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Reactive Hyperemia is Associated with Adverse Clinical Outcomes in Heart Failure

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Abstract

Introduction—Impaired endothelial function, as assessed by brachial artery flow-mediated dilation (FMD), is an established risk factor for cardiovascular events. FMD is impaired in heart failure (HF) patients, but less is known about hyperemic brachial artery flow. We investigated the relationship between FMD and hyperemic flow with adverse clinical outcomes in HF patients.

Methods—Brachial artery FMD and hyperemic flow were assessed in 156 patients (70.5 % Male; 45.5% Caucasian; mean age (± SD) = 56.2 (± 12.4) years) with HF and reduced left ventricular ejection fraction (LVEF). Cox proportional hazard models were used to assess the potential explanatory association of FMD and hyperemic flow with the composite outcome of death or cardiovascular hospitalization over a median 5-year follow-up period.

Results—Both FMD and hyperemic flow were negatively correlated with age, but unrelated to sex, race, body mass index, LVEF or N-terminal pro-B-Type natriuretic peptide (NT-ProBNP). Reduced hyperemic flow, but not FMD, was associated with an increased risk of death or cardiac hospitalization after controlling for traditional risk factors.

Conclusion—The association of reduced hyperemic flow with increased risk of adverse clinical outcomes suggests that micro-vascular function may be an important prognostic marker in patients with HF.

Keywords

flow-mediated dilation; hyperemic flow; endothelial function; heart failure

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INTRODUCTION

Nearly 6 million Americans suffer from heart failure (HF) (1) with a 46% increase projected by 2030 (2). Both the instability of symptoms and deterioration of patients' clinical status can lead to hospitalization for HF, which is estimated to result in an annual cost of $31 billion (1). The vascular endothelium plays an important role in the regulation of vascular tone, coagulation, cell adhesion, and cell proliferation, and endothelial dysfunction is a predictor of atherosclerotic events (3–5). Flow-mediated dilation (FMD) of the brachial artery is a useful non-invasive measure of endothelial function that is considered broadly reflective of the endothelial health of the entire arterial system, including the coronary arteries (6).

Endothelial dysfunction is linked to a wide range of cardiovascular risk factors (7–9) and is a predictor of cardiovascular events, independent of other traditional risk factors (4,10,11). Endothelial dysfunction is evident in HF patients (12–14), and previous studies have suggested that impaired FMD is predictive of cardiac death and hospitalization (12,13,15). The stimulus for the FMD response is increased shear stress, which is evoked by transient forearm occlusion giving rise to hyperemic flow through the brachial artery. There is some evidence that cardiovascular risk is related to hyperemic velocity (14,16), with some studies noting that the hyperemic response to forearm ischemia is a better predictor of adverse cardiovascular outcomes than FMD (17,18).

Although comparatively few studies have examined the relationship between FMD and clinical events in patients with HF, they have shown that impaired FMD is associated with worse clinical outcomes (12,13,15,19,20). However, none of these studies assessed the potential role of hyperemic flow as an independent predictor of adverse outcomes. Therefore, we evaluated whether FMD and reactive hyperemia were associated with risk of death or cardiac hospitalization in patients with HF, in a secondary analysis from a previously reported study (21) .

MATERIAL AND METHODS

Participants

Participants were recruited from a series of patients seen at the heart failure clinics at Duke University Medical Center and the University of North Carolina at Chapel Hill, from January 2000 through December 2002. Approximately 500 patients that met our eligibility criteria (see below) were approached; 219 of these patients consented to participate and were enrolled; for 204 of these participants we obtained a plasma NT-proBNP value necessary to control for HF disease severity in our analyses, as reported in our original paper (21). From this main sample, 156 of these participants were NOT taking nitrates and were included in the current analyses; no participants were lost to clinical follow-up which included 156 of the participants comprising the current sample (N=156). Inclusion criteria were: New York Heart Association class II-III HF; chronic HF of at least 3-months duration; and left ventricular ejection fraction (LVEF) of 40% or less as assessed by echocardiography, radionuclide imaging, or left ventriculography within 6 months of study enrollment. Exclusion criteria were: uncontrolled hypertension (blood pressure (BP) > 180/105 mm Hg);

myocardial infarction or coronary revascularization procedure in the past 3 months; HF due to correctable cause or condition, such as uncorrected primary valvular disease, uncorrected thyroid heart disease, or persistent tachyarrhythmia; pacemaker dependence; use of mechanical assist devices; life limiting or complicated illness including cancer, renal dysfunction, hepatic dysfunction, dementia, and nitrate use. Patients who were pregnant, had atrial fibrillation, reported alcohol or drug abuse within 12 months, or were unable to comply with the assessment procedure or to provide informed consent were excluded. The study complies with the Declaration of Helskinki and was approved locally by the Institutional Review Board at Duke University Medical Center, where all assessments were performed. Written informed consent was obtained from all participants before their participation.

Assessments

Clinical Status and Medications—Heart failure diagnosis, etiology, comorbidities and health behaviors (smoking, alcohol use) were assessed by questionnaires and medical record review. Medication use was documented by participants showing research staff all their current medications. Blood samples for NT-proBNP analysis were taken, stored and analyzed in line with standard procedures and with the associated coefficients as described elsewhere (22).

Blood Pressure—BP was assessed using a Suntech 4240 blood pressure monitor, with measurements of systolic (SBP) and diastolic (DBP) BP acquired during the final 5 minutes of a 30 minute period of quiet relaxation in a seated posture.

Endothelial Function—All participants completed the FMD assessment protocol in the morning, following an overnight fast. Prescribed medications were taken as normal, except for low dose aspirin which participants took with a breakfast provided following the FMD assessment protocol. While others advise that if possible, vasoactive medications should be withheld prior to FMD assessment (23), the maintenance of the medication regimen in this study allows us to ascertain the predictive value of FMD and reactive hyperemia on outcomes, when taking these daily medication regimens, leading us to more generalizable data relevant to our population. Vascular imaging was performed by a single sonographer using an Acuson Aspen ultrasound platform equipped with an Acuson L10 (7–10 MHz) linear array transducer, following guidelines described elsewhere (23). After the participant had rested for 10 min in the supine posture, longitudinal B-mode images of the brachial artery, in the region 4 to 6 cm proximal to the antecubital fossa, were acquired. Images were then captured during the first 120 seconds of reactive hyperemia achieved by inflation of a pneumatic occlusion cuff located around the forearm to supra-systolic pressure (~200 mm Hg) for 5 minutes. Gated end-diastolic images of the artery were stored and arterial diameters were measured as the distance between the proximal and distal arterial wall intima-media interfaces using PC-based software (Brachial Analyzer - Version 5.0, Medical Imaging Applications LLC, Iowa City, Iowa). Peak FMD response was assessed from 10–120 seconds post-deflation of the cuff, with peak arterial diameter quantified using polynomial curve fitting. FMD was expressed as percent increase in arterial diameter (maximum arterial diameter - baseline arterial diameter/baseline arterial diameter × 100)

(23). Because the percent change index may result in bias towards greater vasodilation in smaller arteries (23), baseline arterial diameter was used as a covariate in all analyses. Pulsed Doppler flow signals in the brachial artery were recorded at baseline and for up to 15 seconds after cuff release. The velocity-time integral for baseline and reactive hyperemia was based upon the mean of triplicate pulsed-Doppler flow tracings recorded at each of these phases. Hyperemic velocity was derived by dividing the velocity-time integral by the inter-beat interval, and hyperemic flow was calculated from hyperemic velocity and brachial artery cross-sectional area. All ultrasound image analyses were performed blinded to participants' identities. In our previous work, we have demonstrated excellent reproducibility of the FMD measure (r=0.81) between participants (24).

Follow-up of vital status and hospitalizations—The medical records of participants were reviewed annually, on the anniversary of their baseline assessments, over a median of 5 years (with a range of 4 to 7 years); no participants were lost to follow-up. Each year, patients also were contacted by mail and asked to indicate whether they had been hospitalized during the previous 12 months and provided consent for retrieval of their hospitalization records. The primary end point was defined as the time to cardiovascular hospitalization or death (whichever occurred first) within the follow-up period. Death and instances of cardiac hospitalization were verified through hospital and emergency medical services records and reviewed at semi-annual cause-of-death (COD) committee meetings, whereby COD will be established by consensus. Any hospitalization for myocardial infarction, stroke, worsening HF, coronary artery bypass graft surgery, or heart transplantation was classified as a cardiovascular hospitalization and confirmed by consensus.

Data Analysis—Results are presented as means with standard deviations (SD) or percentages (%) where appropriate. Participants who were taking nitrates were excluded, given the role of nitrates in vasodilation and regulation of vascular tone; thus, 156 participants were included in the final analyses. Pearson's correlations were utilized to determine the univariate relationships between FMD and hyperemic flow with age, sex, race, BMI, LVEF, and N-terminal pro-brain natriuretic peptide (NT-ProBNP).

Cox proportional hazards regression (PHREG) models were used to assess the associations of FMD and hyperemic flow with death or cardiac hospitalization over a 5 year follow up period. In the PHREG models to evaluate the potential explanatory roles of FMD and hyperemic flow, HF etiology (ischemic or nonischemic), LVEF, NT-ProBNP, age, and baseline heart rate (HR) were included in planned models. In order to account for individual differences in vessel diameter, baseline arterial diameter was included in all models. NT-proBNP was expressed as NT-proBNP/1000 and age was expressed as age/10. As a further conservative strategy to confirm the adequacy of the planned models, other possibly relevant factors related to outcome were made available for inclusion in the model by stepwise selection (significance level required for entry into the model was p 0.10). These factors included current alcohol use, current smoking status, defibrillator, diabetes, hypertension, hypercholesterolemia, estimated GFR, as well as usage of the following medication classes: anti-platelet drugs, beta blockers, anti-coagulants, antidepressants, statins, diuretics, ACE-

inhibitors and ARBs. All statistical analyses were conducted using the SAS 9.3 system (SAS Institute, Cary, NC) with significance set at $p = .05$.

RESULTS

Demographic and clinical characteristics of the study sample are summarized in Table 1. Out of the 156 participants in our study, 122 experienced death or a cardiac event (78.2%), with 62 participants dying during our 5 year follow-up (39.7%). The mean FMD (±SD) in our HF population was $4.6 \pm 3.4\%$, and mean reactive hyperemic flow was 0.87 ± 0.42 L/min. Across our sample, 35% had ischemic etiology, an average LVEF of 31%, nearly 10% had a pacemaker and nearly 6% had an ICD. Regarding medication use, our sample were taking beta-blockers (85%), diuretics (90%), ACE inhibitors (89%), statins (40%), anti-coagulants (30%) and antidepressants (nearly 20%). Age was correlated with both FMD (r (155) = −0.24, $p = 0.003$) and hyperemic flow (r (155) = −0.36, $p < 0.001$). No significant associations were observed between FMD or hyperemic flow and other demographic or clinical characteristics.

Cox Proportional Hazards Regression Models

Unadjusted Cox proportional hazards regression models were performed to examine the relationships of vascular function (i.e., FMD and hyperemic flow) to death or cardiac hospitalization. In models which also included HF etiology, LVEF, NT-ProBNP, age, baseline HR, and baseline arterial diameter, hyperemic flow was predictive of death or cardiac hospitalization (HR=0.931; 95% CI 0.873–0.993; $p = 0.030$) whereas FMD was not predictive (HR=1.048; 95% CI 0.981–1.118; $p = 0.17$). Inclusion of both in the model (Table 2), revealed hyperemic flow to still be predictive of death of cardiac hospitalization (HR=0.923; 95% CI 0.867–0.983; $p = 0.013$), whereas FMD was not (HR=1.046; 95% CI 0.980–1.117; $p = 0.17$). In the extended model, hypercholesterolemia was also included by stepwise selection, but the explanatory roles of FMD (HR=1.062; 95% CI 0.993–1.135; $p = 0.08$) and hyperemic flow (HR=0.935; 95% CI 0.878–0.997; $p = 0.04$) remained unchanged, indicating that the planned model was robust. Additional analyses which included all participant characteristics in the extended model did not change the pattern of the results.

Kaplan Meier survival curves illustrating the relationship between hyperemic flow and clinical events are shown in Figure 1, using tertiles of hyperemic flow (High hyperemic flow = flow greater than 0.975 L/min; Intermediate hyperemic flow = less than 0.975 L/min but greater than 0.64 L/min; Low hyperemic flow = flow less than 0.64 L/min). Using the lowest tertile group (i.e., lowest hyperemic flow response) as a reference point, individuals in the highest tertile group (i.e., with the largest reactive hyperemic flow response) had a reduced risk of death or cardiac hospitalization (HR, 0.531; 95% CI 0.278–1.017; $p = 0.056$); risk for individuals in the intermediate tertile was comparable to those in the lowest tertile (HR, 1.016; 95% CI 0.618–1.672; $p = 0.95$).

DISCUSSION

The current study assessed the associations of FMD and reactive hyperemic flow with death or cardiac hospitalization in heart failure patients with reduced LVEF. Reduced reactive

hyperemic flow following forearm occlusion was associated with these adverse outcomes over a follow up period of 5 years, but FMD was not. HF patients with the greatest hyperemic flow were at lowest risk. Our findings are similar to those of Huang et al., who found that reduced reactive hyperemic velocity was predictive of death or cardiac hospitalization in patients with peripheral arterial disease (18). Anderson and colleagues also found that reactive hyperemia was indicative of cardiovascular risk in healthy individuals (17).

In contrast to previous studies, we did not find FMD to be an independent predictor of cardiac death or hospitalization in HF patients (12,13,15,19). Because our study sample was larger than the majority of previous studies, it is unlikely that our null finding for impaired FMD as a risk factor for adverse outcomes in HF could be attributed to lack of statistical power. Indeed, in marked contrast to prior studies, when controlling for hyperemic flow and background characteristics, there was a non-significant trend for a greater FMD response to be associated with worse clinical outcomes. One possible explanation for the discrepancy is that participants in our study were more likely to be taking beta-blockers than in the previously reported studies; 85% of our sample was on beta blockers.

Alternatively, over 60% of patients in our study sample were taking statins, which is higher than the levels observed in the study reported by Huang et al.(18). However, in a similar study reported by Calderaro et al (25), 98% of their high risk vascular patients were taking statins, and hyperemic flow and not FMD was found to be associated with worse clinical outcomes. Given the pleiotropic effects of statins on endothelial function (26,27), higher statin use may promote better endothelial function and thereby possibly negate the potential of FMD (a reflection of endothelial function) to predict risk of death or events in these types of patients. This may be particularly important considering that beta-blockade use enhances the pleiotropic effects of statins on endothelial function (28), and our sample reported a very high use of beta-blockers (85%). Additionally, only Katz et al. (19) asked participants to discontinue nitrate use on the morning of the vascular assessment, whereas the other studies did not appear to control for nitrate use. Given that acute administration of nitrates causes vasodilation, whereas chronic administration can lead to endothelial dysfunction (29,30), the use of nitrates may have been a confounding factor in some of the previous studies. Alternatively, our discrepant findings might imply that impaired microvascular function may be more important as a marker of vascular disease and associated risk than the assessment of FMD in a conduit artery (31), given that hyperemic flow is secondary to vasodilation in microvascular beds.

Hyperemic flow and accompanying shear stress is the stimulus for FMD, with hyperemic velocity often associated with other traditional cardiovascular disease (CVD) risk factors (14,16). In the Framingham study, reactive hyperemia demonstrated more robust associations with traditional CVD risk factors and hyperemic sheer stress than FMD (16,32). Statistical adjustment for hyperemic velocity reduced the strength of the observed associations between FMD and CVD risk factors, further supporting the potential value of hyperemic flow as a marker of risk for CVD events (16). Attenuated hyperemic flow in the coronary circulation can lead to a worsening of myocardial ischemia, and may be a triggering factor for cardiac events (18). Coronary microvascular function has been linked to

adverse CVD outcomes in coronary artery disease patients (33,34), with reduced hyperemic flow or microvascular dysfunction associated with increasing risk of cardiovascular events, as well as the development of HF (35).

Limitations and methodological considerations

With assessment of brachial artery hyperemic responses and FMD, methodological considerations must be discussed. Firstly, FMD has been criticized as a user-dependent measurement in contrast to reactive hyperemia, and should be considered when interpreting FMD results (36). As noted elsewhere, upper arm occlusion is technically challenging for accurate data acquisition as the image can be distorted by collapse of the brachial artery (23), however, forearm occlusion was completed in the current study and we observed no arterial collapse at the imaging site. Further, changes in FMD of the conduit artery could be interpreted as changes in flow (indirectly as a consequence of changes in the microcirculation) rather than endothelial function improvements (23). Thus, as recommended by guidelines (23), our reporting of hyperemic flow should help to determine that our assessment of FMD was not indicative of flow. Finally, while statistical adjustments were made as a part of our analyses, it should be clarified that this cannot account for biological processes. Although our analyses did not show any differences in FMD or hyperemic flow associated with BMI, nor any predictive value of BMI on risk of death or cardiac hospitalization, future work should nonetheless consider the potential effects of obesity on reactive hyperemia and its relationship to clinical outcomes. Better assessments of obesity, including waist circumference, lipid profiles and body composition may prove to be more informative.

In summary, our study of stable HF outpatients with reduced ejection fraction showed that reactive hyperemic blood flow, but not brachial artery FMD, was associated with adverse clinical outcomes. Compared with a strong reactive hyperemic response, moderately or markedly reduced reactive hyperemia were associated with significantly greater risk of hospitalization or death over 5 years of follow-up. These findings are consistent with growing evidence that peripheral microvascular dysfunction may be an important marker of risk for adverse cardiovascular outcomes in patients with coronary artery disease (CAD) and HF.

Acknowledgments

ABBREVIATIONS

HF	Heart Failure
FMD	Flow Mediated Dilation
LVEF	Left Ventricular Ejection Fraction
BP	Blood Pressure

SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
COD	Cause of Death
BMI	Body Mass Index
NT-ProBNP	N-terminal pro-Brain Natriuretic Peptide
HR	Heart Rate
GFR	Glomerular Filtration Rate
ACE	Angiotensin Converting Enzyme
ARB	Angiotensin II Receptor Blockers
CVD	Cardiovascular Disease
CAD	Coronary Artery Disease

References

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart Disease and Stroke Statistics-2015 Update: A Report From the American Heart Association. Circulation. 2015; 131:e29–322. [PubMed: 25520374]

2. Heidenreich PA, Albert NM, Allen LA, et al. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. Circ Heart Fail. 2013; 6:606–19. [PubMed: 23616602]

3. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol. 2003; 23:168–75. [PubMed: 12588755]

4. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation. 2005; 111:363–8. [PubMed: 15668353]

5. Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. Circulation. 2003; 108:2093–8. [PubMed: 14530195]

6. Takase B, Hamabe A, Satomura K, et al. Close relationship between the vasodilator response to acetylcholine in the brachial and coronary artery in suspected coronary artery disease. Int J Cardiol. 2005; 105:58–66. [PubMed: 16207546]

7. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992; 340:1111–5. [PubMed: 1359209]

8. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000; 101:1899–906. [PubMed: 10779454]

9. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol. 2013; 168:344–51. [PubMed: 23041097]

10. Mancini GBJ. Vascular structure versus function: is endothelial dysfunction of independent prognostic importance or not? J Am Coll Cardiol. 2004; 43:624–8. [PubMed: 14975473]

11. Fichtlscherer S, Breuer S, Zeiher AM. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. Circulation. 2004; 110:1926–32. [PubMed: 15451794]

12. Meyer B, Mörtl D, Strecker K, et al. Flow-mediated vasodilation predicts outcome in patients with chronic heart failure: comparison with B-type natriuretic peptide. J Am Coll Cardiol. 2005; 46:1011–8. [PubMed: 16168284]

13. Shechter M, Matetzky S, Arad M, Feinberg MS, Freimark D. Vascular endothelial function predicts mortality risk in patients with advanced ischaemic chronic heart failure. Eur J Heart Fail. 2009; 11:588–93. [PubMed: 19406838]

14. Philpott AC, Lonn E, Title LM, et al. Comparison of new measures of vascular function to flow mediated dilatation as a measure of cardiovascular risk factors. The American journal of cardiology. 2009; 103:1610–5. [PubMed: 19463524]

15. Fischer D, Rossa S, Landmesser U, et al. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. Eur Heart J. 2005; 26:65–9. [PubMed: 15615801]

16. Mitchell GF, Parise H, Vita JA, et al. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. Hypertension. 2004; 44:134–9. [PubMed: 15249547]

17. Anderson TJ, Charbonneau F, Title LM, et al. Microvascular function predicts cardiovascular events in primary prevention: long-term results from the Firefighters and Their Endothelium (FATE) study. Circulation. 2011; 123:163–9. [PubMed: 21200002]

18. Huang AL, Silver AE, Shvenke E, et al. Predictive value of reactive hyperemia for cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery. Arterioscler Thromb Vasc Biol. 2007; 27:2113–9. [PubMed: 17717291]

19. Katz SD, Hryniewicz K, Hriljac I, et al. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation. 2005; 111:310–4. [PubMed: 15655134]

20. Takishima I, Nakamura T, Hirano M, et al. Predictive value of serial assessment of endothelial function in chronic heart failure. Int J Cardiol. 2012; 158:417–22. [PubMed: 21371765]

21. Sherwood A, Blumenthal JA, Trivedi R, et al. Relationship of depression to death or hospitalization in patients with heart failure. Arch Intern Med. 2007; 167:367–73. [PubMed: 17325298]

22. Sherwood A, O'Connor CM, Routledge FS, et al. Coping Effectively with Heart Failure (COPE-HF): Design and Rationale of a Telephone-based Coping Skills Intervention. J Card Fail. 2011; 17:201–207. [PubMed: 21362527]

23. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002; 39:257–65. [PubMed: 11788217]

24. Sherwood A, Bower JK, McFetridge-Durdle J, Blumenthal JA, Newby LK, Hinderliter AL. Age moderates the short-term effects of transdermal 17beta-estradiol on endothelium-dependent vascular function in postmenopausal women. Arterioscler Thromb Vasc Biol. 2007; 27:1782–7. [PubMed: 17541023]

25. Calderaro D, Monachini MC, Vieira CLZ, et al. Reactive Hyperemia Revisited. Arterioscler Thromb Vasc Biol. 2008; 28:e23–e24. [PubMed: 18354086]

26. Liao JK, Laufs U. PLEIOTROPIC EFFECTS OF STATINS. Annu Rev Pharmacol Toxicol. 2005; 45:89–118. [PubMed: 15822172]

27. Blum A, Shamburek R. The pleiotropic effects of statins on endothelial function, vascular inflammation, immunomodulation and thrombogenesis. Atherosclerosis. 2009; 203:325–30. [PubMed: 18834985]

28. Takase B, Hattori H, Tanaka Y, Nagata M, Ishihara M. Anti-sympathetic action enhances statin's pleiotropic effects: the combined effect of rosuvastatin and atenolol on endothelial function. Int Angiol. 2014; 33:27–34. [PubMed: 24452083]

29. Münzel T, Gori T. Nitrate therapy and nitrate tolerance in patients with coronary artery disease. Curr Opin Pharmacol. 2013; 13:251–9. [PubMed: 23352691]

30. Münzel T, Steven S, Daiber A. Organic nitrates: Update on mechanisms underlying vasodilation, tolerance and endothelial dysfunction. Vascul Pharmacol. 2014; 63:105–113. [PubMed: 25446162]

31. Title LM, Lonn E, Charbonneau F, et al. Relationship between brachial artery flow-mediated dilatation, hyperemic shear stress, and the metabolic syndrome. Vasc Med. 2008; 13:263–270. [PubMed: 18940902]

32. Vita JA. Nitric oxide-dependent vasodilation in human subjects. Methods Enzymol. 2002; 359:186–200. [PubMed: 12481571]

33. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation. 2000; 101:948–54. [PubMed: 10704159]

34. Halcox JPJ, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation. 2002; 106:653–8. [PubMed: 12163423]

35. Petersen JW, Pepine CJ. Microvascular coronary dysfunction and ischemic heart disease: where are we in 2014? Trends Cardiovasc Med. 2015; 25:98–103. [PubMed: 25454903]

36. Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial Flow-Mediated Dilation Predicts Incident Cardiovascular Events in Older Adults: The Cardiovascular Health Study. Circulation. 2007; 115:2390–2397. [PubMed: 17452608]
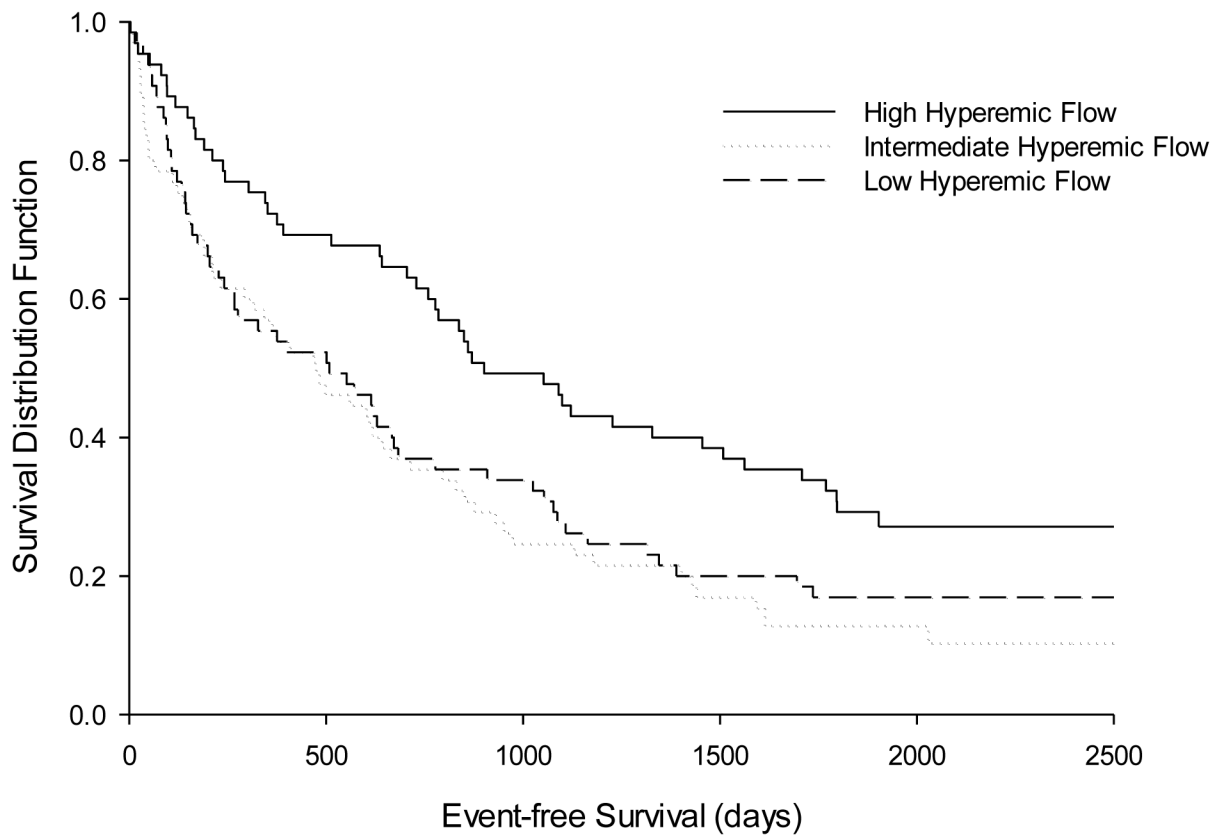
Figure 1.
Kaplan Meier survival curves which illustrate the associated risk between death or cardiac hospitalization and hyperemic flow responses. Note: High hyperemic flow = flow greater than 0.975 L/min; Intermediate hyperemic flow = less than 0.975 L/min but greater than 0.64 L/min; Low hyperemic flow = flow less than 0.64 L/min

Table 1

Characteristics of the study sample, and by hyperemic flow group

Characteristic (N=156)	Mean (SD) or % (n)	High Hyperemic Flow (N=72)	Intermediate Hyperemic Flow (N=71)	Low Hyperemic Flow (N=76)	F value
Age (years)	56.2 (12.4)	53.2 (11.5)	57.5 (12.1)	61.4 (12.2)	8.79, p <.001
Race (% Caucasian)	45.5 (71)	54.2 (39)	50.7 (36)	40.7 (48)	1.44, p=0.24
Sex (% Male)	70.5 (110)	90.3 (65)	63.3 (45)	27.1 (20)	20.07, p <.001
BMI (kg/m²)	31.1 (7.01)	31.8 (6.36)	32.5 (7.71)	29.87 (7.45)	2.53, p=0.08
Etiology (%ischemic)	35.3 (55)	36.1 (26)	49.3 (35)	39.5 (30)	1.38, p=.25
NT-ProBNP (pg./mL)	1571 (2380)	879 (1353)	1824 (2473)	2448 (3623)	5.95, p <.001
LVEF (%)	31 (11)	34 (11)	29 (10)	31 (11)	3.06, p=.049
Baseline SBP (mmHg)	99 (18)	101 (18)	96 (18)	103 (20)	2.85, p=.06
Baseline HR (bpm)	67 (12)	68 (12)	66 (12)	67 (12)	0.45, p=.64
Current smoker (%)	14.1 (22)	23.6 (17)	14.1 (10)	11.8 (9)	2.09, p=.13
Alcohol use (%)	23.8 (37)	26.8 (19)	22.7 (16)	16.4 (12)	1.08, p=.34
Diabetes (%)	39.1 (61)	33.3 (24)	47.8 (34)	51.3 (39)	2.73, p=.07
Hyperlipidemia (%)	48.1 (75)	47.2 (34)	54.9 (39)	44.7 (34)	0.82, p=.44
Pacemaker (%)	9.6 (15)	8.33 (6)	15.5 (11)	7.89 (6)	1.39, p=.25
Implantable Cardioverter Defibrillator (ICD) (%)	5.7 (9)	8.33 (6)	7.04 (5)	7.89 (6)	0.04, p=.96
Beta block use (%)	85.3 (133)	90.3 (65)	81.7 (58)	90.8 (69)	1.75, p=.18
Diuretic use (%)	90.4 (141)	88.9 (64)	93.0 (63)	96.1 (73)	1.41, p=.25
ACE inhibitor use (%)	89.1 (140)	88.9 (64)	90.1 (64)	82.9 (63)	0.99, p=.37
Statin use (%)	40.4 (63)	44.4 (32)	45.1 (32)	44.7 (34)	0.00, p=.99
Anticoagulant use (%)	30.2 (47)	23.6 (17)	35.2 (25)	30.3 (23)	1.16, p=.32
Antidepressant use (%)	19.8 (31)	18.1 (13)	22.5 (16)	22.4 (17)	0.28, p=.76

Note: High hyperemic flow = flow greater than 0.975 L/min; Intermediate hyperemic flow = less than 0.975 L/min but greater than 0.64 L/min; Low hyperemic flow = flow less than 0.64 L/min. BMI = Body Mass Index (BMI); NT-ProBNP = N-terminal pro-brain natriuretic peptide; LVEF = Left Ventricular Ejection Fraction; SBP = Systolic Blood Pressure; HR = Heart Rate; GFR=glomerular filtration rate.

Table 2

Cox proportional hazard regression analyses in relation to the composite outcome of death or cardiac hospitalization

Variable	Planned Model[*] Hazard Ratio (95% CI)	P Value	Extended Model[†] Hazard Ratio (95% CI)	P Value
Etiology	1.773 (1.151–2.730)	**0.009**	2.167 (1.343–3.496)	**0.002**
LVEF	0.973 (0.953–0.993)	**0.009**	0.972 (0.953–0.992)	**0.006**
NT-ProBNP	1.234 (1.114–1.368)	**<0.0001**	1.227 (1.107–1.360)	**<0.0001**
Age	0.914 (0.756–1.103)	0.35	0.920 (0.764–1.107)	0.38
Baseline HR	1.020 (1.000–1.039)	**0.047**	1.020 (1.001–1.040)	**0.040**
Baseline Arterial Diameter	1.232 (0.903–1.680)	0.19	1.284 (0.931–1.171)	0.13
FMD	1.046 (0.980–1.117)	0.17	1.062 (0.993–1.135)	0.08
Hyperemic Flow	0.923 (0.867–0.983)	**0.013**	0.912 (0.854–0.973)	**0.0055**
Hyperlipidemia	-	-	1.528 (0.963–2.426)	**0.07**

Note: CI = confidence interval; LVEF = left ventricular ejection fraction; NT Pro-BNP = N-terminal pro-natriuretic peptide; HR = heart rate; FMD = flow-mediated dilation. NT-proBNP was expressed as NT-proBNP/1000, age was expressed as age/10 and hyperemic flow was expressed as hyperemic flow/1000.

[*] Adjusted for etiology, LVEF, NT-ProBNP, age, baseline HR, baseline arterial diameter, hyperemic flow and FMD.

[†] Adjusted for the variables in the a priori planned model, as well as hyperlipidemia