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Comparative Prognostic and Diagnostic Value of Myocardial Blood Flow and Myocardial Flow Reserve After Cardiac Transplant

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ABSTRACT (262/350 words)

Rationale

Cardiac allograft vasculopathy (CAV) is a major cause of graft failure after cardiac transplantation. CAV is characterized by diffuse involvement of epicardial coronary arteries and the microvasculature. Positron emission tomography (PET) allows quantification of absolute myocardial blood flow (MBF) and myocardial flow reserve (MFR), which may be accurate markers of CAV severity. We compared the diagnostic and prognostic utility of stress MBF and MFR following cardiac transplantation.

Methods

This was a cohort study of consecutive cardiac transplant patients undergoing ^{82}Rb PET scans. Semi-quantitative regional analysis and global measurement of stress MBF and MFR were performed. Associations with all-cause mortality were assessed with multivariable Cox analysis. The diagnostic accuracy for significant CAV (grade 2/3) and prognostic accuracy of stress MBF and MFR, corrected and uncorrected for rate pressure product, were compared.

Results

A total of 99 patients, mean age 68.8 and 75.8% male, were followed for a median of 3.4 years, during which 26 deaths occurred. Stress MBF and MFR had similar diagnostic accuracy for significant CAV. However, uncorrected MFR had improved discrimination for all-cause mortality compared to stress MBF (area under the curve 0.748 vs 0.639, p=0.048). Higher MFR (adjusted hazard ratio (aHR) 0.30, p<0.001), but not stress MBF (aHR 1.14, p=0. 656), was associated with reduced all-cause mortality. Preserved MFR (>2.0) identified relatively low-risk patients (annual mortality 4.7%) while the presence of left ventricular ejection fraction <45% and MFR<1.7 identified high-risk patients (annual mortality 51.6%).

Conclusion

Quantitative PET analysis, and particularly MFR, has diagnostic and prognostic utility. following heart transplantation. Preserved MFR identifies low-risk patients while the presence of multiple abnormal parameters identifies high-risk patients.

Keywords

Heart transplantation, myocardial flow reserve, myocardial blood flow, positron emission tomography

Abbreviations

CAV: cardiac allograft vasculopathy

HR: hazard ratio

MBF: myocardial blood flow

MFR: myocardial flow reserve

PET: Positron emission tomography

^{82}Rb: Rubidium-82

INTRODUCTION

Heart transplantation is a definitive therapy for patients with end-stage heart failure. Due to advances in post-transplant patient care, median post-transplant survival is now over 13 years.(*1*) As long-term survival has increased, the prevalence of cardiac allograft vasculopathy (CAV), characterized by a diffuse arteriopathy involving the epicardial coronary arteries and microvasculature, has increased.(*2*) CAV accounts for over a third of deaths in patients who survive at least 5 years post-transplant and is the most common indication for re-transplantation in patients who survive one year.(*3*)

While invasive coronary angiography (ICA) and intravascular ultrasound (IVUS) have been the gold-standard for diagnosing CAV, they are associated with inherent procedural risk.(*4*) Non-invasive monitoring of CAV may provide valuable diagnostic information while avoiding the risk of invasive studies.(*2*) Noninvasive imaging with stress echocardiography and single photon emission computed tomography have poor sensitivity for diagnosis of CAV(*5, 6*). However, positron emission tomography (PET) myocardial perfusion imaging (MPI) offers the advantage of measurement of absolute myocardial blood flow (MBF) quantification and calculation of myocardial flow reserve (MFR). Stress MBF and MFR provide physiologic assessments of both epicardial coronary artery and coronary microvascular function.(*7*) Thus, PET may provide a more comprehensive assessment for CAV compared to other non-invasive modalities. In previous studies, both MBF and MFR have provided incremental diagnostic and prognostic utility to semi-quantitative assessment of perfusion for the diagnosis of CAV following cardiac transplant.(*8-10*).

While evidence regarding PET for CAV surveillance has grown, it has not been adopted as standard clinical practice. Previous studies have used variable thresholds of MBF and MFR

for diagnosis of CAV. Additionally, there is absence of consensus on whether MFR or stress MBF should be used as the marker adverse cardiovascular outcomes. Our objectives were to compare the diagnostic value of MBF and MFR to detect CAV, to evaluate the prognostic utility of MBF and MFR for prediction of adverse outcomes and to evaluate the performance of previously described thresholds for ^{82}Rb PET MPI parameters in patients following cardiac transplant.

MATERIALS AND METHODS

Study Population

In total, 105 consecutive patients who underwent pharmacological stress ^{82}Rb PET following cardiac transplantation between April 2010 and December 2015 were identified at a single center. Patients without dynamic data were excluded (n=6). In patients with multiple studies, the first study with dynamic data was included. This study was approved by the Institutional Review Board at Cedars-Sinai Medical Center (CR00013886) and written informed consent was obtained from all patients.

Transplant demographics were obtained from medical records. History of acute cellular rejection (ACR) was defined as biopsy showing cellular rejection ≥ 2R or a history of treated ACR. History of antibody mediated rejection (AMR) was defined as a history of treated AMR. ICA which most closely preceded PET was reviewed and graded for CAV according to the International Society of Heart and Lung Transplantation (ISHLT) classification based on the interpretation of the performing cardiologist, blinded to PET results.(*11*)

Image Acquisition and Reconstruction

Patients were imaged with a whole-body PET/CT scanner (Siemens Biograph-64 TruePoint PET/CT with the True V) (*12*). A 6-min list mode rest acquisition was performed simultaneously with injection of 925-1850 MBq of ^{82}Rb. Regadenoson or adenosine stress acquisitions were then performed with the same protocol.(*13*) The heart rate and blood pressure were recorded before ^{82}Rb injection and at peak stress. The 6 minutes rest and stress data were reconstructed into a dynamic imaging series consisting of 16 frames (12 × 10 s, 2 × 30 s, 1 × 60 s, and 1 × 120 s) using the vendor iterative method (Fourier rebinning + 2-dimensional attenuation-weighted ordered-subsets expectation maximization) with 2 iterations, 8 subsets, and 8-mm gaussian postprocessing filter.(*14*). CT attenuation-correction scans were acquired both before rest and after stress imaging. The CT attenuation map registration with the PET images was verified visually by an experienced technologist.

Semi-quantitative assessments of perfusion were performed at stress and rest to derive the summed stress score (SSS), summed rest score (SRS), and summed difference score (SDS).(*15*) Patients were grouped according to abnormalities SRS (≥ 1 or 0), two criteria for SSS (SSS ≥4or <4and >1 or ≤ 1) and ischemia (SDS thresholds >1). Left ventricular ejection fraction (LVEF) was assessed at rest. For quantitative measurements, MBF was computed from the dynamic imaging series with dedicated software (QPET, Cedars-Sinai Medical Center, Los Angeles, California).(*14, 16*) A standard 1-tissue-compartment model and ^{82}Rb extraction fraction derived from the described Renkin-Crone function was used to estimate MBF from K1.(*17*) MFR was calculated as MBF at stress/MBF at rest. Corrected rest MBF was calculated as MBF at rest × rate pressure product/10000 and corrected MFR as stress MBF/corrected MFR. Rate pressure product was calculated as heart rate * systolic blood pressure.

Thresholds for quantitative PET analysis were based on previously published values including: MFR < 2.0,(*8*) or <1.75 (*9*); stress MBF < 3.7 (8) or <1.7.(*10*) Additionally, we investigated combinations of parameters previously described as having prognostic value : SSS≥4, LVEF ≤45, or MFR <1.75;(*9*) and MBF < 1.7 with SSS >1 or LVEF ≤ 45.(*10*) Additionally, cut-offs were derived from the ROC curves generated in this study using the Youden index.(*18*)

Outcomes

The diagnostic outcome was the presence of CAV grade 2 or 3. The primary prognostic outcome was all-cause mortality. Cardiovascular mortality, the secondary prognostic outcome, was determined after review of all available clinical information based on standard definitions.(*19*) No patients in our cohort underwent re-transplantation. Follow-up of all patients was confirmed by trained research coordinators, including patients who transfer to alternate transplant programs.

Statistical Analyses

Continuous variables with normal distributions are presented as mean ± standard deviation (SD) and compared with a student's t-test. Continuous variables which were not normally distributed are presented as median (interquartile range [IQR]) and compared using the Mann-Whitney U test. Categorical variables are summarized as number (proportion) and compared using a Fisher exact test.

The primary outcome was assessed using Kaplan–Meier survival curves and compared using the log-rank test. Univariable and multivariable Cox proportional hazards analyses were

performed to identify associations with all-cause mortality and cardiovascular mortality. Multivariable models included variables identified as significant on univariable analyses ($P < 0.05$).

Receiver operating characteristic (ROC) curves were generated for semi-quantitative and quantitative PET parameters. Area under the curve (AUC) was compared using the method established by Delong et al (*20*). Calibration of quantitative PET measurements was assessed using the Hosmer-Lemeshow goodness of fit test and was found to be adequate for each combination of measurement and outcome (all $p > 0.05$). Event rates were extrapolated from Cox-proportional hazards models. The proportional hazards assumption was assessed with Schoenfeld residuals for each model and found to be valid. We assessed for interactions between each of the variables in the final model, with no significant interactions identified. All statistical tests were two-sided, with p-value <0.05 considered significant. Analysis was performed using SAS (JMP ver. 13, SAS, Cary, NC, USA) and Stata version 13 (StataCorp, College Station, Texas).

RESULTS

Follow-Up and All-Cause Mortality

In total, 99 patients were included with baseline characteristics outlined in Table 1. The mean age was 68.8 ± 10.0 years, and 75 (75.8%) patients were male. Median resting heart rate was 82 (IQR 73 – 90). During a median follow-up of 3.4 year (IQR 2.2–4.0), 26 patients died (26.3 %). Patients who died were older at the time of PET (mean age 74.0 vs. 66.7, p<0.001) and had lower mean LVEF (56.8vs. 65.4%, p<0.001).

Imaging characteristics are outlined in Table 2. There were no significant differences in

SSS, SRS and SDS between patients with and without all-cause mortality. However, stress MBF (2.54 vs. 2.88 ml/min/g, p=0.024) and MFR (1.69 vs. 2.37, p<0.001) were lower in patients who died during follow-up.

Diagnostic Utility of ^{82}Rb PET

Reference angiography occurred at a median of 1.0 (IQR 0.9 – 2.0) years prior to the PET study. MBF, MFR, and corrected MFR demonstrated good discrimination of significant CAV. There were no significant differences in the ability of stress MBF (AUC 0.713), MFR (AUC 0.749), or corrected MFR (AUC 0.714) to identify patients with significant CAV (Figure 1**)**. Optimal cut-offs in our population were: stress MBF <2.83 (sensitivity 73.1%, specificity 56.2%), uncorrected MFR < 2.22 (sensitivity 80.8%, specificity 61.6%), corrected MFR <2.19 (sensitivity 76.9%, specificity 65.8%). SSS alone (AUC 0.706) had similar differentiation compared to stress MBF, uncorrected MFR, and corrected MFR. Addition of abnormal regional perfusion to stress MBF, corrected MFR and uncorrected MFR did not result in a statistically significant improvement in discrimination of patients with CAV compared to quantitative markers alone (Supplemental Figure 1).

Prognostic Utility of ^{82}Rb PET

Kaplan-Meier survival curves for semi-quantitative regional analysis are shown in Figure 2. SSS ≥4 (log-rank p=0.017) was associated with increased all-cause mortality. Abnormal SRS or SDS were not associated with all-cause mortality. Kaplan-Meier survival curves for quantitative PET variables are shown in Figure 3. Abnormal MFR (log-rank p<0.001), corrected MFR (log-rank p<0.001) and stress MBF (log-rank p=0.002) were associated with

increased all-cause mortality.

In a univariable Cox-proportional hazards analysis (results in Table 3), stress MBF (unadjusted HR 0.56, 95% CI 0.35 – 0.90, p=0.017), uncorrected MFR (unadjusted HR 0.34, 95% CI 0.19 – 0.62, p<0.001), and corrected MFR (unadjusted HR 0.44, 95% CI 0.26 – 0.74, p=0.002) were associated with all-cause mortality**.** Higher SSS was also associated with increased all-cause mortality (unadjusted HR 1.09, 95% CI 1.03 – 1.15, p=0.002) as were age and lower LVEF. However, in a multivariable model, only age and MFR continued to be associated with all-cause mortality (uncorrected: adjusted HR 0.30, p=0.017; corrected: adjusted HR 0.43, p=0.025). Addition of uncorrected MFR significantly improved death prediction when added to age, LVEF, SRS, SSS, SDS, and stress MBF (chi-square 6.5, p=0.011), with less improvement by adding corrected MFR (chi-square 5.4, p=0.020).

ROC curves for prediction of all-cause mortality shown in Figure 4. Uncorrected MFR had improved discrimination for all-cause mortality compared to stress MBF (AUC 0.748 vs 0.639, p=0.048). However, there was no significant difference in mortality prediction between MFR and corrected MFR. Prediction of all-cause mortality with SSS alone (AUC 0.593) was significantly worse compared to MFR (p=0.007), and corrected MFR (p=0.019), but not stress MBF (p=0.435). Addition of abnormal regional perfusion to MFR or stress MBF analyses did not significantly improve the prognostic accuracy compared to quantitative markers alone (Supplemental Figure 2).

Cardiovascular Mortality

Cardiovascular mortality occurred in 16 (16.2%) patients. Univariable and multivariable Cox-proportional hazards analysis of the association with cardiovascular mortality are shown in

Supplemental Table 1. Stress MBF was associated with increased cardiovascular mortality in univariable (unadjusted HR 0.37, p=0.002) but not multivariable analyses (adjusted HR 1.08, p=0.848). Uncorrected MFR was associated with increased cardiovascular mortality in univariable (unadjusted HR 0.17, p<0.001) and multivariable analyses (adjusted HR 0.05, p=0.001). ROC curves for prediction of cardiovascular mortality are shown in Supplemental Figure 3.

Comparison of Previously Described Thresholds

Table 4 summarizes the diagnostic and prognostic utility of previously described abnormal thresholds and their combinations. Uncorrected MFR was used since discrimination was numerically superior to corrected MFR in our prior analyses. With respect to diagnosis of CAV, stress MBF performed well among individual variables, but with restrictive thresholds. Stress MBF <3.7 had the highest sensitivity but classified 79.8% of patients as abnormal, while stress MBF <1.7 had the highest specificity with only 10.1% of patients classified as abnormal. Highest overall accuracy was achieved with MFR <2.0 or the combined marker of the presence of any of SSS≥4, LVEF <=45% or MFR <1.75. Regarding prognosis, stress MBF <1.7 was the single predictor which identified patients with the highest risk annualized mortality rate (35.8%). Patients with reduced stress MBF (<1.7) and either SSS>1 or LVEF ≤45% had an annual mortality rate of 60.7%. Patients with preserved MFR (≥2.0) were at the lowest risk (annual mortality rate 4.7%).

DISCUSSION

PET-MPI with MBF quantification has been shown to improve diagnosis of CAV and

provide incremental prognostic value in patients following heart transplant. However, there is no consensus on which marker, stress MBF or MFR, should be applied for either diagnostic or prognostic purposes.(*21*) In this study we demonstrate that stress MBF, uncorrected MFR and corrected MFR were equivalent in discriminating patients with significant CAV. For prognosis, we found that reduced MFR offered superior discrimination for all-cause mortality compared to stress MBF. Finally, we found that correcting for RPP did not improve prognostic or diagnostic accuracy of MFR.

Our study adds to a growing body of literature describing the diagnostic utility of quantitative PET analysis. Bravo et al. reported that in patients imaged by ^{13}N-NH_3 PET MPI, stress MBF and LVEF combined with regional perfusion improved detection of significant CAV with an AUC of 0.88 compared to 0.82.(*10*) Konerman et al. reported that MFR or stress MBF numerically, but not significantly, improved discrimination of CAV compared to regional perfusion when assessed by ^{82}Rb PET.(*8*) Chih et al. found that stress MBF and MFR had similar discrimination of CAV when defined angiographically or by IVUS.(*22*) In our population, stress MBF, uncorrected MFR and corrected MFR had comparable performance for diagnosis of significant CAV. These results suggest that either stress MBF or MFR may improve diagnosis of CAV, and that if differences in discrimination are present, they are likely not clinically significant.

Our study also confirmed the prognostic utility of PET-MPI in patients following heart transplant. McArdle et al. followed 140 cardiac transplant patients who underwent ^{82}Rb PET for a median 18.2 months follow-up during which 14 events occurred, including 9 deaths.(*9*) They demonstrated that MFR, but not stress MBF, was associated with increased adverse events, with MFR <1.5 conferring a 4-fold increase in risk. Konerman et al. reported similar associations with

a combined outcome of death (n=2), acute coronary syndrome (n=5), revascularization (n=8), or heart failure (n=15). Both studies used uncorrected MFR. We expand on this evidence by including a greater number of hard events and observed that only reduced MFR was associated with increased all-cause mortality in adjusted analyses. Additionally, the discriminatory value of MFR was superior compared to stress MBF for identifying patients who died during follow-up. Serial evaluation with PET has been shown to further refine prognostication.(*23*) Our findings provide evidence that quantitative PET blood flow analysis, particularly with MFR, has significant prognostic utility following heart transplantation.

Lastly, we describe the performance of previously described abnormal thresholds in our cohort. While semiquantitative perfusion abnormalities were associated with increased all-cause mortality, they did not significantly improve diagnosis of CAV or prognostication when added to stress MBF or MFR. Further, we found that preserved MFR (defined as ≥ 2.0) identified a group of patients with the lowest rate of all-cause mortality during follow-up. Combining reduced LVEF with reduced MFR (or MBF) identified a group of patients with annual mortality over 50%. Additional patients at high mortality risk were identified by combining the presence of severely reduced stress MBF with abnormal regional perfusion or reduced LVEF. As a point of reference, the ISHLT guidelines suggest that patients with end-stage HF and estimated one-year mortality over 20% be considered for transplant listing.(*24*) Therefore, PET may have a role for identifying patients who may benefit from re-transplantation before they develop significant HF symptoms or recurrent hospitalizations. Physicians should integrate multiple parameters to improve diagnostic sensitivity or risk-stratification based on individual clinical scenarios.

Our study has a few important limitations. This is a retrospective study of patients from a single-center. Our sample size is small, but it is comparable to prior studies. The patients who

were referred for PET represented a high-risk cohort as evidenced by the high annual mortality rates. PET perfusion studies were performed as part of routine clinical practice, therefore the time interval between ICA and PET scan was not standardized and a delay over 2 years was present in one quarter of patients. While this may have impacted the assessment of diagnostic accuracy, most patients were more than 10 years post-transplant at which time CAV tends to progress less rapidly.(*2*) We used a different software package compared to other published studies, which may explain some of the variation in findings. However, the correlation across software packages is excellent,(*25*) Additionally, our results confirm that PET has diagnostic and prognostic utility regardless of the software package used.

CONCLUSIONS

We confirmed the diagnostic and prognostic utility of PET flow quantitation in post-transplant patients. Stress MBF and MFR had similar diagnostic utility and correcting for RPP did not improve diagnostic or prognostic accuracy. However, we found that uncorrected MFR was superior to stress MBF for prognostication. Preserved MFR identifies low-risk patients while the presence of multiple abnormal parameters identifies patients at the highest risk.

KEY POINTS

Question

What are the diagnostic and prognostic utility of MBF and MFR in patients following cardiac transplant?

Pertinent Findings

We specifically compared the performance of MFR with and without correction for rate pressure product, showing that uncorrected values have numerically higher diagnostic and prognostic utility. Additionally, we show that stress MBF and MFR have similar diagnostic utility, while uncorrected MFR has superior prediction of all-cause mortality.

Implications for Patient Care

Physicians should integrate multiple parameters to improve diagnostic sensitivity or risk-stratification based on individual clinical scenarios.

REFERENCES

(1) Lund LH, Khush KK, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant.* 2017;36:1037-46.

(2) Pollack A, Nazif T, Mancini D, Weisz G. Detection and imaging of cardiac allograft vasculopathy. *JACC: Cardiovasc Imaging.*2013;6:613-23.

(3) Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report. *J Heart Lung Transplant*. 2014;33:996-1008.

(4) Stone GW, Maehara A, Lansky AJ, et al. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med.* 2011;364:226-35.

(5) Chirakarnjanakorn S, Starling RC, Popovic ZB, Griffin BP, Desai MY. Dobutamine stress echocardiography during follow-up surveillance in heart transplant patients: Diagnostic accuracy and predictors of outcomes. *J Heart Lung Transplant.* 2015;34:710-7.

(6) Thompson D, Koster MJ, Wagner RH, Heroux A, Barron JT. Single photon emission computed tomography myocardial perfusion imaging to detect cardiac allograft vasculopathy. *Eur Heart J Cardiovasc Imaging.* 2012;13:271-5.

(7) Bravo PE, Di Carli MF, Dorbala S. Role of PET to evaluate coronary microvascular

dysfunction in non-ischemic cardiomyopathies. *Heart Failure Rev*. 2017;22:455-64.

(8) Konerman MC, Lazarus JJ, Weinberg RL, et al. Reduced myocardial flow reserve by positron emission tomography predicts cardiovascular events after cardiac transplantation. *Circ Heart Fail.* 2018;11:1-10.

(9) Mc Ardle BA, Davies RA, Chen L, et al. Prognostic value of rubidium-82 positron emission tomography in patients after heart transplant. *Circ Cardiovasc Imaging.* 2014;7:930-7.

(10) Bravo PE, Bergmark BA, Vita T, et al. Diagnostic and prognostic value of myocardial blood flow quantification as non-invasive indicator of cardiac allograft vasculopathy. *Eur Heart J.* 2018;39:316-23.

(11) Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. J *Heart Lung Transplant.* 2010;29:717-27.

(12) Slomka PJ, Diaz-Zamudio M, Dey D, et al. Automatic registration of misaligned CT attenuation correction maps in Rb-82 PET/CT improves detection of angiographically significant coronary artery disease. *J Nucl Cardiol.* 2015;22:1285-95.

(13) Henzlova MJ, Duvall WL, Einstein AJ, Travin MI, Verberne HJ. ASNC imaging guidelines for SPECT nuclear cardiology procedures: Stress, protocols, and tracers. *J*

Nucl Cardiol. 2016;23:606-39.

(14) Dekemp RA, Declerck J, Klein R, et al. Multisoftware reproducibility study of stress and rest myocardial blood flow assessed with 3D dynamic PET/CT and a 1-tissue-compartment model of 82Rb kinetics. *J Nucl Med.* 2013;54:571-7.

(15) Chow BJ, Ananthasubramaniam K, dekemp RA, Dalipaj MM, Beanlands RS, Ruddy TD. Comparison of treadmill exercise versus dipyridamole stress with myocardial perfusion imaging using rubidium-82 positron emission tomography. *J Am Coll Cardiol.* 2005;45:1227-34.

(16) Nakazato R, Berman DS, Dey D, et al. Automated quantitative Rb-82 3D PET/CT myocardial perfusion imaging: normal limits and correlation with invasive coronary angiography. *J Nucl Cardiol.* 2012;19:265-76.

(17) Lortie M, Beanlands RS, Yoshinaga K, Klein R, Dasilva JN, DeKemp RA. Quantification of myocardial blood flow with 82Rb dynamic PET imaging. *Eur J Nucl Med Mol Imaging.* 2007;34:1765-74.

(18) Youden WJ. Index for rating diagnostic tests. *Cancer.* 1950;3:32-35.

(19) Hicks KA, Mahaffey KW, Mehran R, et al. 2017 Cardiovascular and stroke endpoint definitions for clinical trials. *Circulation.* 2018;137:961-972

(20) DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more

correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* 1988;44:837-45.

(21) Miller RJH, Kobashigawa JA, Berman DS. Should positron emission tomography be the standard of care for non-invasive surveillance following cardiac transplantation? *J Nucl Cardiol.* 2018, in press.

(22) Chih S, Chong AY, Erthal F, et al. PET Assessment of epicardial intimal disease and microvascular dysfunction in cardiac allograft vasculopathy. *J Am Coll Cardiol.* 2018;71:1444-56.

(23) Feher A, Srivastava A, Quail MA, et al. Serial assessment of coronary flow reserve by Rubidium-82 positron emission tomography predicts mortality in heart transplant recipients. *JACC Cardiovasc Imaging.* 2018, in press.

(24) Mehra MR, Canter CE, Hannan MM, et al. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. *J Heart Lung Transplant.* 2016;35:1-23.

(25) Nesterov SV, Deshayes E, Sciagrà R, et al. Quantification of myocardial blood flow in absolute terms using (82)Rb PET imaging: the RUBY-10 Study. *JACC Cardiovasc Imaging.* 2014;7:1119-27.

FIGURE LEGENDS

Figure 1: Receiver operating characteristic curves for diagnosis.

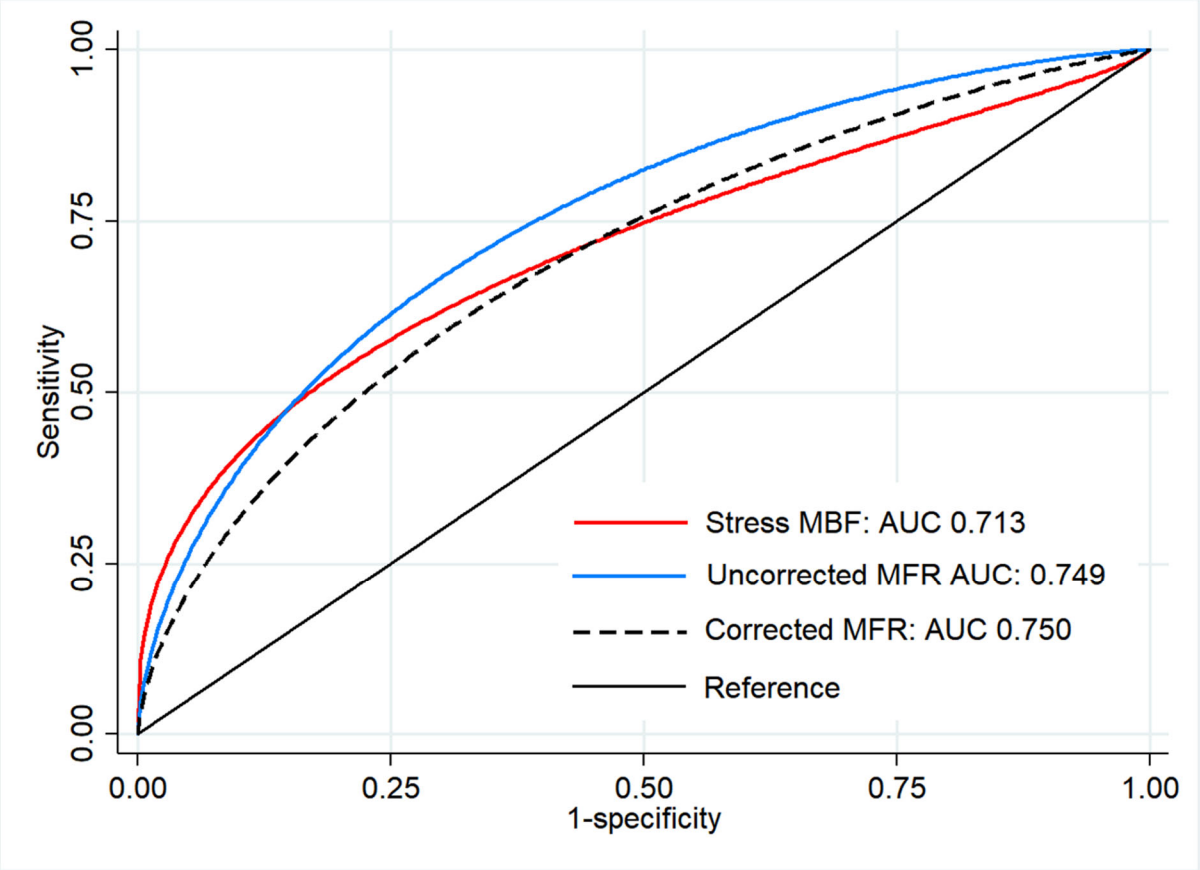


Figure 1: Receiver operating characteristic curves for diagnosing CAV ≥ grade 2. There was no difference in the area under the curve (AUC) of uncorrected MFR compared to stress MBF (p=0.499) or corrected MFR (p=0.310). AUC – area under the curve, MBF – myocardial blood flow, MFR – myocardial flow reserve.

Figure 2. Kaplan-Meier survival curves for semi-quantitative analysis

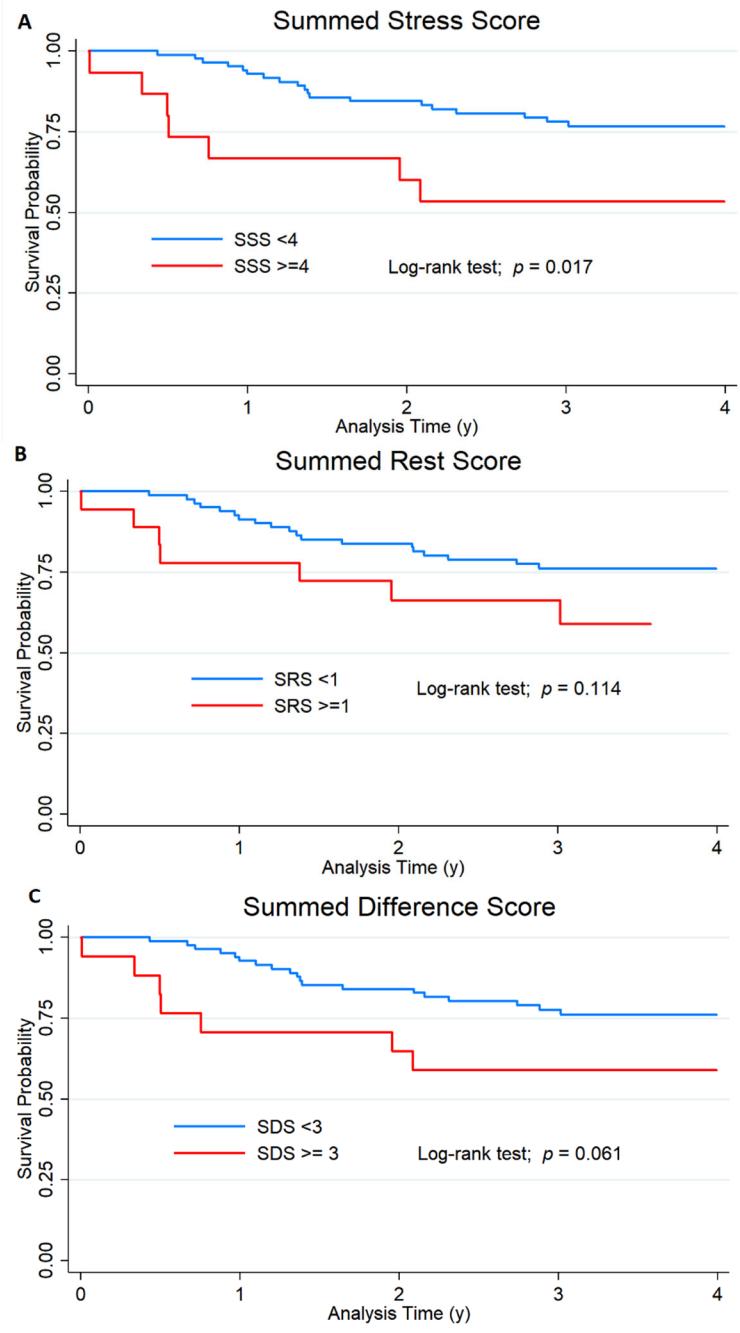


Figure 2. Kaplan-Meier survival curves for all-cause mortality stratified by the presence of abnormal regional perfusion. (A) summed stress score (SSS), (B) summed rest score (SRS), and (C) summed difference score (SDS). Patients with SSS≥4 were more likely to experience all-cause mortality during follow-up (log-rank p=0.017).

Figure 3. Kaplan-Meier survival curves for PET quantitative analysis

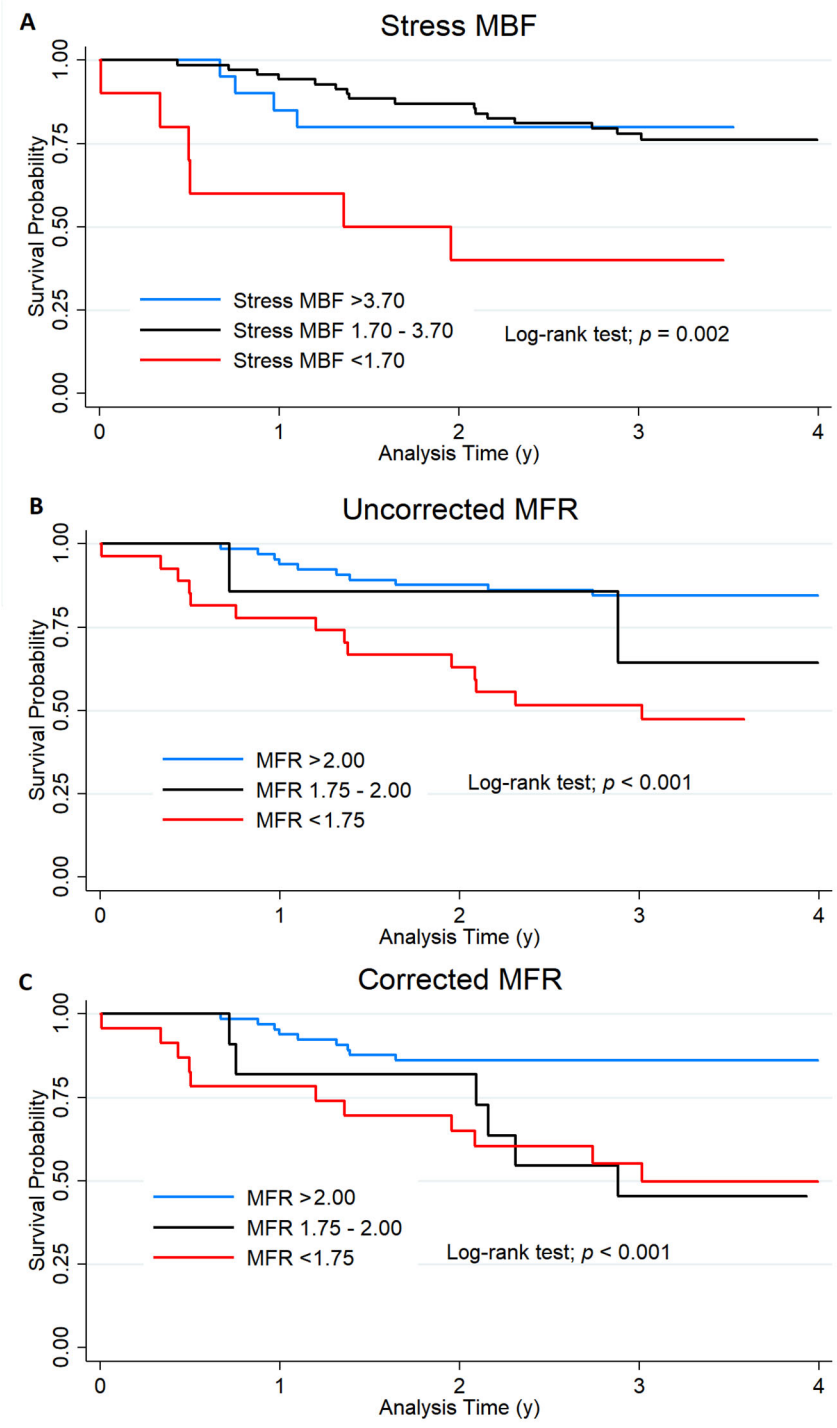


Figure 3: Kaplan-Meier survival curves for all-cause mortality stratified by quantitative positron emission tomography (PET) results. of (A) myocardial blood flow (MBF) at stress, (B) myocardial flow reserve (MFR), and (C) corrected MFR.

Figure 4: Receiver Operating Characteristic Curves for Predicting All-Cause Mortality.

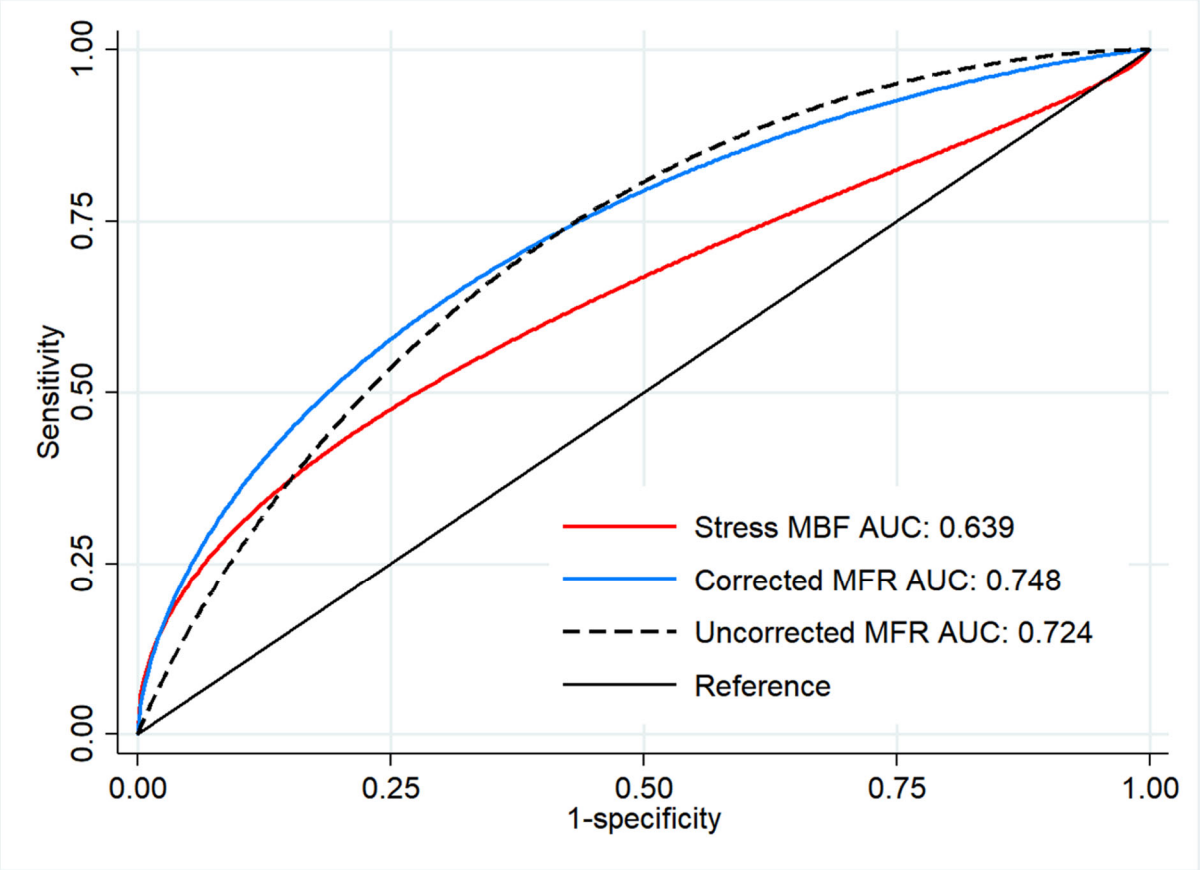


Figure 4: Receiver operating characteristic curves for identifying all-cause mortality during follow-up. Uncorrected myocardial flow reserve (MFR) had a significantly larger area under the curve (AUC) compared to stress myocardial blood flow (MBF) (p=0.047). There was no difference between MFR and corrected MFR (p=0.681).

Table 1: Baseline Population characteristics.

	No death (n=73)	Death (n=26)	*P*-value
Age (years), mean ± SD	66.7 ± 10.5	74.0 ± 7.3	0.001
Male, n(%)	54(74.0)	21(80.8)	0.599
Age at transplant (years), mean ± SD	54.3±11.1	61.9 ±6.5	0.001
Donor Age (years), mean ± SD	30.2±11.9	35.4±10.7	0.089
Time post-transplant (years), mean ± SD	12.5±5.2	12.5±5.4	0.977
Body mass index (kg/m^2), mean ± SD	26.5±5.6	25.8±5.0	0.560
Hypertension, n(%)	62(84.9)	19(73.1)	0.236
Diabetes, n(%)	31(42.5)	15(57.7)	0.252
Dyslipidemia, n(%)	53(72.6)	21(80.8)	0.600
Ex-smoker, n(%)	4(5.5)	2(7.7)	0.651
Renal failure, n(%)	7(9.6)	4(15.4)	0.472
CAV grade* (0/1/2/3)	46/17/5/3	13/6/2/4	0.489
CMV viremia, n(%)	10(13.7)	3(11.5)	1.000
History of ACR, n(%)	10(13.7)	5(19.2)	0.531
History of AMR, n(%)	4(5.5)	4(15.4)	0.154
Medication use, n(%)			
Aspirin	39(53.4)	16(61.5)	0.501
Beta-blockers	32(43.8)	9(34.6)	0.490
ACEi or ARB	36(49.3)	11(42.3)	0.649
Diuretics	16(21.9)	9(34.6)	0.292
Statins	58(79.5)	16(61.5)	0.113
Calcineurin inhibitor	63(86.3)	22(84.6)	1.000
mTOR inhibitor	32(43.8)	9(34.6)	0.490

Table 1: Baseline population characteristics. ACEi; Angiotensin converting inhibitor, ACR; acute cellular rejection, AMR; antibody mediated rejection, ARB; angiotensin receptor blocker, CAV; cardiac allograft vasculopathy, CMV; cytomegalovirus, MI; myocardial infarction, *CAV grade No death (n = 71), Death (n = 25).

Table 2: Imaging characteristics:

	No Death (n=73)	Death (n=26)	*P*-value
Resting heart rate	81.88 ± 12.5	81.5 ± 12.8	0.980
Rate pressure product (bpm*mmHg) mean ± SD	10895 ± 2229	11122 ± 1627	0.635
Resting LVEF (%), mean ± SD	65.4 ± 9.7	56.8 ± 13.1	<0.001
^{82}Rb semi-quantitative imaging, median(IQR)			
Summed rest score	0(0-0)	0(0-1)	0.136
Summed stress score	0(0-2)	0(0-8)	0.102
Summed difference score	0(0-1)	0(0-4)	0.072
^{82}Rb quantitative imaging, median(IQR)			
Rest TPD	0(0 – 0.3)	0.2(0.0 – 1.5)	0.018
Stress TPD	1.1(0.0 – 4.4)	2.1(0.6 – 7.9)	0.111
Ischemic TPD	1.1(0.0 – 3.9)	1.9(0.5 – 5.8)	0.200
Rest MBF (mL/min/g)	1.29(1.06 - 1.44)	1.29(1.14 - 1.56)	0.216
Stress MBF (mL/min/g)	2.88(2.41 - 3.60)	2.54(1.71 - 3.24)	0.024
MFR	2.37(2.01 - 2.80)	1.69 (1.28 - 2.19)	<0.001

Table 2: Imaging characteristics: MBF; myocardial blood flow, MFR; myocardial flow reserve, LVEF; left ventricular ejection fraction, TPD; total perfusion deficit.

Table 3. Univariable and multivariable association with all-cause mortality

Variable	Unadjusted HR (95% CI)	*P*-value	Adjusted HR (95% CI)	*P*-value
Age	1.08(1.03 - 1.14)	<0.001	1.10(1.04 – 1.17)	0.001
Male	1.37(0.52 - 3.65)	0.523	-	-
Body mass index	0.97(0.80 - 1.05)	0.442	-	-
LVEF	0.95(0.92 - 0.98)	0.001	0.98(0.94-1.02)	0.232
Cardiac risk factors				
Hypertension	0.54(0.23 - 1.28)	0.161	-	-
Diabetes	1.83(0.84 – 3.99)	0.129	-	-
Dyslipidemia	1.55(0.58 - .11)	0.380	-	-
Renal failure	1.68(0.58 - 4.88)	0.339	-	-
CMV viremia	0.77(0.23-2.55)	0.666	-	-
ACR	1.43(0.54 - 3.79)	0.474	-	-
AMR	1.88(0.84 – 4.20)	0.124	-	-
PET parameters				
Summed rest score	1.15(1.01 – 1.31)	0.033	0.71(0.20 – 2.54)	0.602
Summed stress score	1.09(1.03 - 1.15)	0.002	1.02(0.29 – 3.54)	0.976
Summed difference score	1.15(1.06 - 1.26)	0.001	1.22(0.35 – 4.19)	0.754
Rest MBF	1.81(0.78 – 4.19)	0.166	-	-
Stress MBF	0.56(0.35 – 0.90)	0.017	1.14(0.64 – 2.05)	0.656
Uncorrected MFR*	0.34(0.19 – 0.62)	<0.001	0.30(0.11 – 0.81)	0.017
Corrected MFR*	0.44(0.26 – 0.74)	0.002	0.43(0.20 – 0.90)	0.025

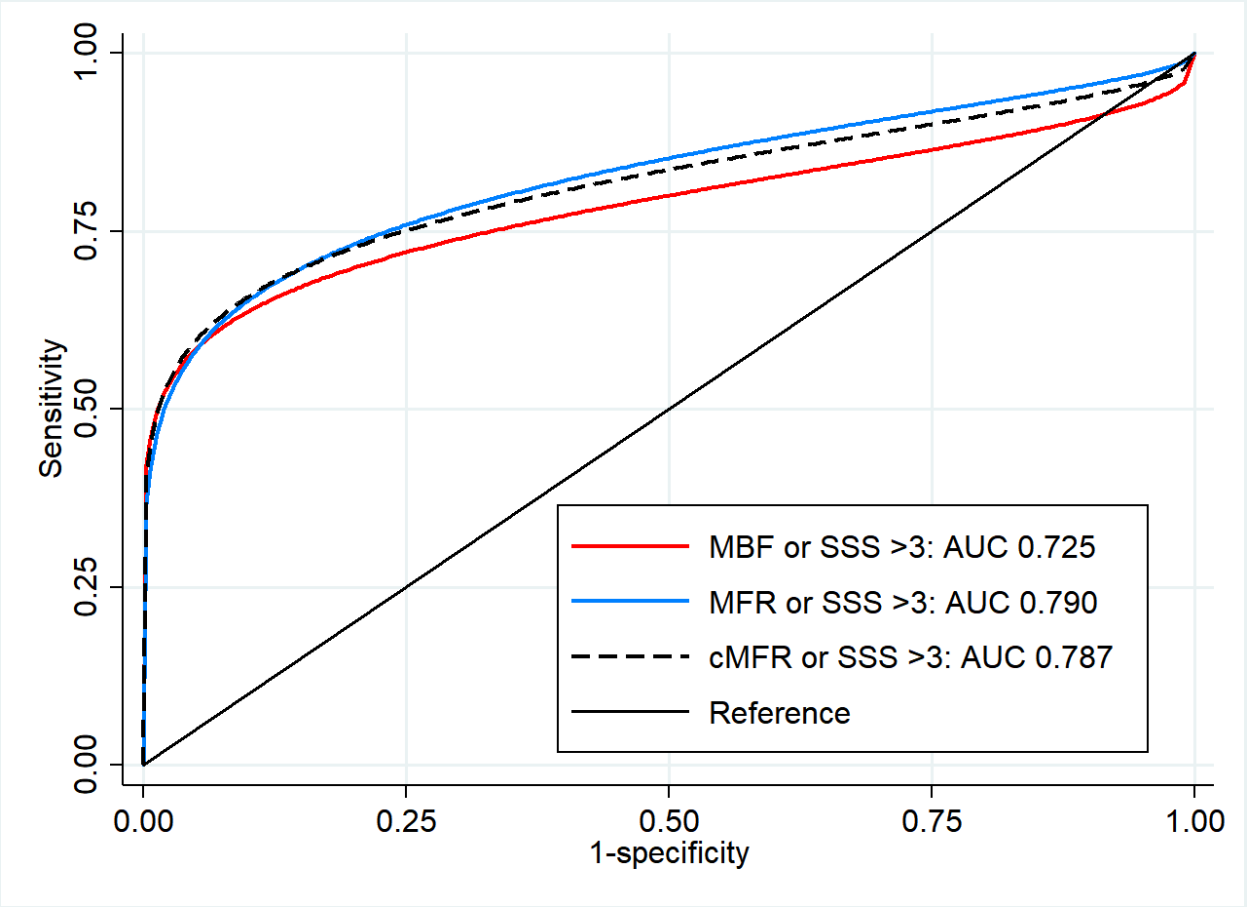
Table 3: Univariable and multivariable analysis of associations with all-cause mortality. *Multivariable analysis performed separately with corrected and uncorrected myocardial flow reserve (MFR). ACR; acute cellular rejection, AMR; antibody mediated rejection, CMV; cytomegalovirus, HR; hazard ratio, LVEF; left ventricular ejection fraction, MBF; myocardial blood flow, MFR; myocardial flow reserve.

Table 4: Diagnostic and Prognostic Accuracy of Established Cut-offs

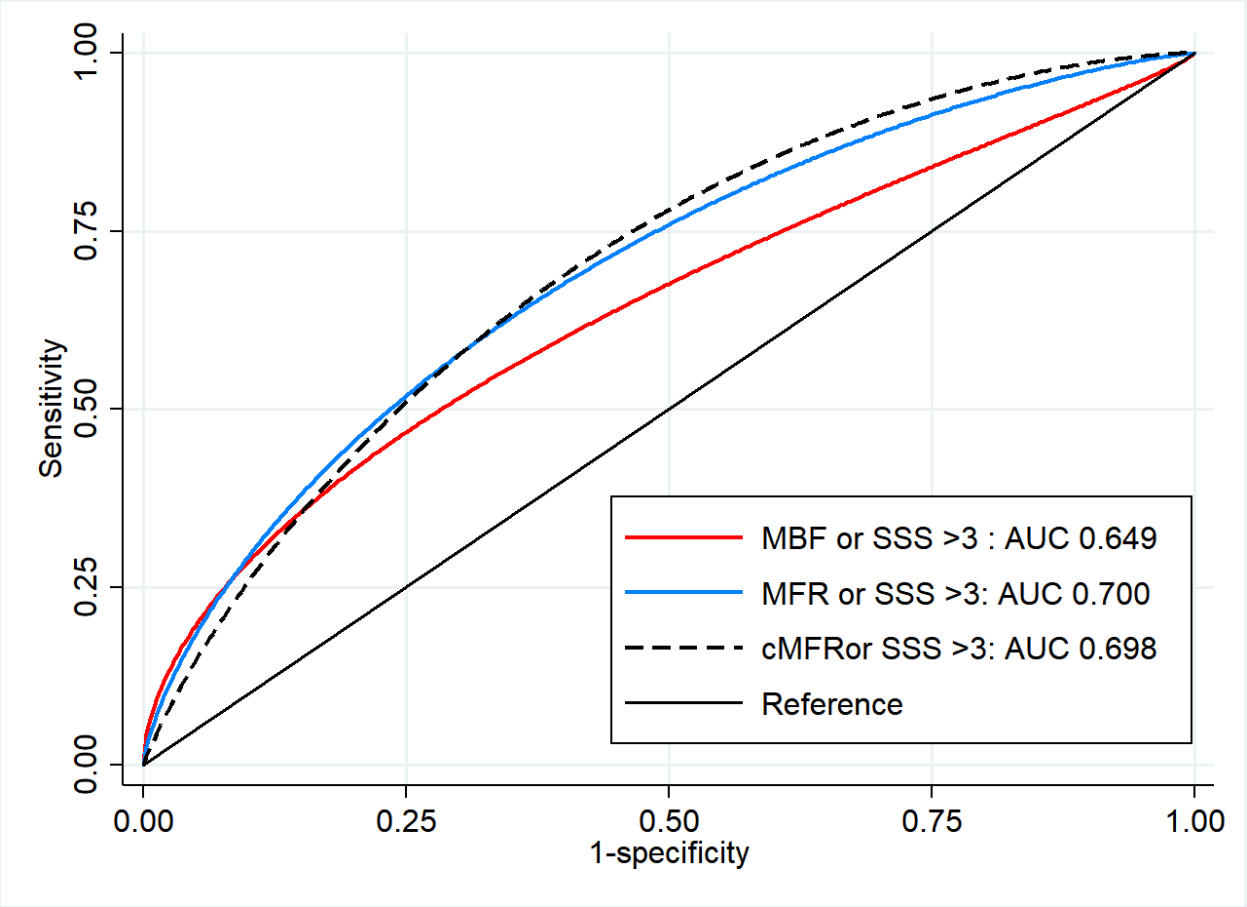
	Patients Abnormal n(%)	Diagnosis of CAV grade 2/3		Annualized All-Cause Mortality Rate	
Cut-off		Sensitivity	Specificity	Abnormal	Normal
MFR < 2.0	34(34.3)	71.4%	71.8%	17.7%	4.7%
MFR <1.75	27(27.3)	57.1%	77.7%	19.6%	5.2%
Stress MBF <3.7	79(79.8)	92.9%	22.4%	9.0%	6.7%
Stress MBF <1.7	10(10.1)	42.9%	95.3%	35.8%	7.0%
SSS > 1	32 (32.2)	64.3%	72.9%	12.3%	7.0%
SSS > 3	15 (15.2)	64.3%	92.9%	18.7%	7.1%
LVEF ≤ 45	10(10.1)	42.9%	95.3%	25.0%	7.2%
MBF < 1.7 and SSS >1 or LVEF ≤ 45	8(8.1)	42.9%	97.7%	60.7%	6.8%
SSS ≥4, LVEF ≤ 45 or MFR <1.75	36(36.4)	71.4%	69.4%	15.4%	5.0%
LVEF ≤ 45% and MFR<1.75*	5(5.1)	35.7%	100.0%	51.6%	7.4%

Table 4: Diagnostic and Prognostic Values of Previously Reported Thresholds. Uncorrected MFR was used because it was numerically superior in all models. CAV; cardiac allograft vasculopathy, LVEF; left ventricular ejection fraction, MACE; major adverse cardiac event, MBF; myocardial blood flow, MFR; myocardial flow reserve, SSS; summed stress score. *The same patients would be identified using MBF<1.7 and LVEF<45%.

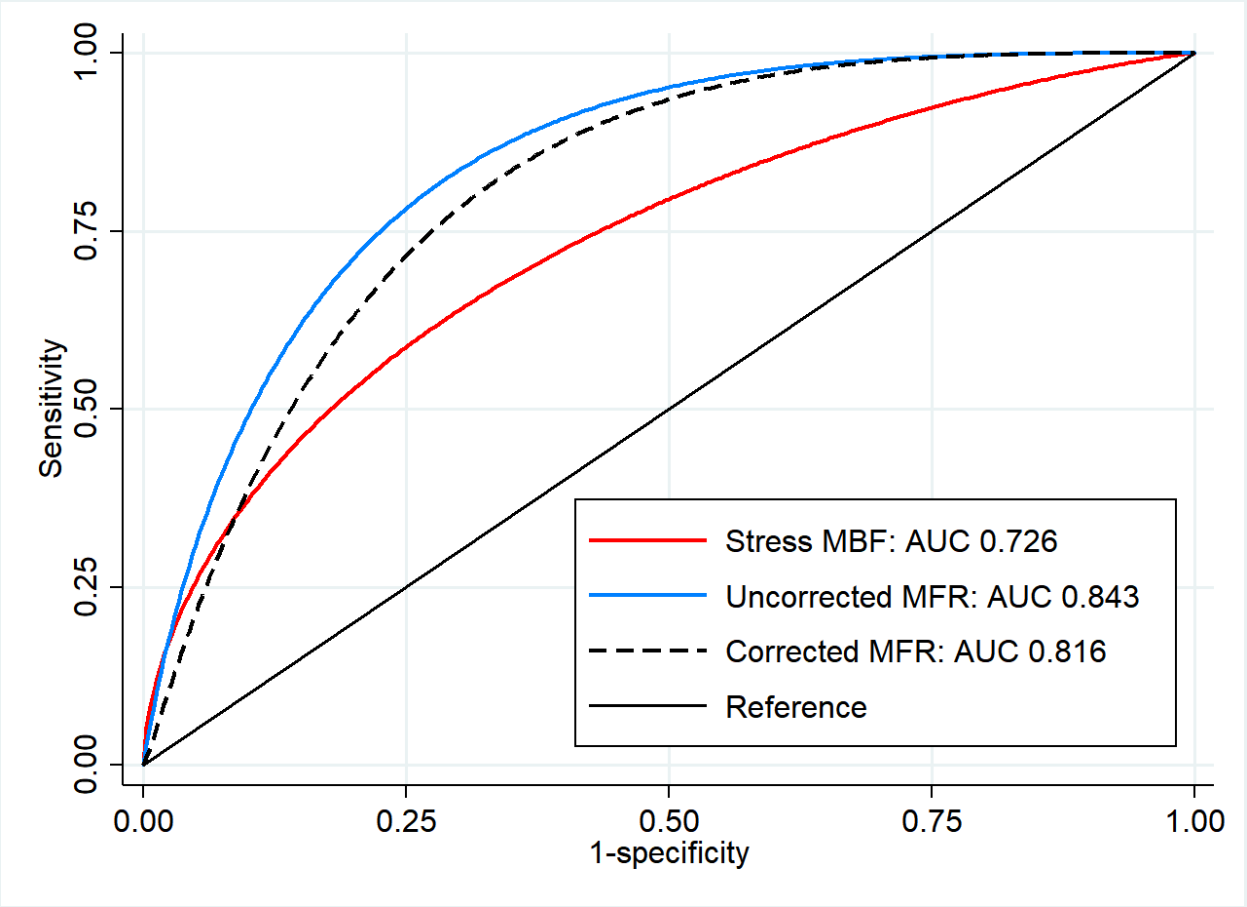
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 1. Univariable and multivariable association with cardiovascular mortality

Variable	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value
Age	1.07 (1.01 – 1.14)	0.030	1.13 (1.05 – 1.22)	0.001
Male	1.42 (0.40 – 4.98)	0.586	-	-
Body mass index	0.94 (0.85 – 1.05)	0.287	-	-
LVEF	0.93 (0.90 – 0.97)	<0.001	0.95 (0.90 – 1.01)	0.079
Cardiac risk factors				
Hypertension	0.60 (0.19 – 1.86)	0.374	-	-
Diabetes	2.20 (0.80 – 6.05)	0.129	-	-
Dyslipidemia	0.81 (0.28 -2.33)	0.694	-	-
Renal failure	2.11 (0.60 – 7.42)	0.243	-	-
CMV viremia	0.85 (0.19 – 3.73)	0.826	-	-
ACR	1.99 (0.64 – 6.18)	0.233	-	-
AMR	2.32 (0.95 – 5.70)	0.066	-	-
PET parameters				
Summed rest score	1.16 (0.99 – 1.36)	0.059	-	-
Summed stress score	1.11 (1.05 – 1.18)	<0.001	0.57 (0.42 – 0.79)	0.001
Summed difference score	1.22 (1.11 – 1.34)	<0.001	2.26 (1.48 – 3.44)	<0.001
Rest MBF	2.33 (0.88 – 6.19)	0.090	-	-
Stress MBF	0.37 (0.20 – 0.69)	0.002	1.08 (0.49 – 2.37)	0.848
Uncorrected MFR*	0.17 (0.08 – 0.36)	<0.001	0.05 (0.01 – 0.30)	0.001
Corrected MFR*	0.25 (0.12 – 0.52)	<0.001	0.22 (0.06 – 0.79)	0.020

Supplemental Table 1: Univariable and multivariable analysis of associations with cardiovascular mortality. *Multivariable analysis performed separately with corrected and uncorrected myocardial flow reserve (MFR). ACR; acute cellular rejection, AMR; antibody mediated rejection, CMV; cytomegalovirus, HR; hazard ratio, LVEF; left ventricular ejection fraction, MBF; myocardial blood flow, MFR; myocardial flow reserve.