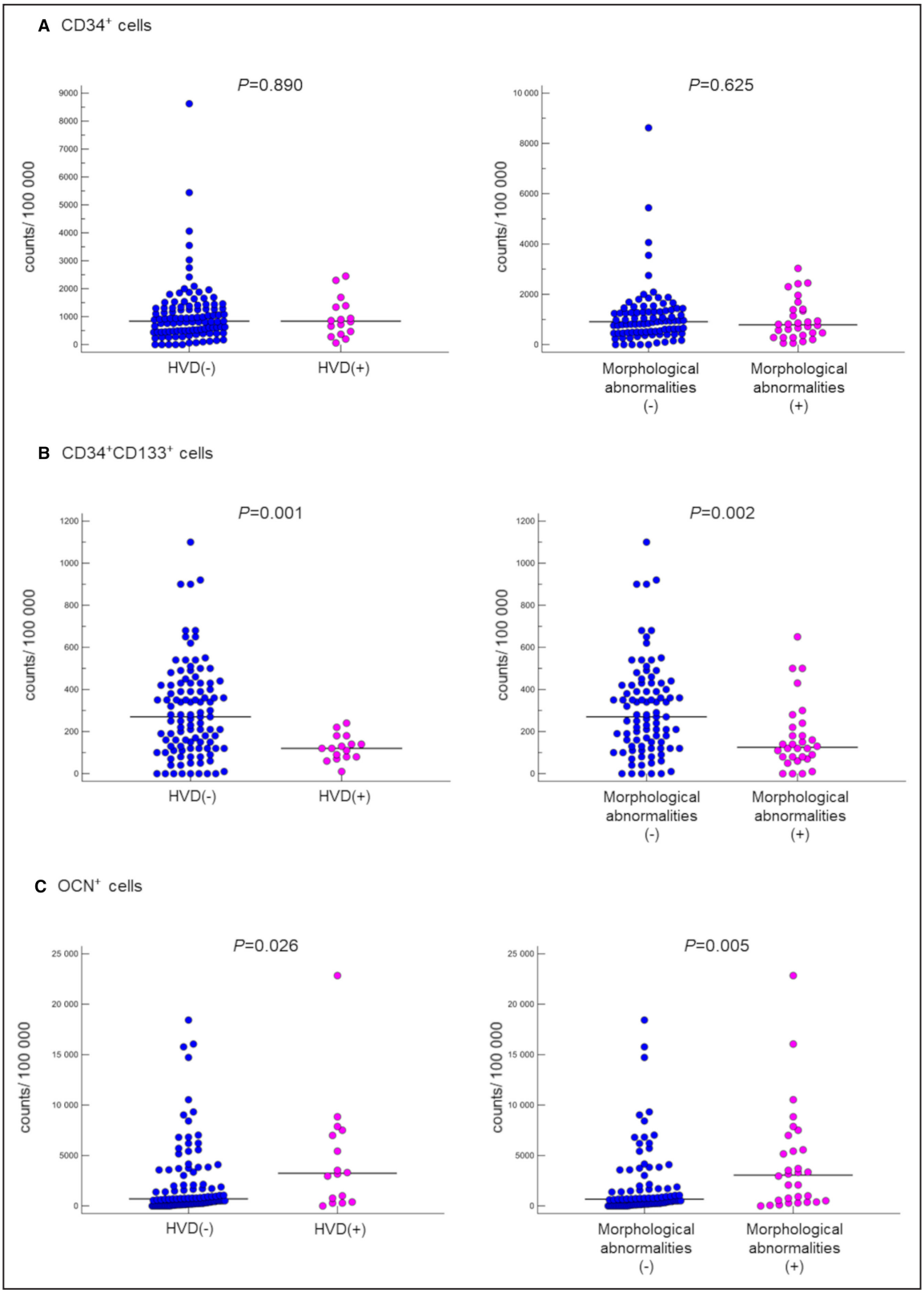

Figure 2. Comparison of circulating progenitor cells levels in patients with or without hemodynamic valve deterioration/morphological valve abnormalities.
A, $CD34^+$ cells. **B**, $CD34^+CD133^+$ cells. **C**, Osteocalcin-positive cells. Patients with morphological valve abnormalities or hemodynamic valve deterioration had significantly lower levels of $CD34^+CD133^+$ cells and a higher level of osteocalcin-positive cells. HVD indicates hemodynamic valve deterioration; and OCN^+, osteocalcin positive.

Table 3. Cox Proportional-Hazard Regression Analysis of HVD

	Univariable analysis			Multivariable analysis*		
	HR	95% CI	*P* value	HR	95% CI	*P* value
$CD34^+$ cells	1.000	0.999–1.001	0.798			
$CD34^+CD133^+$ cells	0.995	0.990–0.999	0.020	0.995	0.990–0.999	0.033
OCN^+ cells	1.000	0.999–1.000	0.456			

HVD indicates hemodynamic valve deterioration; and OCN^+, osteocalcin positive.
*Adjusted for age and diabetes.

the bone marrow progenitor cell capacity.[13,14] These cells might also play a role in re-endothelization and proper function of bioprosthetic valves. In fact, recipient $CD34^+$ and $CD133^+$ progenitor cells are abundant in explanted severely degenerated bioprosthetic aortic valves, suggesting they might be recruited to repair the valve as response to injury and inflammation.[15] Visscher et al further reported that recruited and activated progenitor cells postulated to increased vascularization and recellularization, contributing to maintaining the biohybrid valve prostheses.[16] Although the present study did not show the pathophysiological findings of CPCs on degenerated BVs, our results that showed lower levels of the repair cells $CD34^+CD133^+$ cells in peripheral blood of patients who experienced HVD could be considered in line with these findings. In the present study, there was no significant difference in overall $CD34^+$ cells between patients with HVD and those without. $CD34^+$ cells are a diverse progenitors group consisting of both hematopoietic and nonhematopoietic CPCs,[17] and some previous studies have suggested that the coexpression of CD133 increases the specificity for CPCs because it is not expressed by mature endothelial cells.[18] Therefore, $CD34^+CD133^+$ cells possibly demonstrate a more specific subtype. The inability of the extracellular matrix to remodel effectively plays a crucial function in the development of BVD,[10,19,20] and our results might suggest the role of $CD34^+CD133^+$ cells as a novel biomarker for valve integrity and for predicting BVD.

CPC-OCN in HVD

Previously, we reported that greater levels of CPC-OCNs may increase activation of osteoblastic valve interstitial cells on valve injury, contributing to valve calcification.[3] In the current study, the number of OCN^+ cells was significantly higher in the patients with morphological valve leaflet abnormalities or HVD and were capable of stratifying patients with lower levels of $CD34^+CD133^+$ cells, supporting our previous findings within bioprosthetic valves. Yet, they were not significant in Cox proportional hazard analysis. Calcification and abnormal tissue repair have also been proposed as a major mechanism of BVD, and several studies demonstrated osteocalcin expression in calcific bioprosthetic valves. However, noncalcific mechanisms including protein infiltration, glycation, oxidative and mechanical stress are considered non-negligible pathways of structural valve degeneration,[19] and it has been reported that >25% of reoperations caused by BVD attributed to structural valve degeneration with no or minimal leaflet calcification.[21] Considering the relatively small study population in the present study, the statistical analyses might be underpowered to classify the differences between calcific and noncalcific mechanisms in BVD. Therefore, future large studies are needed to clarify the role of CPC-OCN in BVD.

Potential Clinical Implications

Currently, the use of BVs in patients aged >50 years has been increasing as a result of its favorable hemodynamic profile generating the risk for recurrent tissue valve degeneration and repeat procedures such as repeated valve in valve. Thus, extended durability with the recent bioprosthesis design and low risk of thrombosis remain a priority and an unmet need. Therefore, there is an increasing need to explore mechanisms, predict BVD development, and provide novel treatment modalities for BVD management. The other risk factors previously reported, such as younger age, renal insufficiency, diabetes, PPM, and higher postoperative mean gradient, were not significant in univariate analysis, possibly because of a small number of study populations and events in the present study. Although the associations between these factors and the levels of CPCs remain undetermined, our results suggest CPCs can be used as a risk stratification tool for patients undergoing AVR, providing important information about the risk of BVD after bioprosthetic valve implantation. The use of BVs in younger patients with low $CD34^+CD133^+$ cells level and higher OCN^+ cells level might need caution, and they may need closer follow-up starting at the early period after BV replacement. Furthermore, future therapeutic strategies directed toward reduced CPC that represent the vascular dysfunction may potentially provide a novel management option for improving prognosis in patients with BV replacement. Several investigators have reported the significance of the CPC level in cardiovascular disease,[6,22] and treatment strategies using CPC have been of interest in the recent

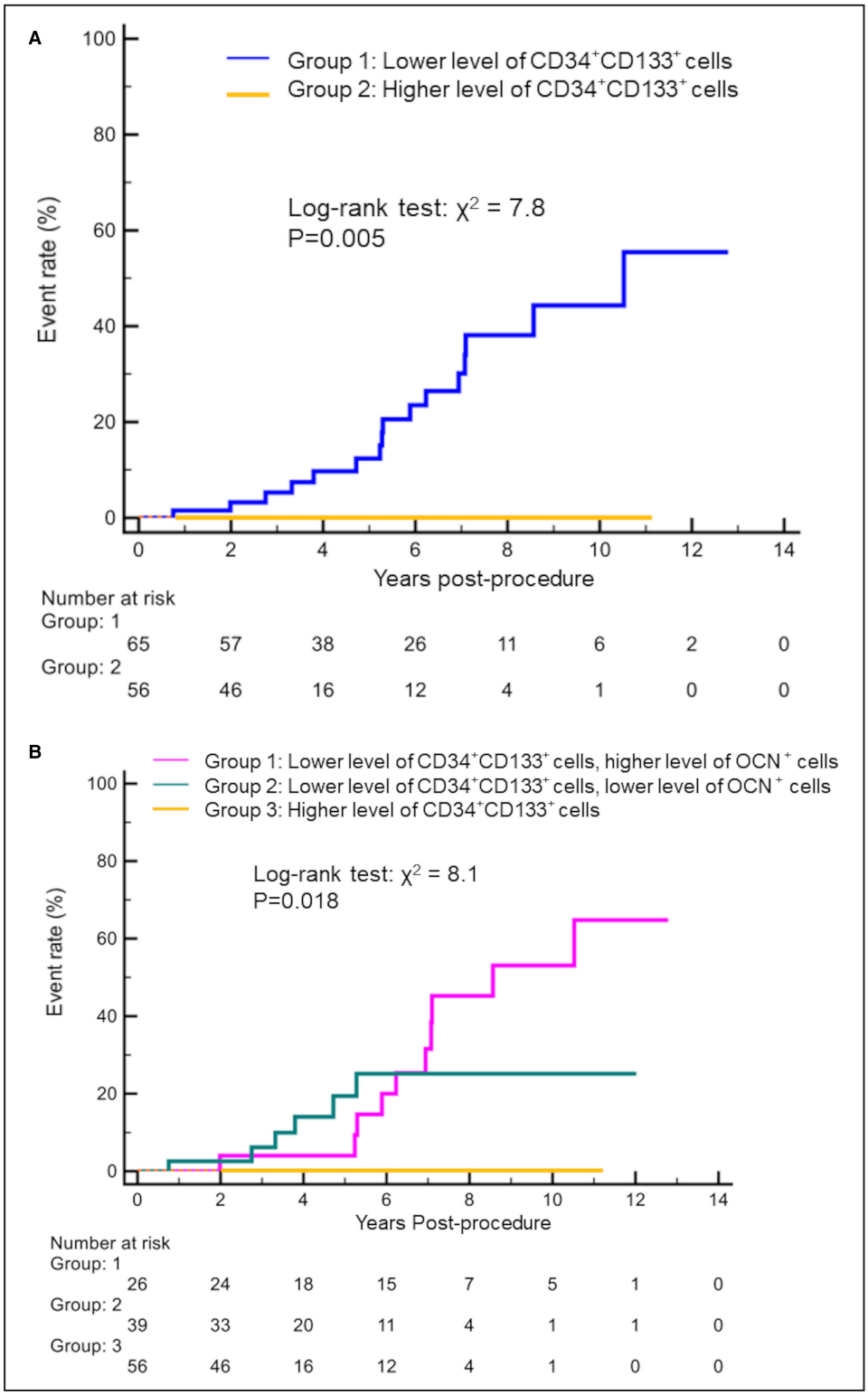

Figure 3. Kaplan–Meier analysis for the incidence of hemodynamic valve deterioration stratified by (A) the level of CD34+CD133+ cells.
(B), the level of CD34+CD133+ cells and osteocalcin-positive cells. Event-free survival was significantly worse in patients with a lower level of CD34+CD133+ cells and patients with a lower level of CD34+CD133+ cells and a higher level of osteocalcin-positive cells. The thresholds are defined by the optimal cut-off value by receiver-operating characteristic curve analysis for predicting hemodynamic valve deterioration (245 counts/100 000 for CD34+CD133+ cells, 2550 counts/100 000 for osteocalcin-positive cells).

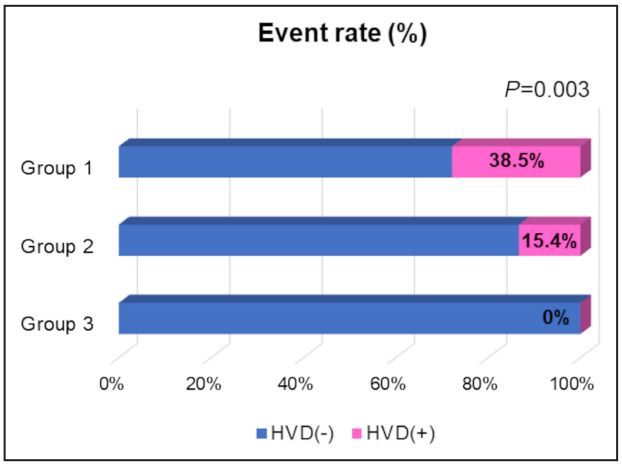

Figure 4. Frequency of hemodynamic valve deterioration stratified by the level of CD34⁺CD133⁺ cells and the level of CD34⁺CD133⁺ cells and osteocalcin-positive cells.
Group 1: Lower level of CD34+CD133+ cells, higher level of osteocalcin-positive. Group 2: Lower level of CD34+CD133+ cells, lower level of osteocalcin-positive. Group 3: Higher level of CD34+CD133+ cells. The event rate was significantly worse in the patients with lower levels of CD34⁺CD133⁺ cells and higher levels of osteocalcin-positive cells (Group 1 versus Group 2; *P*=0.044, Group 2 versus Group 3; *P*=0.009). HVD indicates hemodynamic valve deterioration.

years.[23–25] Nevertheless, this hypothesis, based on our study, is merely speculative and should be tested in further large prospective studies.

Study Limitations

The results of the present study should be interpreted with consideration for several limitations. This study included a relatively small number of patients from a single center, which may not allow extensive subgroup analyses or more reliable multivariable analyses.

Furthermore, the thresholds of the levels of CPCs might be debatable because they were calculated by ROC analysis in the current study population, and their sensitivity is relatively low. The results of this study should therefore be confirmed and validated in further large studies. Second, rigorous exclusion criteria and patients lost to echocardiogram follow-up potentially created a selection bias in the population eligible for final analysis. Further studies evaluating the relationship between the longitudinal change in CPC levels and BVD progression would provide further important insights into valve deterioration.

CONCLUSIONS

Lower levels of circulating CD34⁺CD133⁺ cells and higher OCN⁺ cells evaluated before valve replacement were significantly associated with the incidence of HVD in patients who underwent bioprosthetic aortic valve replacement. Decreased levels of CD34⁺CD133⁺ cells emerged as a significant predictor of HVD. CPCs might play a vital role in HVD progression and provide additional potential insight for the risk stratification and therapeutic target of patients undergoing aortic valve replacement.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Table S1–S3
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics at the procedure and procedural data with and without MACE

	Study population N = 121	Patients with early mortality N = 20	Patients with incomplete echocardiographic follow-up N = 11	p value	p value
Demographics					
Age, y	73 ± 8	79 ± 8	74 ± 5	<0.001	0.650
Female, n (%)	34 (28.1)	9 (37.5)	3 (27.3)	0.499	1
Body surface area, m^2	2.03 [1.87, 2.16]	1.89 [1.69, 2.15]	2.08 [1.92, 2.25]	0.104	0.254
Medical history, n (%)					
Hypertension, n (%)	93 (76.9)	17 (70.8)	10 (90.9)	0.712	0.486

Hyperlipidemia, n (%)	91 (75.2)	18 (75.0)	8 (72.7)	1	1
Diabetes mellitus, n (%)	27 (22.3)	9 (37.5)	5 (45.5)	0.189	0.178
Smoker, n (%)	44 (36.4)	10 (41.7)	4 (36.4)	0.795	1
History of CAD, n (%)	69 (57.0)	18 (75.0)	5 (45.5)	0.157	0.672
Laboratory data					
T-chol, mg dL^{-1}	167 [140, 193]	149 [134, 179]	171 [147, 190]	0.182	0.865
HDL-chol, mg dL^{-1}	49 [40, 61]	52 [43, 63]	53 [47, 57]	0.566	0.386
LDL-chol, mg dL^{-1}	85 [69, 108]	78 [57, 94]	87 [69, 107]	0.133	0.88
TG, mg dL^{-1}	113 [79, 157]	98 [61, 140]	121 [100, 159]	0.160	0.536
Creatinine, mg dL^{-1}	1.00 [0.80, 1.20]	1.10 [0.90, 1.30]	1.00 [0.84, 1.25]	0.119	0.747

eGFR, ml min^{-1} 1.73 m^{-2}	62.0 [51.0, 74.0]	55 [39, 75]	55 [48, 78]	0.093	0.559
NT-proBNP, ng L^{-1}	417 [161, 1159]	1340 [761, 4230]	816 [343, 2033]	0.001	0.199
Procedural data					
Concomitant CABG	33 (27.3)	6 (30.0)	2 (18.2)	1	0.766
Valve size, mm	23 [23, 26]	23 [23, 25]	25 [24, 26.50]	0.546	0.142
Type of valve				0.264	0.888
stent less	3 (2.5)	0 (0.0)	0 (0.0)		
stented porcine	26 (21.5)	2 (8.3)	3 (27.3)		
stented pericardial	75 (62.0)	16 (66.7)	6 (54.5)		

TAVI	17 (14.0)	6 (25.0)	2 (18.2)

CAD: coronary artery disease; CABG: coronary artery bypass grafting; eGFR: estimated glomerular filtration rate; estimated glomerular filtration rate; HDL-chol: high density lipoprotein cholesterol; LDL-chol: low density lipoprotein cholesterol; NT-proBNP: N-terminal pro-B-type natriuretic peptide; TAVI: transcatheter aortic valve implantation; T-chol: total cholesterol

Table S2. Preoperative and postoperative echocardiogram data

	Total N = 121	Patients with early mortality N = 20	Patients with incomplete echocardiographic follow-up N = 11	P value*	P value †
Preoperative data					
LVDd, mm	50 [47, 53]	49 [47, 55]	52 [49, 56]	0.841	0.299
LVDs, mm	31 [29, 34]	33 [28, 41]	32 [30, 35]	0.421	0.670
EF	64 [60, 68]	62 [53, 68]	65 [59, 67]	0.169	0.737
LV Mass Index	111 [100, 124]	122 [113, 140]	113 [94, 122]	0.038	0.925

Peak velocity	4.20 [3.90, 4.60]	4.40 [3.90, 4.85]	3.90 [3.26, 4.40]	0.253	0.138
Mean gradient	44 [37, 52]	48 [37, 56]	38 [25, 45]	0.547	0.095
Aortic valve area, cm^2	0.94 [0.82, 1.14]	0.87 [0.77, 1.04]	1.05 [0.90, 1.19]	0.098	0.351
Postoperative data					
Peak velocity	2.40 [2.00, 2.70]	2.45 [2.02, 2.98]	2.30 [1.95, 2.85]	0.551	0.884
Mean gradient	12 [9, 16]	13 [7, 19]	12 [9, 17]	0.873	0.714
Effective orifice area, cm2	2.12 [1.70, 2.49]	2.12 [1.48, 2.64]	2.10 [1.83, 2.33]	0.751	0.965
Effective orifice area index, cm2/m2	1.05 [0.87, 1.29]	1.11 [0.84, 1.26]	1.07 [0.91, 1.13]	0.900	0.997
Patient-prosthesis mismatch, n (%)				0.953	0.789

None	93 (76.9)	18 (75.0)	9 (81.8)		
Moderate	20 (16.5)	4 (16.7)	1 (9.1)		
Severe	8 (6.6)	2 (8.3)	1 (9.1)		
Prosthetic regurgitation				0.963	0.714
None	110 (90.9)	21 (91.3)	11 (100)		
Mild	11 (9.1)	2 (8.7)	0 (0.0)		

P* value for comparison of study population versus patients who died without echocardiography follow-up.

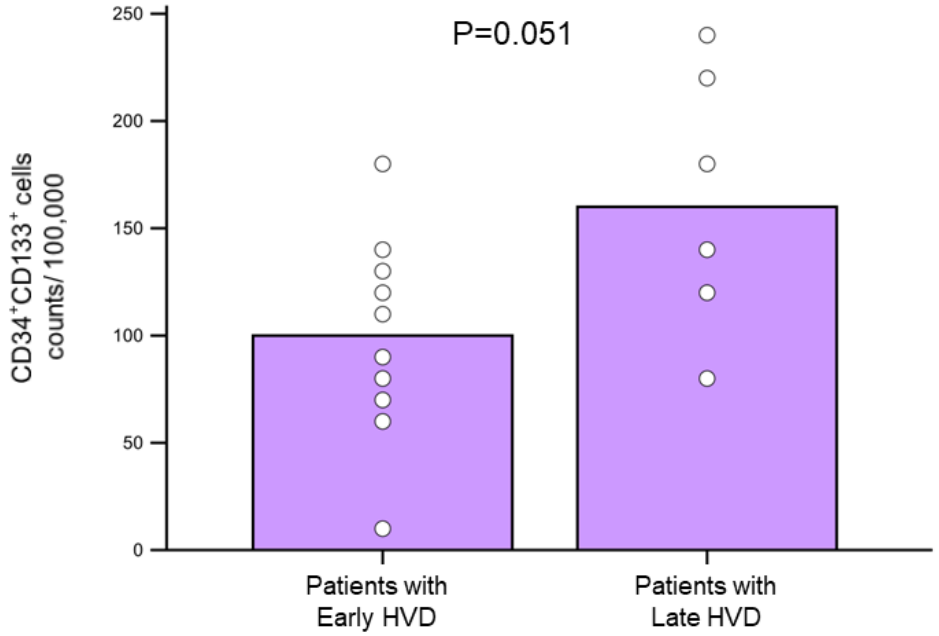
P† value for comparison between study population and patients with incomplete echocardiography follow-up.

EF: ejection fraction; LV: left ventricle; LVDd: left ventricular end-diastolic diameter; LVDs: left ventricular end-systolic diameter

Table S3. The association between CPC levels and preoperative mean gradient, annualized change in mean gradient

	Preoperative mean gradient		Annualized change in mean gradient	
	Correlation coefficient	**P**	**Correlation coefficient**	**P**
$CD34^+$ cells	0.168	0.068	0.004	0.964
$CD34^+CD133^+$ cells	0.032	0.726	-0.106	0.261
OCN^+ cells	0.032	0.726	-0.002	0.987

Figure S1. Comparison of time to event in patients with early and late HVD



The level of CD34+CD133+ cells tended to be higher in the patients with late valve degeneration than those with early HVD when divided into two groups by the median follow-up duration (5.9years).