Novel Cytokine Score and Cardiac Allograft Vasculopathy

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To date, there are no established noninvasive biomarkers available for prediction of cardiac allograft vasculopathy (CAV) after orthotopic heart transplantation (OHT). Inflammatory processes are supposed to play a central role in the pathogenesis of CAV. Recent studies have suggested that immune mediators could serve as biomarkers for cardiovascular diseases. We hypothesized particular cytokines or a combination thereof may serve as noninvasive biomarkers for CAV. Plasma cytokines were screened from 27 patients with CAV and 27 patients without CAV after OHT. The concentrations of interleukins-4, -6, -10, -21, -23, -31, -33, interferon gamma, tumor necrosis factor alpha, and the soluble activation marker CD40 ligand were determined using Luminex-based multiplex analyses. Although concentrations of all cytokines except interferon gamma were on average higher in the CAV group, there were no significant differences between the groups for any 1 cytokine. Using a binary logistic regression model, we were able to develop a probability score for detecting patients at elevated risk for advanced CAV with a sensitivity of 92.31% and a specificity of 60.71% (receiver-operating characteristic area under the curve 0.799 ± 0.06; $p < 0.0001$). In conclusion, analyzing the concentration of specific inflammatory cytokines could be meaningfully included in evaluation of CAV after OHT. **(Am J Cardiol 2019;123:1114−1119)**

Cardiac allograft vasculopathy (CAV) is one of the most common and challenging long-term complications after orthotopic heart transplantation (OHT). Lesions detected by coronary angiography are present in 7.8% of patients within the first year after OHT, increasing from 30% in a 5-year follow-up to 50% within 10 years.[1] The registry of the International Society for Heart and Lung Transplantation (ISHLT) reveals CAV as a leading cause of death beyond the first year after OHT that implicates decline in overall survival.[2] Repetitive performance of routine coronary angiography remains the recommended procedure for evaluation of CAV.[3,4] However, in addition to high cost and stress for the patient, coronary angiography is an invasive procedure that involves various risks.[5,6] Therefore, it is of great importance to find an alternative, noninvasive diagnostic method for detection of CAV. Several risk factors of coronary artery disease play a role in the development of CAV, but in general it is primarily triggered by inflammatory processes reflecting chronic rejection. We hypothesized that the concentration of particular inflammatory mediators in plasma could be detected and serve as noninvasive diagnostic biomarkers of CAV.

Methods

All 293 patients after OHT currently treated in our outpatient clinic were screened for inclusion in our pilot study. Patients with signs of severe heart failure or acute infection at the time of blood sampling, and patients with a history of cancer or severe renal failure (serum creatinine >200 μmol/L) were excluded. According to these criteria and results of routinely performed coronary angiography, a total of 27 patients with confirmed advanced CAV defined as ISHLT CAV_2 or CAV_3 were included in the study. The control group was composed of 27 patients without angiographic signs of CAV (ISHLT CAV_0). Minimum postoperative follow-up time for the patients assigned to the non-CAV group was set at 5 years (Figure 1). Diagnosis of CAV was made by coronary angiography performed according to current recommendations of the ISHLT.[7] CAV severity was assessed according to the standardized international nomenclature defined as ISHLT CAV_0 (not significant), CAV_1 (mild), CAV_2 (moderate), and CAV_3 (severe).[8] Further information on the patients' clinical histories such as initial cardiac disease, cardiovascular risk factors, cytomegalovirus infection, rejection periods, and demographics were documented. Hypertension was defined according to current guidelines of the European Society of Cardiology.[9] Blood samples were drawn from a peripheral vein during regular follow-up visits. Blood was collected in standard tubes and stored immediately at 4°C. Within

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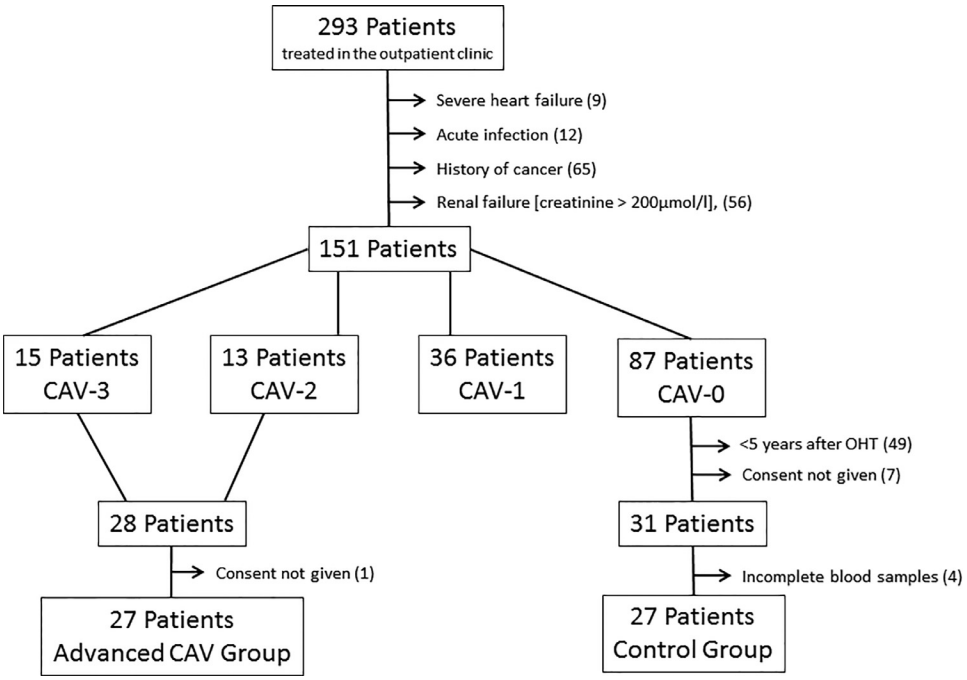

Figure 1. Study flow chart.

2 hours of blood withdrawal, each plasma sample was centrifuged for 15 minutes at 300g at room temperature. Supernatants were stored in ribonuclease/deoxy-ribonuclease free cryovials at −80°C until analyzed. Plasma samples were subjected to Luminex-based multiplex analyses for the quantification of soluble immune mediators by the human Th17-plex (BioRad, Hercules, California) according to the manufacturer's instructions. In brief, 25-μl plasma was diluted with 25-μl sample diluent and incubated with magnetic beads loaded with capture antibodies for 15 soluble factors, that is, interleukins-1β, -4, -6, -10, -17A, -17F, -21, -22, -23, -25, -31, -33, interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), and the soluble activation marker CD40 ligand. Streptavidin-phycoerythrin was used for detection and quantification. For each sample and each cytokine, 50 to 100 beads were measured and the mean fluorescence intensity of these beads was used for quantification of each cytokine using individual standard curves. All concentrations were determined as pg/ml and the dynamic range was between 1 pg/ml and 40 ng/ml. The 10 proteins with clear detectable values within detection limits (i.e., interleukins-4, -6, -10, -21, -23, -31, and -33, IFNγ, TNFα, and soluble activation marker CD40 ligand) were included in further analyses.

Statistical analyses were performed using MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) and SPSS 24 (Armonk, New York). Normal distribution of values was tested according to Kolmogorov-Smirnov. Values are expressed as means ± standard error for normally distributed values and as interquartile ranges for those that were not normally distributed. A chi-square test or, if appropriate, a Fisher's exact test was used to assess differences among variables with dichotomous, categorical, or normally distributed values. The nonparametric Mann-Whitney *U* test was performed to evaluate differences between non-normally distributed continuous values between the 2 groups. The concentrations of all measured cytokines were entered into a binary logistic regression model yielding the probability for having CAV. The validity of the resulting CAV-cytokine score was assessed with the Hosmer-Lemeshow test, and the area under the curve of the resulting receiver-operating characteristic was determined. The regression equation, including the constant, the coefficients, and a calculation instruction are given in Figure 2. Differences were considered either not significant ($p \geq 0.05$) or significant ($p < 0.05$). The study was approved by the Ethics Committee of Hannover Medical School, Hannover, Germany (no. 1610-2012). Written informed consent was obtained from all participants.

$$\text{CAV probability} = \frac{1}{1 + 2.71828^{-(\sum_a^j - 3.10403)}}$$

a = - 0.03909 x IL4 [pg/ml]
b = 0.08784 x IL6 [pg/ml]
c = - 0.00129 x IL10 [pg/ml]
d = 0.02397 x IL21 [pg/ml]
e = 0.02365 x IL23 [pg/ml]
f = 0.08847 x IL31 [pg/ml]
g = 0.02139 x IL33 [pg/ml]
h = - 0.00550 x sCD40L [pg/ml]
i = - 0.31795 x IFNγ [pg/ml]
j = 0.10802 x TNFα [pg/ml]

Figure 2. Representation of CAV-cytokine score: calculation of CAV cytokine score estimated from the concentrations of cytokines using a logistic regression equation.

Table 1.
Characteristics of patients with cardiac allograft vasculopathy (CAV) and controls

	Number (%) or median (IQR)		
	CAV (n = 27) %	Controls (n = 27)	p value
Age [years] (mean ± SD)	60 ± 13	54 ± 16	0.436
Men			
Active smoking	23 (85%)	21 (78%)	0.728
Left ventricular ejection fraction	60 (60. . .65)%	62.5 (60. . .65)%	0.164
Years after heart transplantation	12.0 (7.2. . .16.6)	11.8 (5.6. . .14.8)	0.401
Assessment of CAV (Angiography)	100%	100%	
Cyclosporine A	14 (52%)	20 (74%)	0.158
Tacrolimus	12 (44%)	7 (26%)	0.254
Mycophenolate	21 (78%)	14; (52%)	0.086
Everolimus	5 (19%)	9 (33%)	0.352
Low-dose corticosteroids	5 (2.5. . .5)	2.5 (2.5. . .5)	0.058
Double therapy	7 (26%)	10 (37%)	0.559
Triple therapy	20 (74%)	17 (63%)	0.559
Hypertension	11 (41%)	11 (41%)	1
Early rejection	7 (26%)	3 (11%)	0.293
			0.395
Human leukocyte antigen mismatch (mean ± SD)	5.8 ± 2.4	5.6 ± 2.6	0.828
Human leukocyte antigen antibodies	0	0	1
Cytomegalovirus infection	4 (15%)	5 (19%)	1
Body mass index	28.0 (25.7. . .30.5)	27.4 (24.0. . .31.1)	0.952
Diabetes mellitus	8 (30%)	1 (4%)	0.024
Hyperlipidemia	26 (96%)	23 (85%)	0.351
Dilatative cardiomyopathy	19 (70%)	21 (78%)	0.757
Ischemic cardiomyopathy	6 (22%)	3 (11%)	0.467
Congenital heart disease	1 (3.7%)	2 (7.4%)	1
Hypertrophic obstructive cardiomyopathy	1 (3.7%)	0	1
Valvular heart disease	0	1 (3.7%)	1

Results

Both groups were comparable concerning age, gender, follow-up after OHT, prevalence of previous acute rejection, human leukocyte antigens mismatch and antibodies as well as cytomegalovirus infection, body mass index, hypertension, or hyperlipidemia. Only one of the known nonimmunologic risk factors—diabetes—was significantly more prevalent in the CAV group (Table 1, Supplementary Table 3A and B). All patients were nonsmokers. There were no significant differences between CAV and control patients concerning the administration of immunosuppression. Although the patients in the CAV group received on average more mycophenolate and low-dose corticosteroids than patients without CAV, the differences did not reach level of significance. The underlying cardiovascular pathologies before OHT were dilated cardiomyopathy (74%), ischemic cardiomyopathy (16.7%), and congenital heart disease (5.6%). One patient had valvular heart disease and 1 had hypertrophic obstructive cardiomyopathy. At the time of blood sampling, all patients had C-reactive protein and white blood cell values within the normal range. The distribution of affected vessels in the CAV-group was as follows: single left anterior descending (LAD) (n = 5; 18.5%), left circumflex (LCX) (n = 2; 7.4%), right coronary artery (RCA) (n = 6; 22.2%), left main coronary artery (LMCA) (n = 1; 3.7%), 2-vessel disease (n = 9; 33.3%), and 3-vessel disease (n = 4; 14.8%). Plasma concentrations of immune mediators revealed slightly higher activities in patients with advanced CAV for all tested cytokines except IFNγ (Figure 3, Supplementary Table 4), whereas none of these differences were statistically significant (Table 2). Merely the differences in concentrations of interleukin-6 and TNFα were close to statistical significance (p = 0.06 and p = 0.08,

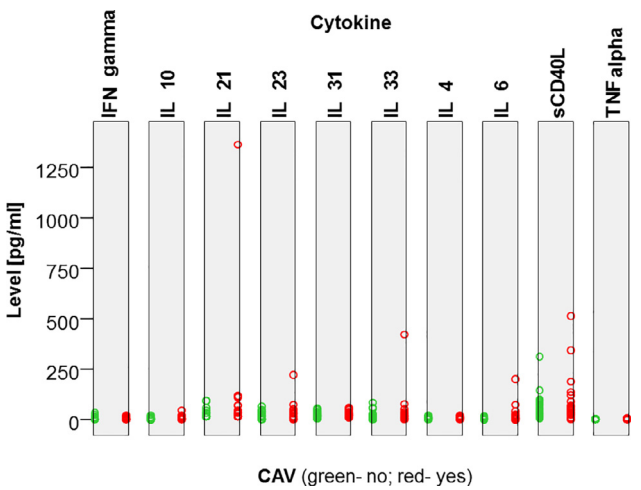

Figure 3. Overview of the plasma concentrations of 10 selected cytokines: analysis of concentrations of selected immune mediators in the plasma of patients with CAV after OHT (n = 27) compared with a control group consisting of patients without any angiographic signs of CAV at least 5 years after OHT (n = 27).

Table 2
Cytokine values of patients with CAV and controls

	Mean ± standard error [pg/ml]		
Cytokine	CAV (n = 27) %	Controls (n = 27)	p value
Interferon gamma	4.3 ± 1.1	4.8 ± 1.6	0.756
Interleukin 10	8.4 ± 1.8	5.4 ± 0.9	0.155
Interleukin 21	85.7 ± 49.5	28.0 ± 4.2	0,312
Interleukin 23	26.4 ± 8.4	17.6 ± 3.5	0.618
Interleukin 31	28.3 ± 2.5	24.2 ± 2.7	0.208
Interleukin 33	33.5 ± 15.3	17.4 ± 3.9	0.380
Interleukin 4	8.9 ± 1.1	8.5 ± 0.9	0.808
Interleukin 6	20.6 ± 7.6	6.9 ± 0.9	0.061
Soluble activation marker CD40 Ligand	78.9 ± 21.6	62.9 ± 11.7	0.742
Tumor necrosis factor alfa	3.1 ± 0.4	2.4 ± 0.2	0.080

respectively). Implementation of analyzed cytokine concentrations into a binary logistic regression model allowed us to set a score for assessment of probability for having CAV. Calculation of receiver-operating characteristic yielded statistical significance of sensitivity and specificity for the score with an area under the curve of 0.799 ± 0.06 ($p < 0.0001$; Figure 4). Applying the score revealed a clear differentiation among CAV patients with a calculated probability of 62.14% (confidence interval 55.0 to 69.3) compared with non-CAV patients with a calculated probability of 35.16% (confidence interval 27.5 to 42.9; Figure 5). The difference tested by a 2-sided *t* test for unpaired samples was highly significant ($p < 0.00001$).

Discussion

Several efforts have been made to find a noninvasive biomarker of CAV. Brain natriuretic peptide and C-reactive protein have been suggested as biomarkers for the development of CAV.[10] Although both show a certain sensitivity and involvement in the development of CAV, they lack sufficient specificity.[11,12] Recently, Singh et al reported that endothelium-enriched microRNAs could be used as biomarkers for CAV and our group published promising results concerning micro-RNA 155 and 628-5p.[4,13] Other studies have indicated the central role of inflammatory processes in the pathogenesis of CAV[14] or detected proinflammatory immune mediators in the context of coronary artery disease and myocardial infarction.[15] The current view of post-transplant graft vasculopathy is based on a landmark publication from Tellides et al showing that IFNγ, even in the absence of leukocytes, is able to mediate arterial intima proliferation leading to luminal obstruction.[3] Because lymphocytes are the major source of IFNγ, we hypothesized that other T cell-derived cytokines also could be associated with the development of CAV. Although the connection between proinflammatory mediators and the development of CAV has already been described, to our knowledge, there is no detailed analysis of the concentrations of circulating T-helper 1 and T-helper 2 cytokines in patients with angiographically confirmed CAV. We focused on the analysis of specific mediators that are involved in inflammatory processes, such as the T-helper cytokines IFNγ, TNFα, and interleukin-23; the T-helper 2 cytokines interleukins-4, -10 and -31; the T cell activation marker soluble activation marker CD40 ligand; as well as other proinflammatory cytokines such as interleukins-6, -21, and -33. Although we did not identify any singular immune mediator, we were able to develop a probability score for detecting patients at elevated risk for advanced CAV using a binary logistic regression model with 10 clear detectable immune mediators. This score revealed highly significant cumulative differences between patients with advanced CAV and without it (Figures 4 and 5), and is thus a candidate for a novel, noninvasive test. Elevated concentrations of IFNγ were rather less apparent in patients with CAV. So while there is evidence that IFNγ plays a role at atherosclerosis,[3] our results indicate a rather minor contribution of IFNγ to the development of CAV. This is in line with the

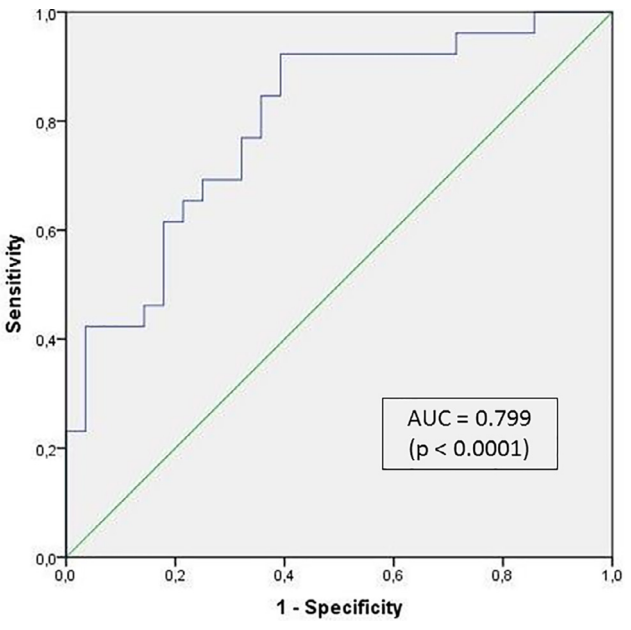

Figure 4. Receiver-operating characteristic and area under the curve analysis of CAV-cytokine score.

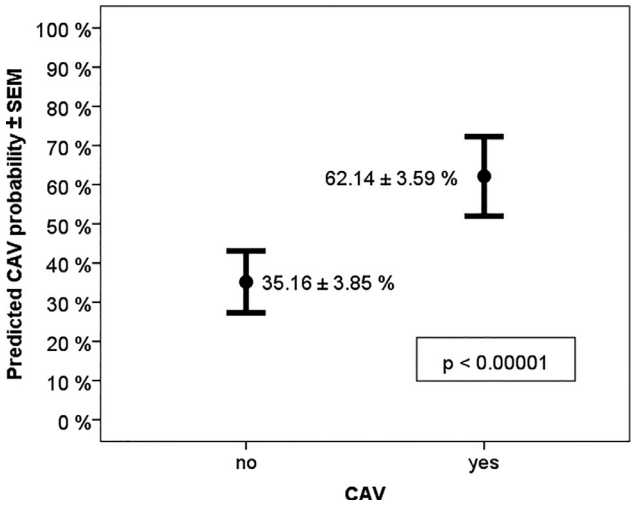

Figure 5. Representation of the predicted probability for CAV: prediction of CAV after OHT by cytokine score in the whole study group.

current view that although CAV resembles atherosclerosis, there are some important differences.[16,17] According to the study published by Roldan et al. showing an involvement of the humoral immune system and alloreactive T cells in the development of CAV, the ratio of these may also be a marker for risk of CAV.[18] In our study, all cytokines except IFNγ showed on average higher concentrations in the blood of CAV patients. However, only interleukin-6 and TNFα, which are likely important players in the inflammatory reaction during CAV, showed nearly significant differences. Both immunological and nonimmunological factors have been implicated in the development of CAV. The immunological reaction of the recipient causes fundamental pathophysiological changes in the transplanted organ and nonimmunological factors such as hypertension, hyperlipidemia, hyperglycemia, smoking, and viral infections contribute to the damaging process of CAV.[17,19] In addition to donor age, various "sterile" (e.g., ischemia and reperfusion time of the transplanted heart) and pathogenic (e.g., CMV infection) insults can result in endothelial damage in coronary vessels. As a consequence of the endothelial damage, inflammatory mediators, cytokines, chemokines, as well as the complement system are locally activated.[20] This immunological reaction leads to local inflammation, thrombosis, vasoconstriction, intimal hyperplasia, and smooth muscle cell proliferation. Initial endothelial damage seems to be the determining factor in the development of CAV.[16] Recipients receiving a transplanted heart induce an innate immune response. Myeloid cells, natural killer cells, and interleukin-6 are the main mediators of the innate immune response and therefore influence the progression of CAV.[21] Recent investigation of different cytokines that are involved in the activity of natural killer cells have shown higher interleukin-6 concentrations in transplanted hearts with evident CAV lesions, whereas transplanted hearts with no signs of CAV expressed little interleukin-6.[22] In agreement with these reports, our data revealed nearly significant higher concentrations of interleukin-6 in patients with CAV. Of note, the upregulation of the proinflammatory monocyte-derived interleukin-6 indicates that monocyte activation may play a major role in CAV development. In addition, endothelial cells can secrete interleukin-6 upon stimulation with damage-associated cytokines like interleukin 1β (data not shown), arguing for a combined inflammatory response of the endothelial system and innate immune cells. Our study has some limitations. First, this is a single-center study. Second, most patients in this study were male. As male gender has been described as a risk factor for CAV, this may have influenced our results. Third, there was more diabetes among patients with CAV. Although the impact of diabetes on concentration of cytokines is not clear, it is associated with chronic inflammation and could therefore influence the result. We did not include patients with early or developing CAV (ISHLT CAV_1). We chose to compare patients with extreme phenotypes, to increase the probability of discovering differences. Our study did not examine cytokine levels at different time points after OHT. Patients with advanced kidney impairment (creatinine >200 μmol/L) were excluded from the study. Because many patients after OHT simultaneously develop renal insufficiency and CAV, our results cannot be transferrable to all patients after OHT. In conclusion, assessment of a single cytokine or immune mediator does not allow identification of patients with CAV. Combining the concentrations of interleukins-4, -6, -10, -21, -23, -31, and -33, IFNγ, TNFα, and soluble activation marker CD40 ligand led to the development of a cytokine score with high sensitivity and specificity, which seems to be associated with established CAV. Please follow https://www.mh-hannover.de/ambulanz.html to test the score.

Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.amjcard.2018.12.034.

1. Lund LH, Edwards LB, Dipchand AI, Goldfarb S, Kucheryavaya AY, Levvey BJ, Meiser B, Rossano JW, Yusen RD, Stehlik J. International Society for Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-third adult heart transplantation report-2016; focus theme: primary diagnostic indications for transplant. *J Heart Lung Transplant* 2016;35:1158–1169.
2. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, Levvey BJ, Lund LH, Meiser B, Rossano JW, Stehlik J. The registry of the International Society for Heart and Lung Transplantation: Thirty-second official adult lung and heart-lung transplantation report-2015; focus theme: early graft failure. *J Heart Lung Transplant* 2015;34:1264–1277.
3. Tellides G, Tereb DA, Kirkiles-Smith NC, Kim RW, Wilson JH, Schechner JS, Lorber MI, Pober JS. Interferon-gamma elicits arteriosclerosis in the absence of leukocytes. *Nature* 2000;403:207–211.
4. Singh N, Heggermont W, Fieuws S, Vanhaecke J, Van Cleemput J, De Geest B. Endothelium-enriched microRNAs as diagnostic biomarkers for cardiac allograft vasculopathy. *J Heart Lung Transplant* 2015;34:1376–1384.
5. Kass M, Allan R, Haddad H. Diagnosis of graft coronary artery disease. *Curr Opin Cardiol* 2007;22:139–145.
6. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation* 2008;117:2131–2141.
7. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, Fedson S, Fisher P, Gonzales-Stawinski G, Martinelli L, McGiffin D, Smith J, Taylor D, Meiser B, Webber S, Baran D, Carboni M, Dengler T, Feldman D, Frigerio M, Kfoury A, Kim D, Kobashigawa J, Shullo M, Stehlik J, Teuteberg J, Uber P, Zuckermann A, Hunt S, Burch M, Bhat G, Canter C, Chinnock R, Crespo-Leiro M, Delgado R, Dobbels F, Grady K, Kao W, Lamour J, Parry G, Patel J, Pini D, Towbin J, Wolfel G, Delgado D, Eisen H, Goldberg L, Hosenpud J, Johnson M, Keogh A, Lewis C, O'Connell J, Rogers J, Ross H, Russell S, Vanhaecke J. International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010;29:914–956.
8. Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, Madsen J, Parameshwar J, Starling RC, Uber PA. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010;29:717–727.

9. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F. Task force for the management of arterial hypertension of the European Society of Hypertension and the European Society of Cardiology. 2013 ESH/ESC Practice Guidelines for the management of arterial hypertension. *Blood Press* 2014;23:3–16.
10. Lin D, Cohen Freue G, Hollander Z, John Mancini GB, Sasaki M, Mui A, Wilson-McManus J, Ignaszewski A, Imai C, Meredith A, Balshaw R, Ng RT, Keown PA, Robert McMaster W, Carere R, Webb JG, McManus BM. Plasma protein biosignatures for detection of cardiac allograft vasculopathy. *J Heart Lung Transplant* 2013;32:723–733.
11. Shaw SM, Williams SG. Is brain natriuretic peptide clinically useful after cardiac transplantation? *J Heart Lung Transplant* 2006;25:1396–1401.
12. Labarrere CA, Lee JB, Nelson DR, Al-Hassani M, Miller SJ, Pitts DE. C-reactive protein, arterial endothelial activation, and development of transplant coronary artery disease: a prospective study. *Lancet* 2002;360:1462–1467.
13. Neumann A, Napp LC, Kleeberger JA, Benecke N, Pfanne A, Haverich A, Thum T, Bara C. MicroRNA 628-5p as a novel biomarker for cardiac allograft vasculopathy. *Transplantation* 2017;101:e26–e33.
14. Szygula-Jurkiewicz B, Szczurek W, Gasior M, Zembala M. Risk factors of cardiac allograft vasculopathy. *Kardiochir Torakochirurgia Pol* 2015;12:328–333.
15. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res* 2002;53:31–47.
16. Avery RK. Cardiac-allograft vasculopathy. *N Engl J Med* 2003;349:829–830.
17. Valantine H. Cardiac allograft vasculopathy after heart transplantation: risk factors and management. *J Heart Lung Transplant* 2004;23:S187–S193.
18. Roldan C, Mirabet S, Cecilia C, Brossa V, Roig E, Martin C, Gelpi C. CD4+CD45RO+CD25-/lowCD127+: CD4+CD45RO+CD25hiCD127-/low ratio in peripheral blood: a useful biomarker to detect cardiac allograft vasculopathy in heart transplanted patients. *Transplantation* 2015;99:1521–1528.
19. Caforio AL, Tona F, Fortina AB, Angelini A, Piaserico S, Gambino A, Feltrin G, Ramondo A, Valente M, Iliceto S, Thiene G, Gerosa G. Immune and nonimmune predictors of cardiac allograft vasculopathy onset and severity: multivariate risk factor analysis and role of immunosuppression. *Am J Transplant* 2004;4:962–970.
20. Heim C, Gocht A, Weyand M, Ensminger S. New targets for the prevention of chronic rejection after thoracic organ transplantation. *Thorac Cardiovasc Surg* 2018;66:20–30.
21. Millington TM, Madsen JC. Innate immunity and cardiac allograft rejection. *Kidney Int Suppl* 2010;119:S18–S21.
22. Zhang ZX, Huang X, Jiang J, Lian D, Min WP, Liu W, Haig A, Jevnikar AM. Natural killer cells play a critical role in cardiac allograft vasculopathy in an interleukin-6-dependent manner. *Transplantation* 2014;98:1029–1039.