Biomarkers and Donor Selection in Heart Transplantation

Anne Vorlat[a,*], Nicolas De Hous[a], Alexander J. Vervaecke[a], Tom Vermeulen[a], Emeline Van Craenenbroeck[a], Hein Heidbuchel[a], Inez Rodrigus[b], Walter Van Donink[b], Arnaud Ancion[c], Johan Van Cleemput[d], Viviane O. Van Hoof[e,f], and Marc J. Claeys[a]

[a]Department of Cardiology, University Hospital of Antwerp, Edegem, Belgium; [b]Department of Cardiac Surgery, University Hospital of Antwerp, Edegem, Belgium; [c]Department of Cardiology, University Hospital Sart Tilman, Liege, Belgium; [d]Department of Cardiology, University Hospital of Leuven, Leuven, Belgium; [e]Department of Clinical Chemistry, Antwerp University Hospital, Edegem, Belgium; and [f]Translational Pathophysiological Research Group, University of Antwerp, Edegem, Belgium

ABSTRACT

Background. Previously, we showed that B-type natriuretic peptide (BNP) measured in the donor was related to cardiac performance after cardiac transplantation. The present study assesses the value of 3 biomarkers in the selection of donor hearts in a larger cohort.

Methods. Blood samples were prospectively obtained in 105 brain-dead patients scheduled for heart donation. BNP, soluble suppressor of tumorigenicity 2 (ST2), and troponin of heart donors were correlated with hemodynamic parameters early after transplantation as well as with the mortality of the recipients.

Results. A significant inverse relationship was found between donor BNP measured at the time of donation and recipient cardiac index and cardiac output at day 13 post-transplantation ($r = -0.31$, $P = .005$, and $r = -0.34$, $P = .0016$, respectively). Logistic regression analysis—including BNP, ST2, and troponin—showed that donor BNP was a predictor of a poor cardiac index (< 2.2 L/min/m^2) in the recipient ($P = .04$). A donor BNP > 132 pg/mL has a sensitivity of 56% (95% confidence interval 21–86) and a specificity of 86% (95% confidence interval 77–93) to predict poor cardiac performance in the recipient. When the donor BNP is ≤ 132 pg/mL, the risk of a poor cardiac function in the recipient is very low (negative predictive value 94%). Mortality at 30 days was also correlated to donor BNP ($r = 0.29$, $P = .0029$). Long-term survival of the recipient was not correlated to the biomarkers measured in the donor.

Conclusion. Donor BNP, but not donor ST2 or high-sensitivity troponin, provides information on the donor heart and early post-transplant performance, including 1-month mortality.

SINCE the first heart transplantation in 1967, the selection of suitable donor hearts has been a critical step in the success of heart transplantation. Primary graft failure and other complications, such as right-sided heart failure due to pulmonary hypertension and acute rejection, are major reasons for early mortality [1–3]. Ample evidence exists that the selection of suboptimal donor hearts increases the risk of primary graft failure [4]. However, with the increasing need for donor hearts, marginal donor hearts have been used more frequently to bridge the gap between supply and demand [5]. Until recently, risk stratification of donor hearts has been mainly based on clinical and echocardiographic assessments [6]. The limitations of this approach have been previously documented and are related to inaccuracies of echocardiographic cardiac function evaluation to predict outcome in brain-death donors at high sympatic stress [6–8].

*Address correspondence to Anne Vorlat, MD, PhD, Department of Cardiology, University Hospital of Antwerp, Wilrijkstraat 10, B-2650 Edegem, Belgium. Tel: +3238214769. E-mail: anne.vorlat@uza.be

0041-1345/19
https://doi.org/10.1016/j.transproceed.2019.04.041

Recently, the value of cardiac biomarkers as objective tools in donor assessment has been investigated. The cardiac troponins as markers of myocardial necrosis, the B-type natriuretic peptide (BNP) as a marker of cardiac function, and soluble suppression of tumorigenicity 2 (ST2), a marker of myocardial fibrosis, have all been considered of interest for the assessment of potential donor hearts. The objective of the present work is to investigate if any of these markers measured in the donor are related to early cardiac performance and prognosis in the transplant recipient. In the past, we have shown in a small cohort that donor BNP was related to cardiac function in the recipient and could be of use in the donor selection procedure [7]. This finding needed to be confirmed in a larger cohort.

METHODS

Study Population and Donor Selection

All donors hospitalized at the Antwerp University Hospital and its affiliated hospitals were considered eligible for this study. A total of 105 brain-dead patients were prospectively included in the study between November 2005 and August 2016. Brain death was confirmed using a standardized protocol approved by the ethics committee of the Antwerp University Hospital and according to international guidelines. Three independent medical doctors, not involved in the donor procedure, confirmed the diagnosis. All data were obtained in accordance with Belgian laws concerning organ procurement and after authorization of the ethics committee of the Antwerp University Hospital.

Evaluation of the suitability for heart donation was based on various parameters recorded in all brain-dead donors: age, sex, body mass index, cause of death, time of brain death, blood pressure, diuresis, medical history, use of inotropic agents or other medications, and use of resuscitation measures. Risk factors such as hypertension, diabetes mellitus, smoking, and coronary heart disease were registered. Cardiac status was assessed by echocardiography and electrocardiography. If necessary, coronary angiography was added to the assessment (eg, due to older age and/or severe risk factors for coronary artery disease). Abdominal ultrasound was performed to determine other possible comorbidities. Serum samples were collected to evaluate the renal, hepatic, and cardiac functions and to determine the presence of tissue necrosis.

Additionally, cold and warm ischemic time and extracorporeal circulation were recorded.

Cardiac Biomarkers

At the time of donor evaluation and confirmed brain death, plasma on EDTA was collected in all 105 patients and immediately centrifuged and stored at -80°C for future biomarker analysis. BNP was measured with an immunoassay instrument (Access 2, Beckman Coulter, Inc, Analis, Namur, Belgium). BNP reagents obtained from Biosite (San Diego, Calif, United States) were used, with chemiluminescent detection after separation of magnetic particles coated with mouse anti-human BNP antibodies. For the first 56 patients, troponin I was measured with the Access 2 immunoassay instrument (Beckman Coulter, Inc, Analis, Namur, Belgium) with troponin I reagents (Beckman Coulter). For the remaining 41 patients, measurement of high-sensitivity troponin T was performed using a modular immunoassay instrument (Cobas 6000, Roche Diagnostics, Inc, Vilvoorde, Belgium) with Roche reagents. To achieve a uniform troponin assay, a conversion factor was applied to calculate high-sensitivity troponin T from troponin I, based on previous work [9]. ST2 was determined with the Aspect-PLUS ST2 Rapid Test (Critical Diagnostics Inc, Dublin, Ireland). Importantly, biomarker results were not available at the time of donor evaluation and selection.

Cardiac Performance of Heart Recipients

For heart recipients, cardiac performance was evaluated during right-sided heart catheterization (Swan-Ganz catheter, thermodilution method) at day 13 (range 10–16) after transplantation. A cardiac hemodynamic evaluation was available for analysis in 83 of the cardiac transplant recipients. The remaining 22 of the 105 recipients were excluded because of the following reasons: unavailability of hemodynamic data due to the donor hearts being exported abroad (n = 13), early death (n = 7), and histologic rejection shown by endomyocardial biopsy at the time of the first hemodynamic evaluation (n = 2). The following parameters were registered: cardiac output (CO), cardiac index (CI), mean pulmonary artery pressure, and mean pulmonary capillary wedge pressure. A poor cardiac function was defined as a CI < 2.2 L/min/m^2 at the time of the first biopsy after transplantation. To evaluate the independent predictors of a poor CI (<2 .2 L/min/m^2) in the recipient, donor BNP, ST2, and troponin, donor body mass index, donor age, donor sex, and recipient age were used in a logistic regression analysis.

Clinical Outcome

Length of hospital stay was registered. In a multivariate stepwise linear regression analysis, age of the recipient, urgency state of the recipient on the transplantation waiting list, an assist device prior to heart transplantation, and donor BNP were evaluated as determinants. Long-term survival was assessed by calculating the number of days from the transplantation date until February 1, 2017. All-cause mortality was assessed at 1 month and during the follow-up period. One month mortality of the recipient was correlated to the individual biomarkers in the donor. For long-term mortality, donor age, sex, biomarkers (BNP, ST2, and troponin), and age of the recipient were considered.

Statistical Methods

Variables were expressed as the median with their 25th and 75th percentile (interquartile) range. A Pearson correlation coefficient was calculated to describe the correlation between biomarkers and cardiac function. Intergroup analysis was done using the Mann-Whitney U-test. Logistic regression was used to assess the predictor variable of poor cardiac function (CI < 2.2 L/min/m^2) in the recipient. The cut-off value for BNP to predict a poor cardiac performance in the recipient was calculated using a receiver operating characteristic curve. A Cox proportional-hazards regression analysis was performed to analyze the independent predictors of long-term survival. A P value $< .05$ was considered statistically significant. Statistical analyses were performed with MedCalc, version 17.5.5, software (MedCalc Software bvba, Ostend, Belgium).

RESULTS

Baseline Characteristics of Heart Donors

Between November 5, 2005, and August 25, 2016, 105 heart transplantations were included. Baseline characteristics of the 105 heart donors are depicted in Table 1.

Table 1. Baseline Characteristics of the Heart Donors

Parameter	Results
Age (y)	38 (27–45)
Male (%)	65
BMI (kg/m^2)	24.0 (22.8–27.0)
Hypertension (%)	11
Smoking (%)	35
Cardiac arrest (%)	14
Inotropic support (%)	81
Norepinephrine dose (μg/kg/min)	0.14 (0.06–0.24)
LVEF > 60% (echocardiography) (%)	97
Creatinine (mg/dL)	0.77 (0.61–1.01)
CRP (mg/L)	75.0 (20.1–156.5)
BNP (pg/mL)	56.2 (26.0–120.5)
ST2 (ng/mL)	221.1 (113.5–341.8)
hsTnT (ng/mL) (n = 41)	14.17 (4.65–35.70)

Results are presented as median (interquartile range).
Abbreviations: BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; hsTnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; ST2, suppression of tumorigenicity 2.

Echocardiography at the time of cardiac evaluation established a good systolic left ventricle function (defined as an ejection fraction > 60%) in 102 heart donors (97%) and a slightly diminished left ventricle function (defined as an ejection fraction < 60% but >49%) in 3 donors (2.9%). The results from electrocardiography showed only minor disturbances, with 1 donor having a right bundle branch block, 1 donor showing a prolonged QT-interval, and 1 donor showing ST abnormalities with a negative T wave in V_1 to V_4. For the results of the biomarkers in the donors, see Table 1. Importantly, none of the biomarker results were available at the time of donor selection.

Post-transplant Cardiac Performance in the Recipient

A cardiac hemodynamic evaluation was available for analysis in 83 of the cardiac transplant recipients and was carried out at a median of 13 days (range 10–16) after transplantation. The hemodynamic measurements revealed a median CO of 5.6 L/min (range 4.5–6.3), a median CI of 2.9 L/min/m^2 (range 2.5–3.1), a median pulmonary artery pressure of 24 mm Hg (range 18–30), and a mean pulmonary capillary wedge pressure of 17 mm Hg (range 11–22).

In the univariate analyses, BNP measured at the time of heart donation showed a significant inverse relation with CI and CO measured in the recipient (r = −0.31, P = .005 and r = −0.34, P = .0016, respectively), as shown in Figs 1 and 2. Donor BNP was significantly higher for recipients with a poor cardiac function (CI < 2.2 L/min/m^2) (n = 9) than for those with good cardiac performance: BNP 134 pg/mL (range 31–306) vs 60 pg/mL (range 27–115) (P = .002). As opposed to donor BNP, neither donor ST2 nor donor troponin was significantly related to CI in the recipient (r = 0.01, P = .9385, and r = −0.03, P = .81, respectively).

Logistic regression analysis—including BNP, ST2, and troponin—showed that donor BNP was an independent predictor of a poor cardiac index (CI < 2.2 L/min/m^2) in the recipient (P = .04).

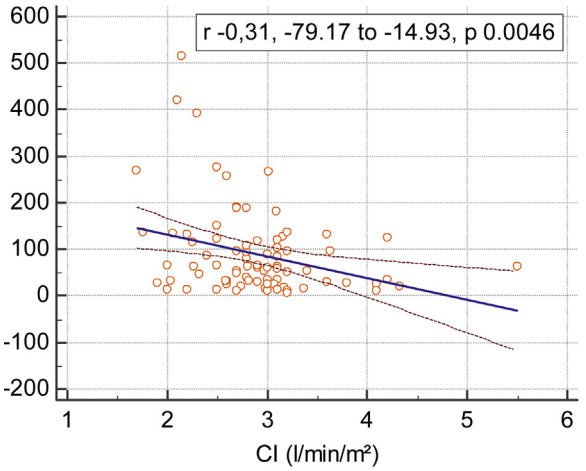

Fig 1. Donor BNP (pg/mL) and cardiac index (L/min/m^2) in the recipient.

A donor BNP value of > 132 pg/mL has a sensitivity of 56% (95% confidence interval 21–86), a specificity of 86% (95% confidence interval 85–98), a negative predictive value (NPV) of 94% (95% confidence interval 85–98), and a positive predictive value of 33% (95% confidence interval 12–61) for the prediction of poor cardiac function in the recipient. Patients with a BNP value ≤132 pg/mL had a 6% risk of having a poor cardiac function in the recipient, and this risk increased to 33% in patients with a BNP value of > 132 pg/mL.

Post-transplant Outcome and Relation with Cardiac Biomarkers

Data concerning mortality within 1 month after transplantation were available in all 105 patients. A total of 8 patients died within 1 month, due to early graft failure (n = 4), sepsis with multiple organ failures (n = 2), acute rejection (n = 1), or hemodynamic complications during the

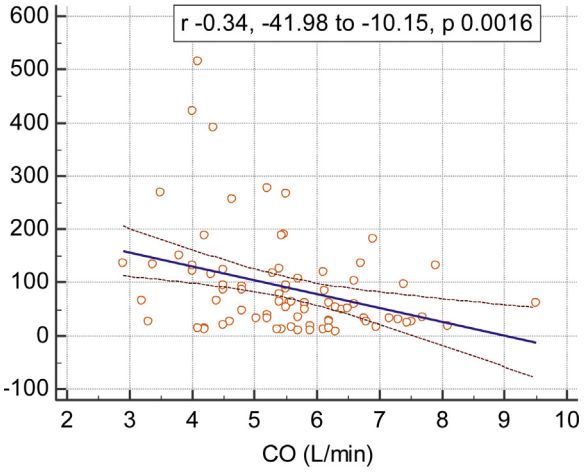

Fig 2. Donor BNP (pg/mL) and cardiac output in the recipient.

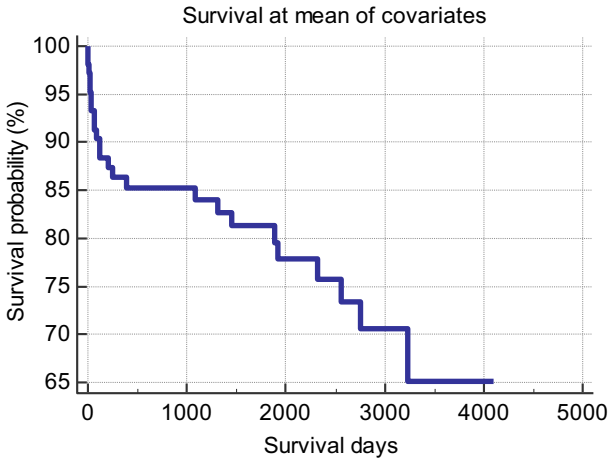

Fig 3. Cox proportional-hazards regression analysis for long-term survival.

transplant procedure (n = 1). Death within 1 month after transplantation was significantly correlated to BNP levels in the donor (r = 0.29, $P = .0034$). For those 8 patients, mean donor BNP ± 1 SD was 375 (± 868) pg/mL as compared to 93 (± 111) pg/mL for the surviving patients ($P = .0029$); median donor BNPs were 52 (range 29–152) and 56 (range 26–120), respectively.

Data for long-term survival were available in 96 transplant recipients. The overall mortality rate was 15% at 1 year and 35% at 10 years (see Fig 3). Median survival was 4.3 years (range 1.2–7.6). A Cox proportional-hazards regression analysis with donor age, sex, biomarkers (BNP, ST2, troponin), and age of the recipient established only the age of the recipient (odds ratio 1.05, 95% confidence interval 1.00–1.10) as a significant determinant.

We also looked at the length of hospitalization (LOS) in recipients who survived for more than 1 month. The median length of hospitalization was 22 days (range 18–36) days. In a univariate analysis, donor BNP was significantly correlated with the length of hospital stay for transplant recipients (r = 0.2472, $P = .039$). In a multivariate stepwise linear regression analysis, BNP was not significantly related with LOS. Only urgency state on the waiting list turned out to be the most important determinant of LOS ($P = .009$, $R^2 = 0.01$).

DISCUSSION

In cardiology, biomarkers such as BNP, troponin, and recently ST2 are common markers for diagnosis and prognosis of many cardiovascular diseases such as coronary artery disease and heart failure. A combination of biomarkers has been applied to improve diagnosis and prognosis, as each biomarker reflects a different pathophysiological pathway [10]. Whether biomarkers, single or combined, can be used to improve the selection of donor hearts is unknown and was the focus of the present study. Our study found that BNP provides incremental information on the donor heart performance, and thus BNP may be helpful in the decision process for a donor heart, whereas ST2 and high-sensitivity troponin have no added value for this decision.

BNP and its precursor N-terminal pro-brain-type natriuretic peptide have consistently been shown to be related to cardiac dysfunction and worse hemodynamic parameters in heart donors [11]. The observed increased levels of BNP in heart donors reflect the physiological changes that occur in the heart after brain death [12]. The present study demonstrates that BNP measured in heart donors at the time of donor evaluation is inversely related to early cardiac performance in transplant recipients (expressed as CI). When the donor BNP is $\leq$ 132 pg/mL, the chances of a poor cardiac function in the recipient are low (NPV 94%). In contrast, donor BN$P > 132$ pg/mL is associated with a moderately increased risk for a poor cardiac function in the recipient (positive predictive value 33%). This information could be of particular interest for those patients in whom the standard clinical evaluation shows equivocal results.

Additional analyses revealed that donor BNP is also related to several important post-transplant outcome parameters, including mortality within 1 month and length of hospitalization. It is might be assumed that this higher morbidity/mortality is related to lower pre-existing cardiac performance as evidenced by higher levels of donor BNP. However, a recent publication of Chen et al showed that a lower ejection fraction in the donor was not associated with increased mortality [13]. More research is needed to unravel the relationship between pre-existing myocardial dysfunction of the donor heart and outcome of the recipient.

Troponin has been studied only marginally for the selection of heart donors. In a study of potential heart donors, elevated conventional troponin I values were associated with a worse cardiac function on echocardiography [14]. The present study could not find a relationship between donor troponin and cardiac performance of the recipient. It should be emphasized that all donors in the present study had a normal (or near normal) cardiac function and had no clinical evidence of ischemic heart disease. Troponin seems not to be of added value to predict cardiac performance post-transplantation in such patients.

Assessment of ST2, in addition to N-terminal pro-brain-type natriuretic peptide, was proven to substantially improve the risk stratification for death in patients with heart failure in an extended model that included established risk factors [15]. This promising result concerning the use of ST2 assessment in both acute and chronic heart failure represents the fundamental motivation to investigate its possible value in heart transplantation. As a cardiac biomarker, ST2 reflects myocardial strain, remodeling, inflammation, and fibrosis. The membrane-bound form of ST2 takes part in a signaling pathway that elicits a cardioprotective effect by reducing reactive hypertrophy and fibrosis [16,17]. In addition to the good performance of ST2 in heart failure, increased ST2 levels are reported in patients with inflammatory disorders such as sepsis and rheumatoid arthritis, suggesting that ST2

can also be seen as a marker of inflammation in general [18,19]. The ST2 expression is not limited to the myocardium, since virtually all immune cell types, and cells from various other organs, are shown to express the membrane-bound receptor form of ST2. The release of large amounts of catecholamines that accompanies brain death results in general vasoconstriction, a reduction in peripheral blood flow, and eventually tissue hypoxia. Therefore, brain death in organ donors is associated with the activation of multiple pro-inflammatory mediators, resulting in a state of general inflammation [20]. These mechanisms could explain the high ST2 levels in our studied heart donors. The median value for ST2 was 221.1 ng/mL (Table 1). An adverse prognosis in chronic heart failure is expected with values above 35 ng/mL. The pro-inflammatory state of brain death subjects seems to have obscured the relationship between ST2 and cardiac performance, as ST2 levels of donors could not predict cardiac performance in the recipient in our study.

Given the lack of correlation between early cardiac performance and ST2 and troponin, the combination of biomarkers (BNP, troponin, ST2) seems to have no incremental value above the use of BNP as a stand-alone strategy for the selection of appropriate donor hearts. Whether other biomarkers, such as some circulating microRNAs, might improve the selection remains to be studied [21].

Limitations

Although the present study is one of the largest involving the use of biomarkers for donor selection in heart transplantation, the study population remains relatively small. Therefore, the results should be interpreted with caution. Heart transplantation remains a relatively infrequent procedure.

Additionally, this study implies a highly selective study population because all heart donors were already approved according to the current standards of donor evaluation. This selection process may potentially mask relations between biomarkers and outcome measures. Furthermore, we must also bear in mind that graft function after transplantation is not determined only by donor cardiac function but that recipient and procedural determinants are also important.

CONCLUSIONS

BNP provides incremental information on donor heart status and early post-transplant performance in the recipient after heart transplantation. BNP could, therefore, be used as an additional parameter for the evaluation of potential heart donors. Although ST2 is useful in the prognosis of cardiac disease, it appears to have no value in the evaluation of potential heart donors.

REFERENCES

[1] International Society for Heart and Lung Transplantation. ISHLT Transplant Registry: overall heart and adult heart transplantation statistics 2010. http://www.ishlt.org/registries/slides.asp?slides=heartLungRegistry.

[2] Cosio C, Gómez Bueno M, Almenar L, Delgado JF, Arizón JM, González Vilchez F, et al. Primary graft failure after heart transplantation: characteristics in a contemporary cohort and performance of the RADIAL risk score. J Heart Lung Transplant 2013;32:1187–95.

[3] Mihaljevic T, Jarrett CM, Gonzalez-Stawinski G, Smedira NG, Nowicki ER, Thuita L, et al. Mechanical circulatory support after heart transplantation. Eur J Cardiothorac Surg 2012;41:200–6.

[4] Iyer A, Kumarasinghe G, Hicks M, Watson A, Gao L, Doyle A, et al. Primary graft failure after heart transplantation. J Transplant 2011;2011:175768.

[5] Bassi N, Jeevanandam V. Choosing the right heart: proper donor selection in cardiac transplantation. Curr Opin Cardiol 2017;32:286–91.

[6] Kilic A, Emani S, Sai-Sudhakar CB, Higgins RS, Whitson BA. Donor selection in heart transplantation. J Thorac Disease 2014;6:1097–104.

[7] Vorlat A, Conraads VM, Jorens PG, Aerts S, Van Gorp S, Vermeulen T, et al. Donor B-type natriuretic peptide predicts early cardiac performance after heart transplantation. J Heart Lung Transplant 2012;31:579–84.

[8] Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, et al. J Heart Lung Transplant, 2014;33:327–40.

[9] Vorlat A, Van Hoof VO, Hammamani R, van Kerckhoven S, Van der Heijden CM, Coenen D, et al. Usefulness of early rule-in and rule-out biomarker protocols to estimate ischemia-induced myocardial injury in early chest pain presenters. Am J Cardiol 2015;115:1667–71.

[10] Lassus J, Gayat E, Mueller C, Peacock WF, Spinar J, Harjola VP, et al. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) Study. Int J Cardiol 2013;168:2186–94.

[11] Dronavalli VB, Ranasinghe AM, Venkateswaran RJ, James SR, McCabe CJ, Wilson IC, et al. N-terminal pro-brain-natriuretic peptide: a biochemical surrogate of cardiac function in the potential heart donor. Eur J Cardiothorac Surg 2010;38:181–6.

[12] Szabo G. Physiologic changes after brain death. J Heart Lung Transplant 2004;23(9 Suppl):S223–6.

[13] Chen CW, Sprys MH, Gaffey AC, Chung JJ, Margulies KB, Acker MA, et al. Low ejection fraction in donor hearts is not directly associated with increased recipient mortality. J Heart Lung Transplant 2017;36:611–5.

[14] Boccheciampe N, Audibert G, Rangeard O, Charpentier C, Perrier JF, Lalot JM, et al. Serum troponin Ic values in organ donors are related to donor myocardial dysfunction but not to graft dysfunction or rejection in the recipients. Int J Cardiol 2009;133:80–6.

[15] Bayes-Genis A, de Antonio M, Galán A, Sanz H, Urrutia A, Cabanes R, et al. Combined use of high-sensitivity ST2 and NTproBNP to improve the prediction of death in heart failure. Eur J Heart Fail 2012;14:32–8.

[16] Daniels LB, Bayes-Genis A. Using ST2 in cardiovascular patients: a review. Future Cardiol 2014;10:525–39.

[17] Chow SL, Maisel AS, Anand I, Bozkurt B, de Boer RA, Felker GM, et al. Role of biomarkers for the prevention, assessment, and management of heart failure: a scientific statement from the American Heart Association. Circulation 2017;135:e1054–91.

[18] Kuroiwa K, Arai T, Okazaki H, Minota S, Tominaga S. Identification of human ST2 protein in the sera of patients with autoimmune diseases. Biochem Biophys Res Commun 2001;284:1104–8.

[19] Brunner M, Krenn C, Roth G, Moser B, Dworschak M, Jensen-Jarolim E, et al. Increased levels of soluble ST2 protein and IgG1 production in patients with sepsis and trauma. Intensive Care Med 2004;30:1468–73.

[20] Pratschke J, Wilhelm MJ, Kusaka M, Hancock WW, Tilney NL. Activation of pro-inflammatory genes in somatic organs as a consequence of brain death. Transplant Proc 1999;31:1003–5.

[21] Scrutinio D, Conserva F, Passantino A, Iacoviello M, Lagioia R, Gesualdo L. Circulating microRNA-150-5p as a novel biomarker for advanced heart failure: a genome-wide prospective study. J Heart Lung Transplant 2017;36:616–24.