Endostatin a Potential Biomarker for Heart Failure with Preserved Ejection Fraction

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Abstract

Background: Endostatin is a circulating endogenous angiogenesis inhibitor preventing neovascularization. Previous studies demonstrated the prognostic value of Endostatin among patients with heart failure with reduced ejection fraction (HFrEF). However, the role of Endostatin among patients with heart failure with preserved ejection fraction (HFpEF) remains unclear.

Objective: This study aimed to investigate the association between serum Endostatin levels, natriuretic peptide levels and the severity of left ventricular diastolic dysfunction and the diagnosis of HFpEF.

Methods: Endostatin serum concentrations were measured in 301 patients comprising 77 HFpEF patients, 169 patients with asymptomatic left ventricular diastolic dysfunction (ALVDD), and 55 controls with normal cardiac function.

Results: Endostatin serum levels were significantly elevated in patients with HFpEF (median/interquartile range 179.0 [159-220]) and ALVDD (163.8 [145.4-191.3]) compared to controls (149.1 [130.6-176.9]), p < 0.001 and p = 0.004, respectively) and significant correlated with N-terminal pro B-type natriuretic peptide (NT-proBNP).

Conclusions: This hypothesis-generating pilot study gives first evidence that Endostatin correlates with the severity of diastolic dysfunction and may become a novel biomarker for HFpEF. We hypothesize a rise in Endostatin levels may reflect inhibition of adaptive angiogenesis and adverse cardiac remodeling. (Arq Bras Cardiol. 2017; 109(5):448-456)

Keywords: Heart Failure; Endostatins; Natriuretic Peptides; Biomarkers; Stroke Volume.

Introduction

The patient population affected by heart failure (HF) is growing in a constant manner. This is because of an aging society, western lifestyle and improved acute clinical care (e.g. after myocardial infarction). Although, the treatment of chronic conditions improved over the last decades, mortality and morbidity rates in this patient population are amongst the highest for western healthcare systems. In the United States (US) HF is the leading cause for hospitalization for patients older > 65 years of age. In 2030 the direct costs for heart failure will reach 70 billion US$ in the US alone. Half of the patients affected by HF present with a diastolic dysfunction and a preserved ejection fraction (HFpEF), with this proportion increasing. Clinical data proves that those patients suffering from a reduced ejection fraction (HFrEF) show better outcomes compared to HFpEF patients. A reasonable idea might be that no therapy has been shown to improve outcomes in HFpEF. Current therapeutic options including fluid management, blood pressure control and physical exercisetoe relief patients’ symptoms. A major drawback regarding the development of new therapies for HFpEF, is the absence of clear diagnostic criteria. This makes the definition of patient populations for clinical studies difficult. At present, the diagnosis is solely based echocardiography. Especially, the separation between HFpEF and HFrEF is even more challenging and misleading in patients with newly diagnosed HF. Therefore new strategies for disease phenotyping in HF are urgently needed. New biomarkers may achieve better disease phenotyping. Although, many reports have been published on HF biomarkers over the last decades, the impact on clinical decision making is still limited. BNP/NTproBNP demonstrated high clinical utility to identify patients at high risk for heart failure hospitalization and death. However, in this context these markers for clinical studies are only applicable in relatively stable patients and not in terminal HF patients. Furthermore, the use of BNP/NTproBNP in clinical practice to optimize therapy with drugs, which are known to improve patient’s outcome is suitable. However, BNP/NTproBNP is not accepted as surrogate endpoint and can only exploratorily be used as endpoint in clinical trials. The appraisal of clinical utility of BNP/NTproBNP manifests in the current guidelines for the management of heart failure.
propose Endostatin, a potent angiogenesis inhibitor, known mostly from oncology, as a potential new HF biomarker candidate.16-18 Most importantly Gouya et al. reported in a prospective observational cohort study in 151 HF patients, a correlation between elevated circulating Endostatin levels and mortality. Furthermore, this study showed a clear association between Endostatin levels and progressing diastolic dysfunction, the key characteristic of HFpEF.19 This is why we hypothesize that Endostatin could potentially be a biomarker suitable to diagnose and disease phenotype HFpEF patients. In the present study, we aimed to investigate the sole role of Endostatin as a biomarker for HFpEF and diastolic dysfunction.

Methods

The study protocol was approved by the Ethics Committee of the Private University of Witten/Herdecke, Germany (project no. 91/08) and conducted in accordance with the Declaration of Helsinki. Signed written informed consent was obtained from all patients.

Study population

Participants of the prospective observational cohort study were patients contacting the HELIOS Klinikum Wuppertal Heart Center (Wuppertal, Germany) for elective coronary angiography or diagnostic work-up of heart failure. Patients with a stable or suspected coronary artery disease (CAD) and/or a diagnostic workup of CHF were included in the study. The exclusion criteria were: left ventricular ejection fraction (EF) < 50%, known CAD with progressive chest pain within the last month, coronary angioplasty or myocardial infarction within 6 weeks, hypertrophic cardiomyopathy, moderate-to-severe valvular heart disease, uncontrolled hypertension, atrial fibrillation or other severe arrhythmias, serum-creatinine > 2.0 mg/dl. Patients selected for the control group had to have no history or symptoms of CHF, a normal ejection fraction > 55%, a ratio of the early diastolic transmural velocity (E) and the early diastolic tissue Doppler velocity (E') of < 8, and normal NTproBNP values. A total of 301 patients were recruited and assigned to three groups based on echocardiographic diagnostic criteria as recommended by the European Society of Cardiology.20 The control group consisted of 55 patients (29 males) with normal diastolic function (DF). The group with asymptomatic left ventricular diastolic dysfunction (ALVDD) contained 169 patients (95 males) with E medial < 8 cm/s, E/E' medial ratio 8-15 and NT-proBNP levels < 220 pg/ml. The group with HFpEF comprised 77 patients (46 females, 31 males) displaying ALVDD Grad II - III with E/E' ratio > 15, NT-proBNP levels > 220 pg/ml and current or previous signs or symptoms of heart failure.

Echocardiography

Echocardiography was performed using a standard ultrasound system (Vivid 7, General Electric, Milwaukee, Wisconsin). A complete transthoracic study was performed including 2D, M-mode, spectral and colour Doppler techniques following current recommendations and guidelines.21,22 The left atrium volume index (LAVI) was calculated using the biplane area-length method. Left ventricular EF was measured by means of the modified biplane Simpson’s method.23 Left ventricular mass index (LVMI) was computed with the Devereux formula indexed to the body surface.24 HFpEF was defined in accordance with the EAE/ASE recommendation, based on the assessment of left ventricular diastolic function.24 Primary measurements included mitral inflow peak early (E-wave) and late (A-wave) diastolic filling velocities as well as systolic (S) and early diastolic (E') mitral annular velocities whereat in each case three consecutive beats were measured and averaged. Conventional transmural flow was measured with Pulse-wave Doppler (PW). PW tissue Doppler imaging (DTI) was performed at the junction of the septal and lateral mitral annulus in the apical 4-chamber view. Based on primary measurements E/A and E'/E' ratios were calculated.

Laboratory analysis

Peripheral venous whole blood samples were taken after 5 minutes at rest for routine laboratory testing (OGTT, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, creatine, leucocytes, hemoglobin, creatin kinase, TSH, hsCRP, GOT, GPT). Blood was drawn into pyrogen-free tubes without any additives, centrifuged at room temperature, aliquoted and stored at –80°C. All laboratory analyses were outsourced to Roche Diagnostics (Penzberg, Germany) and performed on blinded samples. For analysis of plasma NT-proBNP the Elecsys 2010 NT-proBNP assay (Roche Diagnostics, Mannheim, Germany) was used. For measurement of Endostatin the ELISA assay of R&D Systems (Minneapolis, MN USA) was used. All assays were performed according to manufacturer’s recommendations.

Statistical analysis

All analyses were performed using SPSS statistical software (SPSS 19.0, Chicago, IL, USA). The data are presented as median with 25th/75th percentiles (interquartile range) for continuous variables or as absolute numbers and corresponding percentages for categorical variables unless otherwise specified. Log transformed values were used for analysis as appropriate. A p-value < 0.05 was considered statistically significant. We used the Kolmogorov-Smirnov test as appropriate to test for normal distribution. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups. Fisher’s Test was used for the comparison of two sets of binary variables and the χ2 test to evaluate differences in proportions in more than 2 sets of categorical variables. Endostatin and NT-proBNP levels were compared across subjects with normal diastolic function, mild ALVDD and HFpEF by the Mann-Whitney U-test, and Jonckheere-Terpstra test. Spearman rank correlation was used to identify variables associated with Endostatin. A multivariable model was included to predict the presence of HFpEF and included the following covariates: Endostatin, age, gender, diabetes, hypertension, coronary artery disease and body mass index. Due to the exploratory nature of this study, there is no minimum required sample size.
Table 1 – Baseline characteristics of the study population. Values are median (25-75 interquartile range) or absolute numbers and percentage (%)

| Clinical variables (median/interquartile range or %) | Control (n = 55) | mild ALVDD (n = 169) | HFpEF (n = 77) | p value |
|------------------------------------------------------|------------------|----------------------|----------------|---------|
| Age (years) | 54 (48-61) | 66 (58-71) | 73 (68-77) | < 0.001* |
| BMI (kg/m²) | 25.5 (24.1-29.1) | 27.8 (25.6-32.3) | 27.5 (25.7-32.0) | 0.001* |
| Waist circumference (cm) | 98 (86-107) | 102 (94-114) | 102 (98-111) | 0.002* |
| Hip circumference (cm) | 98 (94-103) | 103 (96-111) | 105 (98-114) | 0.003* |
| Systolic BP (mmHg) | 125 (110-136) | 134 (127-140) | 136 (130-140) | 0.001* |
| Diastolic BP (mmHg) | 80 (70-80) | 80 (76-84) | 80 (72-84) | 0.12 |
| Resting HR (beats/min) | 70 (68-76) | 72 (69-76) | 70 (65-76) | 0.51 |
| CAD, n (%) | 21 (38.2) | 99 (58.6) | 49 (63.6) | 0.009* |
| CABG, n (%) | 1 (2.0) | 5 (3.0) | 9 (11.7) | 0.007* |
| PCI, n (%) | 15 (27.3) | 75 (44.4) | 33 (42.9) | 0.074 |
| History of MI, n (%) | 8 (14.5) | 36 (21.3) | 17 (22.1) | 0.501 |
| History of stroke, n (%) | 1 (2.0) | 5 (3.0) | 3 (3.9) | 0.824 |
| Cardiovascular risk factors | | | | |
| Treated hypertension, n (%) | 38 (69.1) | 148 (88.1) | 73 (96.1) | < 0.001* |
| Diabetes mellitus, n (%) | 4 (7.3) | 45 (26.6) | 22 (28.6) | < 0.001* |
| Medication | | | | |
| ACE inhibitor, n (%) | 26 (47.3) | 110 (65.1) | 42 (54.5) | 0.042* |
| AT1 receptor blocker, n (%) | 6 (10.9) | 17 (10.1) | 23 (29.9) | < 0.001* |
| Diuretics, n (%) | 8 (14.5) | 45 (26.6) | 36 (49.8) | < 0.001* |
| Ca²⁺ blocker, n (%) | 6 (10.9) | 23 (13.6) | 21 (27.3) | 0.013* |
| B-blocker, n (%) | 28 (50.9) | 103 (60.9) | 57 (74.0) | 0.021* |

*a statistically significant (p < 0.05). BMI: body mass index; BP: blood pressure; HR: heart rate; CAD: coronary artery disease; CABG: coronary artery bypass graft; PCI: percutaneous coronary intervention; MI: myocardial infarction; DF: diastolic function; ALVDD: left ventricular diastolic dysfunction; HFpEF: heart failure with preserved ejection fraction; NS: non-significant. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups.
Table 2 – Laboratory data and echocardiographic parameters. (25-75th interquartile range) or absolute numbers and percentage (%) X² test was used as appropriate

| Clinical variables | Control (n = 55) | Mild ALVDD (n = 169) | HFpEF (n = 77) | p value |
|-------------------|----------------|---------------------|----------------|---------|
| **Biomarker**     |                |                     |                |         |
| Endostatin (ng/ml)| 149.1 (130.6-176.9) | 163.8 (145.4-191.3) | 179.0 (159-220) | < 0.001* |
| NT-pro-BNP (pg/ml)| 90.1 (45.8-129.2)   | 96.7 (43.7-170.7)    | 343.6 (151.7-703.4) | < 0.001* |
| **Routine parameter** |               |                     |                |         |
| Total cholesterol (mg/dl) | 189 (163-228) | 193 (171-221) | 191 (170-210) | NS |
| LDL-cholesterol (mg/dl)  | 107 (89-135) | 109 (89-135) | 109 (86-129) | NS |
| HDL-cholesterol (mg/dl)  | 53 (46-64) | 50 (38-62) | 48 (41-61) | NS |
| Triglyceride (mg/dl)  | 119 (83-185) | 142 (100-206) | 131 (104-189) | NS |
| Lp (a) (mg/dl)       | 8 (5-27) | 18 (6-39) | 15 (6-52) | NS |
| TSH (mU/l)           | 1.20 (0.94-2.09) | 1.42 (0.824-2.08) | 1.315 (0.80-1.90) | NS |
| Creatinine (mg/dl)   | 0.8 (0.7-0.9) | 0.9 (0.7-0.9) | 0.9 (0.75-1.10) | NS |
| hsCRP                | 0.1 (0.1-0.3) | 0.3 (0.1-0.6) | 0.3 (0.2-0.69) | 0.005* |
| Glucose              | 89 (84-97) | 100 (91-111) | 97 (89-103) | 0.020* |
| Hb (mg/dl)           | 14.3 (13.3-15.1) | 14.1 (13.2-15.0) | 13.6 (12.5-14.5) | 0.004* |
| CK (U/l)             | 76 (58-105) | 78 (60-114) | 72 (55-104) | NS |
| SGOT (U/l)           | 25 (21-31) | 25 (21-31) | 26 (21-32) | NS |
| **LV geometry**      |                |                     |                |         |
| IVS (mm)             | 10 (9-11) | 12 (10-13) | 12 (11-14) | < 0.001* |
| PLW (mm)             | 10 (9-11) | 12 (10-13) | 12 (11-14) | < 0.001* |
| LVEDD (mm)           | 44 (42-47) | 44 (39-48) | 45 (41-50) | NS |
| LVESD (mm)           | 30 (28-34) | 29 (25-34) | 31 (27-36) | NS |
| LVM (g/m²)           | 72 (62-84) | 81 (67-102) | 91 (77-119) | < 0.001* |
| **Systolic function**|                |                     |                |         |
| EF (%)               | 68 (62-72) | 67 (61-71) | 67 (63-73) | NS |
| S’max (cm/s)         | 7.2 (6.3-8.0) | 6.3 (5.7-7.5) | 6.1 (5.4-6.7) | < 0.001* |
| **Diastolic function**|              |                     |                |         |
| LA-Index (m²/m²)     | 25.4 (21.8-28.7) | 29.8 (25.7-33.3) | 39.3 (36.7-49.1) | < 0.001* |
| E (cm/s)             | 60 (60-80) | 60 (50-70) | 80 (70-90) | < 0.001* |
| A (cm/s)             | 60 (50-70) | 80 (70-90) | 80 (70-90) | < 0.001* |
| E/A ratio            | 1.14 (0.68-1.25) | 0.75 (0.67-0.86) | 1.11 (0.85-1.25) | < 0.001* |
| E’ septal (cm/s)     | 8.4 (7.3-9.4) | 5.9 (5.2-6.8) | 5.4 (4.6-6.3) | < 0.001* |
| E’ lateral (cm/s)    | 10.7 (8.5-13.0) | 8.2 (6.9-9.5) | 6.9 (5.6-8.4) | < 0.001* |
| Average E’ (cm/s)    | 9.8 (8.6-11.0) | 7.2 (6.1-8.1) | 6.2 (5.2-7.2) | < 0.001* |
| E/E’ septal ratio    | 8.0 (6.9-9.0) | 10.2 (8.3-11.9) | 15.1 (12.5-17.1) | < 0.001* |
| E/E’ average ratio   | 7.0 (6.0-7.7) | 8.4 (6.8-10.1) | 13.3 (11.1-14.8) | < 0.001* |

*statistically significant (p < 0.05); NT-proBNP: N-terminal fragment of the prohormone B-type natriuretic peptide; LDL: low density lipoprotein; HDL: high density lipoprotein; Lp (a): lipoprotein (a); TSH: thyroid stimulating hormone; hsCRP: high sensitive C-reactive protein; Hb: hemoglobin; CK: creatinkinase; SGOT: serum glutamic oxaloacetic transaminases; IVS: interventricular septum; PLW: posterior lateral wall; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; EF: ejection fraction; LA: left atrial; E: early diastolic transmitral velocity; A: late diastolic transmitral velocity; E’: early diastolic tissue Doppler velocity; DF: diastolic function; LVDD: left ventricular diastolic dysfunction; HFpEF: heart failure with preserved ejection fraction; NS: non-significant. The Mann-Whitney U-test was used to analyze differences between the medians of two groups and the Kruskal-Wallis test to test the equality of medians among more than two distinct groups.
of Endostatin serum levels showed more advanced cardiac remodeling (LV hypertrophy and left atrial enlargement) as well as more severe diastolic function abnormalities reflecting increasing left ventricular filling pressures (Figure 1B). Consistently, there was a significant positive moderate correlation between Endostatin and NT-proBNP levels \((r = 0.32, p < 0.001\); Figure 1C).

Discussion

We hypothesized that circulating Endostatin levels are altered in patients with ALVDD and HFpEF. Furthermore, elevated levels are associated with the presence and severity of diastolic function abnormalities. To verify the hypothesis we performed a clinical observational study including 301 patients, which were assigned based on their echocardiographic characteristics to three different groups. To our knowledge, this is the first published report linking increased circulating Endostatin levels to the presence and severity of diastolic function abnormalities and HFpEF in a well phenotyped cohort of patients with normal systolic function. In the present study, Endostatin showed a graded increase from controls over ALVDD to HFpEF. Furthermore, higher Endostatin levels were significantly associated with established markers of structural cardiac abnormalities including the LAVi and increased LV mass as well as functional abnormalities like E/E’ ratio. Particularly, an increased LAVi without concomitant mitral valve disease reflects a chronic remodeling process typical for HFpEF. Consistently, we found that elevated Endostatin levels were associated with elevated NT-proBNP levels, a well-recognized prognostic marker and indicator of elevated ventricular filling pressures among patients, independent from LVEF.

Endostatin, a 20-kDa proteolytic fragment from the C-terminal domain of collagen XVIII, was shown to have an inhibitory effect on tumor growth working as an antiangiogenic growth factor. Endostatin plays a role in the local balance of angiogenesis and shows potent anti-angiogenic activity by inhibiting proliferation and migration of endothelial cells in addition to inducing endothelial cell apoptosis. Endostatin is produced by the proteolytic cleavage of the C-terminal domain of collagen XVIII, a component of the extracellular matrix. The precise mechanism of conversion from collagen XVIII to Endostatin has not yet been fully elucidated.
patients with coronary artery disease (CAD) demonstrate that Endostatin protein levels correlate significantly with reduced angiogenesis and poorly developed cardiac collateral vasculature.\(^{18,10}\)

The results from our study fit well to the pathophysiological model used to explain the development of HFrEF. In general, HFrEF is a complex disease involving an interplay of various factors. There is the hypothesis that a failure of oxygen delivery to the cardiomyocytes triggers a pro-angiogenic response in patients suffering from heart failure.\(^{35-37}\) Nonetheless, angiogenic and antiangiogenic growth factors often co-exist in tissues with angiogenesis.\(^{34}\) Thus, the status of endothelial cells and endothelial function is determined by a balance between these positive and negative factors on angiogenesis, and the balance may in inappropriate shifted towards antiangiogenic factors in patients with HF. It was shown that the role of microvascular dysfunction and microvascular inflammation is especial for patients with the diagnosis of HFrEF.\(^{13,35,36}\) A new pathophysiologic model presented by Redfield et al.\(^{6}\) points from pro-inflammatory coexisting conditions to systemic endothelial inflammation and impaired oxygen delivery.\(^{5}\) Global ventricular performance is highly dependent on oxygen supply and thus myocardial perfusion, and an essential component of myocardial perfusion during ventricular hypertrophy is the myocyte–microvascular balance and the myocyte/capillary ratio. In cardiac autopsy specimens, it has recently been shown that microvascular rarefaction is a downstream phenomenon in HFrEF.\(^{37}\)

Furthermore, Kitzman et al.\(^{38}\) has demonstrated that HFrEF patients display significant abnormalities in the skeletal muscle as well as an abnormal capillary-to-fiber ratio, probably building the basis for severe exercise intolerance in HFrEF patients.\(^{38}\) In addition, Gouya et al.\(^{19}\) have shown in a relatively small HFrEF study population that high levels of serum Endostatin were associated with all-cause mortality and concluded that the effect of increased angiogenesis is HF may be blunted by an overspill of anti-angiogenic factors such as Endostatin.\(^{19}\) Thus, we hypothesize that similar pathophysiological concepts may be involved in patients with HFrEF, were a high proportion of patients has a coincidence of coronary artery disease and diabetes, both damaging the endothelial structure.\(^{39}\) This was also true for our patient population as shown in table 1. Endostatin could be moderator of the microvascular effects seen in these patients.\(^{40}\)

Several limitations of this study must be acknowledged. The observational nature of the present study prohibits definitive determination of cause and effect relationships. Second, the present study was a single-center experience

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**Figure 1**— (A) The boxplot graphics show serum Endostatin levels for the ALVOD, HFrEF patients and the control group. (B) The correlation between Endostatin levels and the E/E’ ratio as surrogate for increased left ventricular filling pressures. (C) The logarithmic dot blot displays the correlation of Endostatin serum levels with NT-proBNP.
with a relatively small number of subjects. Third, longitudinal
follow-up data were not available to test associations
between the Endostatin serum levels and clinical outcomes.
Moreover, we enrolled consecutive patients referred for
elective coronary angiography and echocardiography, which
may not represent a general population cohort without
evidence or suspicious for cardiovascular diseases.

Further studies should include more patients from a
broader population and capture longitudinal data including
information about hospitalization and mortality.

Conclusion

In this exploratory hypothesis-generating study, we
provide first evidence that Endostatin correlates with the
presence and severity of diastolic dysfunction and HFpEF
and may become a novel biomarker for the diagnosis and
stratification of HFpEF. Increased Endostatin levels may
reflect deterioration of diastolic function caused by adverse
remodeling. Further prospective studies are needed to
determine the causal relationship as well as the diagnostic
and prognostic value of Endostatin in HFpEF and the
potential role as a therapeutic target.

Author contributions

Conception and design of the research: Barroso MC,
Dinh W; Acquisition of data: Barroso MC, Gükler JE, Dinh W;
Analysis and interpretation of the data: Barroso MC, Boehme P,
Kramer F, Gükler JE, Mondriztik T, Koehler T, Karoff M, Dinh W;
Statistical analysis: Dinh W; Writing of the manuscript: Barroso
MC, Boehme P, Dinh W; Critical revision of the manuscript
for intellectual content: Boehme P, Kramer F, Mondritzik T,
Koehler T, Gükler JE, Karoff M, Dinh W.

Potential Conflict of Interest

No potential conflict of interest relevant to this article
was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

References

1. Writing Group M, Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha
ML, et al. Heart disease and stroke statistics-2016 Update: a report from the
American Heart Association. Circulation. 2016;133(4):e38-360. doi:
10.1161/CIR.0000000000000350

2. Ambrosy AP, Fonarow GC, Butler J, Chioncel O, Greene SJ, Vaduganathan
M, et al. The global health and economic burden of hospitalizations for heart
failure: lessons learned from hospitalized heart failure registries. J Am Coll
Cardiol. 2014;63(12):1123-31. doi: 10.1016/j.jacc.2013.11.053

3. Desai AS, Stevenson LW. Rehospitalization for heart failure: predict or prevent?
Circulation. 2012;126(4):501-6. doi: 10.1161/CIRCULATIONAHA.112.125435

4. Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC,
et al. Forecasting the impact of heart failure in the United States: a policy
statement from the American Heart Association. Circulation Heart Failure.
2013;6(3):606-19. doi: 10.1161/HHF01013e318291329a

5. Gladderd JD, Linke WA, Redfield MM. Heart failure with preserved ejection fraction.
Ph:gers-Archiv : Eur J Physiol. 2014;466(6):1037-53. doi: 10.1007/
s00424-014-1480-8

6. Chan MM, Lam CS. How do patients with heart failure with preserved
ejaculation fraction die? Eur J Heart Fail. 2013;15(6):604-13. doi: 10.1039/
eurj/hft06062

7. Lee DS, Gona P, Albano J, Larson MG, Benjamin EJ, Levy D, et al. A
systematic assessment of causes of death after heart failure onset in the
community: impact of age at death, time period, and left ventricular
systolic dysfunction. Circ Heart Fail. 2011;4(1):36-43. doi: 10.1161/
CIRCHEARTFAILURE.110.957480

8. Redfield MM. Heart failure with preserved ejection fraction. N Engl J Med.
2016;375(19):1868-77. doi: 10.1056/NEJMmp1511175

9. Redfield MM. Heart failure with preserved ejection fraction. N Engl J Med.
2017;376(9):897. doi: 10.1056/NEJMct1615918

10. Ponikowski P, Voors AA, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)
Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016;37(27):2129-200. doi: 10.1093/
eurheartj/ehw128

11. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr., Drazner MH, et al.
2013 ACCF/AHA guideline for the management of heart failure: executive
summary: a report of the American College of Cardiology Foundation/American
Heart Association Task Force on practice guidelines. Circulation. 2013;
128(16):1810-52. doi: 10.1161/CIR.0b013e31829e8807

12. Garg A, Virmani D, Agarwal S, Agarwal C, Sharma A, Stefani C, et al. Clinical
application of biomarkers in heart failure with a preserved ejection fraction: a
review. Cardiology. 2017;136(3):192-203. doi: 10.1159/000450573

13. Januzzi JL Jr, Felker GM. Surfing the biomarker tsunami. JACC Heart Fail.
2013;1(3):213-5. doi: 10.1016/j.jchf.2013.03.007

14. Balion C, McKelvie R, Don-Wauchope AC, Sontaquida PL, Oremus M,
Keshavarz H, et al. B-type natriuretic peptide-guided therapy: a systematic
review. Heart Fail Rev. 2014;19(4):553-64. doi: 10.1007/j.10159.2013.03.007

15. 2016 ACC/AHA/HFSA Focused Update on New Pharmacological
Therapy for Heart Failure: An Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Card Fail.
2016;22(9):659-69. doi: 10.1016/j.cardfail.2016.07.001

16. Ueland T, Aukrust P, Nymo SH, Kjekshus J, McMurray JJ, Wikstrand J,
et al. Clinical application of biomarkers in heart failure with a preserved ejection fraction: a review. Cardiology. 2017;136(3):192-203. doi: 10.1159/000450573

17. Ponikowski P, Voors AA, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)
Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J. 2016;37(27):2129-200. doi: 10.1093/
eurheartj/ehw128
17. Motiwala SR, Szymonikia J, Belcher A, Weiner RB, Bagghal AL, Gaggin HK, et al. Measurement of novel biomarkers to predict chronic heart failure outcomes and left ventricular remodeling. J Cardiovasc Transl Res. 2014;7(2):250-61. doi: 10.1007/s12265-013-9522-8

18. Mitsuma W, Kodama M, Hanawu H, Ito M, Ramadan MM, Hiroto S, et al. Serum endostatin in the coronary circulation of patients with coronary heart disease and its relation to coronary collateral formation. Am J Cardiol. 2007;99(4):494-8. doi: 10.1016/j.amjcard.2006.09.095

19. Gouya G, Siller-Matula JM, Fritzner-Szekeers M, Neuhof S, Storka A, Neuhof LM, et al. Association of endostatin with mortality in patients with chronic heart failure. Eur J Clin Invest. 2014;44(2):125-35. doi: 10.1111/eci12197

20. Paulus WJ, Tschoppe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J. 2007;28(20):2539-50. doi: 10.1093/eurheartj/ehm037

21. Evangelista A, Flachskampf F, Lancellotti P, Badano L, Aguilar R, Monaghan M, et al. European Association of Echocardiography recommendations for standardization of performance, digital storage, and reporting of echocardiographic studies. Eur J Echocardiogr. 2008;9(4):438-48. doi: 10.1093/ejechoc/jen174

22. LangRM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18(12):1440-63. doi: 10.1016/j.echo.2005.10.005

23. Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B, et al. Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. J Am Coll Cardiol. 2004;43(8):1399-404. doi: 10.1016/j.jacc.2003.10.062

24. Nagaei SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic dysfunction by echocardiography. Eur J Echocardiogr. 2009;10(2):165-93. doi: 10.1093/ejechocard/jept007

25. Rossi A, Chonghiade M, Triposkiadis F, Solomon SD, Pieske B, Butler J. Primary structural and functional abnormalities in left atrium in heart failure with preserved ejection fraction: structure, function, and significance. Circ Heart Fail. 2014;7(6):1042-9. doi: 10.1161/CIRCHEARTFAILURE.114.001276

26. van Veldhuisen DJ, Lissen GC, Jaarsma T, van Gilst WH, Hoes AW, Tijssen JG, et al. B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. J Am Coll Cardiol. 2013;61(14):1498-506. doi: 10.1016/j.jacc.2012.12.044

27. O’Reilly MS, Boehm T, Shing Y, Fukai N, Vasis G, Lane WS, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997;88(2):277-85. PMID:9008168

28. Saarelä J, Rehn M, Oikarinen A, Audio-Harainen H, Pihlajaniemi T. The short and long forms of type XVII collagen show clear tissue specificities in their expression and location in basement membrane zones in humans. Ann J Pathol. 1998;151(2):611-26. doi: 10.1016/S0002-9440(10)65603-9

29. Saarelä J, Ylikarppa R, Rehn M, Purmonen S, Pihlajaniemi T. Complete primary structure of two variant forms of human type XVII collagen and tissue-specific differences in the expression of the corresponding transcripts. Matrix Biol. 1998;16(6):319-28. PMID:9503365

30. Panchal VR, Rehman J, Nguyen AT, Brown JW, Turriente MW, Mohamed Y, et al. Reduced pericardial levels of endostatin correlate with collateral development in patients with ischemic heart disease. J Am Coll Cardiol. 2004;43(8):1383-7. doi: 10.1016/j.jacc.2003.10.063

31. Ohtsuka T, Inoue K, Hara Y, Morikoa N, Ohshima K, Suzuki J, et al. Serum markers of angiogenesis and myocardial ultrasonic tissue characterization in patients with dilated cardiomyopathy. Eur J Heart Fail. 2005;7(4):689-95. doi: 10.1016/j.ejheart.2004.09.011

32. Patel JV, Abraham A, Chackathayil J, Gunning M, Creager J, Hughes EA, et al. Circulating biomarkers of angiogenesis as indicators of left ventricular systolic dysfunction amongst patients with coronary artery disease. J Intern Med. 2009;265(5):562-7. doi: 10.1111/j.1365-2796.2008.02057.x

33. Friese I, Margossian RE, Moran AM, Cao-Danh H, Mose MA, Del Nido PJ. Vascular endothelial growth factor delays onset of failure in pressure-overload hypertrophy through matrix metalloproteinase activation and angiogenesis. Basic Res Cardiol. 2006;101(3):204-13. doi: 10.1007/s00395-005-0581-0

34. Toyoda E, Matsunaga T, Chilian WM. Myocardial angiogenesis. Mol Cell Biochem. 2004;264(1-2):35-44. doi: 10.1016/j.mcb.2004.09.011

35. Hoening MR, Bianchi C, Rosenzweig A, Sellke FW. The cardiac microvasculature in hypertension, cardiac hypertrophy and diastolic heart failure. Curr Vasc Pharmacol. 2008;6(4):292-300. PMID: 18855717

36. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, et al. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest. 2005;115(8):2108-18. doi: 10.1172/JCI24682

37. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Malezewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. Circulation. 2015;131(6):550-9. doi: 10.1161/CIRCULATIONAHA.114.009625

38. Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, et al. Measurement of novel biomarkers to predict chronic heart failure. J Intern Med. 2009;265(5):292-300. PMID: 18855717

39. Sharma K, Kass DA. Heart failure with preserved ejection fraction: assessment by echocardiography and tissue Doppler imaging. J Am Coll Cardiol. 2004;43(8):1399-404. doi: 10.1016/j.jacc.2003.10.062

40. Nagaei SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, et al. Recommendations for the evaluation of left ventricular diastolic dysfunction by echocardiography. Eur J Echocardiogr. 2009;10(2):165-93. doi: 10.1093/ejechocard/jept007

41. Rossi A, Chonghiade M, Triposkiadis F, Solomon SD, Pieske B, Butler J. Primary structural and functional abnormalities in left atrium in heart failure with preserved ejection fraction: structure, function, and significance. Circ Heart Fail. 2014;7(6):1042-9. doi: 10.1161/CIRCHEARTFAILURE.114.001276

42. van Veldhuisen DJ, Lissen GC, Jaarsma T, van Gilst WH, Hoes AW, Tijssen JG, et al. B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. J Am Coll Cardiol. 2013;61(14):1498-506. doi: 10.1016/j.jacc.2012.12.044

43. O’Reilly MS, Boehm T, Shing Y, Fukai N, Vasis G, Lane WS, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997;88(2):277-85. PMID:9008168
