Relation of Lymphangiogenic Factor Vascular Endothelial Growth Factor-D to Elevated Pulmonary Artery Wedge Pressure

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Lymphatic flow is augmented in states of chronic heart failure (cHF). However, the biological mechanism driving increased lymphatic flow capacity (lymphangiogenesis) in cHF is unknown. Recent studies have indicated that vascular endothelial growth factors (VEGF-A, -C, and -D) are involved in lymphangiogenesis. This study examined the association between VEGF-A, -C, and -D levels, invasively measured hemodynamics, and heart failure symptoms. Subjects who underwent clinically indicated right heart catheterization at Medical University of South Carolina between 12/2016 and 7/2018 were eligible for inclusion. These subjects underwent clinical assessment of cHF severity (including 6MWT and KCCQ), hemodynamic assessment with right heart catheterization, laboratory studies including B-type natriuretic peptide, and concomitant measurement of VEGF-A, -C, and -D. Fifty-six patients were included for analysis. Subjects with elevated pulmonary artery wedge pressure (PAWP) had significantly higher VEGF-D levels (263 ± 415 pg/ml vs 65 ± 101 pg/ml; p = 0.02). PAWP was not associated with VEGF-A or VEGF-C levels. When stratified by VEGF-D, subjects with elevated VEGF-D had clinical and hemodynamic characteristics associated with worse HF severity (lower ejection fraction, higher b-type natriuretic peptide, higher PAWP, lower cardiac output), but were not more symptomatic by Kansas City Cardiomyopathy Questionnaire scores and had similar 6-minute walk test distance compared with subjects with lower VEGF-D. Subjects with an elevated VEGF-D were more likely to have a diagnosis of heart failure for >3 years. In conclusion, VEGF-D is associated with elevated PAWP in cHF, and elevated VEGF-D may mitigate cHF symptoms. **(Am J Cardiol 2019;124:756−762)**

Patients with chronic heart failure (cHF) may have a paucity of pulmonary edema despite elevated intracardiac filling pressures.[1−2] An increase in pulmonary lymphatic drainage capacity is an oft-cited explanation for this observation.[3] The lymphatic system's ability to markedly increase its capacity for interstitial fluid removal may abrogate pulmonary congestion despite elevated pulmonary venous pressure. Dogs with cHF are able to increase their lymphatic flow >10X normal and demonstrated only mild congestive signs.[4] Similarly in humans, a markedly increased lymphatic flow rate was observed via direct thoracic duct cannulation in patients with cHF.[5] However, the biological mechanism by which pulmonary lymphangiogenesis occurs in the state of cHF is unknown. The vascular endothelial growth factors (VEGFs) A, C, and D have been implicated in lymphangiogenesis,[6,7] and recently elevated VEGF-D was shown to correlate with the incident diagnosis of heart failure in dyspneic patients.[8] However, the association of VEGF-A, C, and D with invasive hemodynamic, echocardiographic and clinical characteristics in heart failure are unclear. In this study, we prospectively examined the contemporaneous relations between VEGF-A, -C, and -D levels, invasively measured hemodynamics, and heart failure symptoms. Our hypothesis was that VEGF-D levels would be elevated in subjects with elevated pulmonary arterial wedge pressure (PAWP).

Methods

This study complies with the Declaration of Helsinki, was approved by the Medical University of South Carolina institutional review board, and all subjects signed informed consent. All adult patients who underwent right heart catheterization (RHC) at Medical University of South Carolina were screened for participation. Exclusion criteria included the inability to provide informed consent, active pregnancy, and use of medications or a history of conditions which may be associated with altered lymphangiogenic VEGF levels (e.g., Medications: thalidomide, lenalidomide, pomalidomide, bevacizumab, any cytotoxic chemotherapy agents; Medical Conditions: active cancer within 365 days, diagnosis

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See page 761 for disclosure information.

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0002-9149/
https://doi.org/10.1016/j.amjcard.2019.05.056

of familial or secondary lymphedema, treatment of active bacterial infection within 7 days, active inflammatory rheumatologic disease requiring anti-inflammatory therapy, lymphangioleiomyomatosis).[9–11] After inclusion and consent, clinical and demographic subject data were recorded from the medical record. Heart failure diagnosis duration was established by chart review preferentially, or patient recall if chart review was inadequate, and assigned to 1 of 5 temporal categories (0 to 6 months, 6 months to 1 year, 1 year to 3 years, 3 to 5 years, or >5 years). On the day of the RHC, a clinical assessment was performed including New York Heart Association (NYHA) class assessment, 6-minute walk test (6MWT) with pre- and post-6MWT dyspnea assessed by Likert scale, and Kansas City Cardiomyopathy Questionnaire (KCCQ).

During RHC, B-type natriuretic peptide was drawn from the superior vena cava. Estimated glomerular filtration rate (eGFR) was calculated via the Modification of Diet in Renal Disease Study equation.[12] For lymphangiogenic factor analysis, 3 separate samples were drawn from distinct anatomic sites confirmed by hemodynamic waveforms and/or fluoroscopy: superior vena cava , pulmonary artery (PA), and PA wedge position. All invasively-obtained pressures were measured during spontaneous respiration at end-expiration and were confirmed or adjudicated by the first author. At the time of hemodynamic adjudication, the first author was blinded to the clinical assessment (e.g., NYHA, KCCQ, 6MWT). Hemodynamics and clinical assessment were both adjudicated before analysis of lymphangiogenic factor levels. PAWP position was confirmed by PAWP oxygen saturation >90% as per guidelines.[13] Cardiac output was measured by the thermodilution method averaged in triplicate. Subjects were nil per os or nothing by mouth after the midnight before RHC per institutional protocol, but they continued medications including diuretic therapy.

Samples drawn for assessment of lymphangiogenic factors were placed in Ethylenediamine tetraacetic acid collecting tubes on ice. Each sample was prepared via serial centrifugation and then stored at −80˚C. VEGF analysis was performed by EveTechnologies via multiplex ELISA analysis (EveTechnologies catalogue #: HDAGP17) with a single freeze-thaw cycle.

The distributions of demographic and clinical characteristics were compared using the chi-square test. Continuous parameters are expressed as either mean ± standard deviation or median [IQR] and compared with Student's *t* test or Wilcoxon signed-rank test as appropriate. A 1-way between-subjects ANOVA was conducted to compare the association of HF duration categories with VEGF-D levels. A $p \leq 0.05$ was used as statistically significant; all p values were assessed as 2-sided. Statistical analysis was performed by SPSS (IBM, Version 25.0. Armonk, NY: IBM Corp).

Results

Fifty-eight subjects were enrolled in the study. One subject was excluded after enrollment when they were later found to have systemic sarcoidosis, and 1 subject's samples were contaminated after collection leaving 56 subjects for analysis. Baseline demographics are shown in Table 1. Forty-four (79%) subjects carried a diagnosis of heart failure before RHC, and 26 (46%) subjects had an EF ≤40%. There was no significant difference in VEGF-A, -C or -D in comparison between anatomic sites (superior vena cava, PA, or PAWP), and so an average of all 3 sites was used for each analyte (Supplemental Table 1).

Table 1
Baseline demographic and clinical data

Variable	
Age (years)	55.3 ± 13.6
Men	35/56 (63%)
Weight (kg)	96.8 ± 22.5
BMI (kg/m^2)	32.5 ± 7.7
Race	
• White	32/56 (57%)
• Black	23/56 (41%)
• Hispanic	1/56 (2%)
Pre-RHC heart failure diagnosis	44/56 (79%)
• Ischemic heart disease	10/44 (23%)
• Nonischemic cardiomyopathy	30/44 (68%)
• Congenital heart disease	2/44 (4%)
• Familial cardiomyopathy	2/44 (4%)
Duration heart failure diagnosis (years)	
• 0-0.5	7/44 (14%)
• 0.5-1	5/44 (12%)
• 1-3	6/44 (14%)
• 3-5	5/44 (12%)
• >5	21/44 (48%)
Indication for RHC	
• Evaluation for advanced therapies	24/56 (43%)
• Diagnostic evaluation	29/56 (52%)
• Pulmonary hypertension evaluation	3/56 (5%)
Diabetes mellitus	20/56 (36%)
Hypertension	26/56 (46%)
COPD	8/56 (14%)
NYHA class	2.8 ± 0.6
Left ventricular ejection fraction (%)	43 ± 21
eGFR (ml/min/1.73 m^2)	58.6 ± 27
Sodium (mEq/L)	139 ± 2.2
Medications	
• ACEi/ARB/ARNI	32/56 (57%)
• Beta blocker	40/56 (71%)
• Aldosterone inhibitor	31/56 (55%)

ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor blocker and neprilysin inhibitor; BMI = body mass index; eGFR = estimated GFR calculated from MDRD equation; NYHA = New York Heart Association; RHC = Right heart catheterization.

Subjects with a PAWP below the median (<15 mm Hg) had a significantly lower VEGF-D level than those with PAWP above the median (65 ± 101 pg/ml vs 263 ± 415 pg/ml; p = 0.016). Similar analysis of VEGF-C for those below and above median PAWP showed no difference (135 ± 268 pg/ml vs 78 ± 54 pg/ml respectively; p = 0.28). VEGF-A was also not different comparing those with PAWP below or above median (98 ± 182 pg/ml vs 67 ± 51 pg/ml respectively; p = 0.39). Analysis of average VEGF-D levels stratified by quartiles of PAWP revealed a step-wise relation between VEGF-D and PAWP; illustrated in Figure 1. Similar analyses of VEGF-C and VEGF-A showed no relation (Figures 2 and 3).

When stratified by VEGF-D, subjects with VEGF-D levels above the median had a higher b-type natriuretic peptide

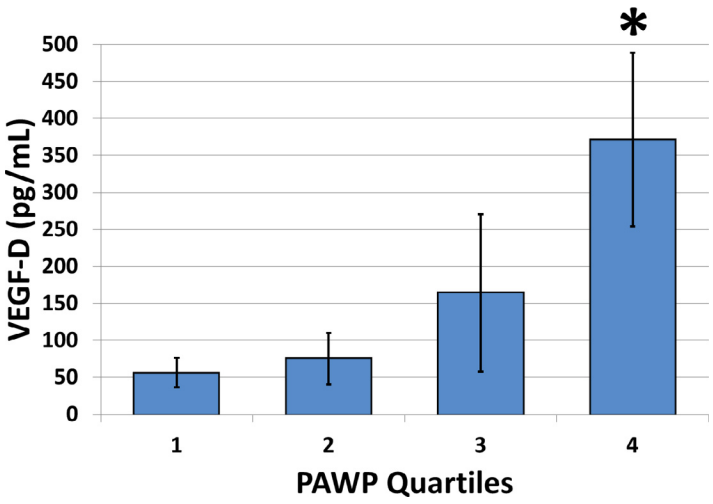

Figure 1. Average VEGF-D levels and SEM stratified by PAWP quartiles. *= $p < 0.05$ compared with quartile 1 and quartile 2. For PAWP quartiles: Q1 = 0 to 10mm Hg; Q2 = 11 to 14mm Hg; Q3 = 15 to 19mm Hg; Q4 = ≥20 mm Hg.

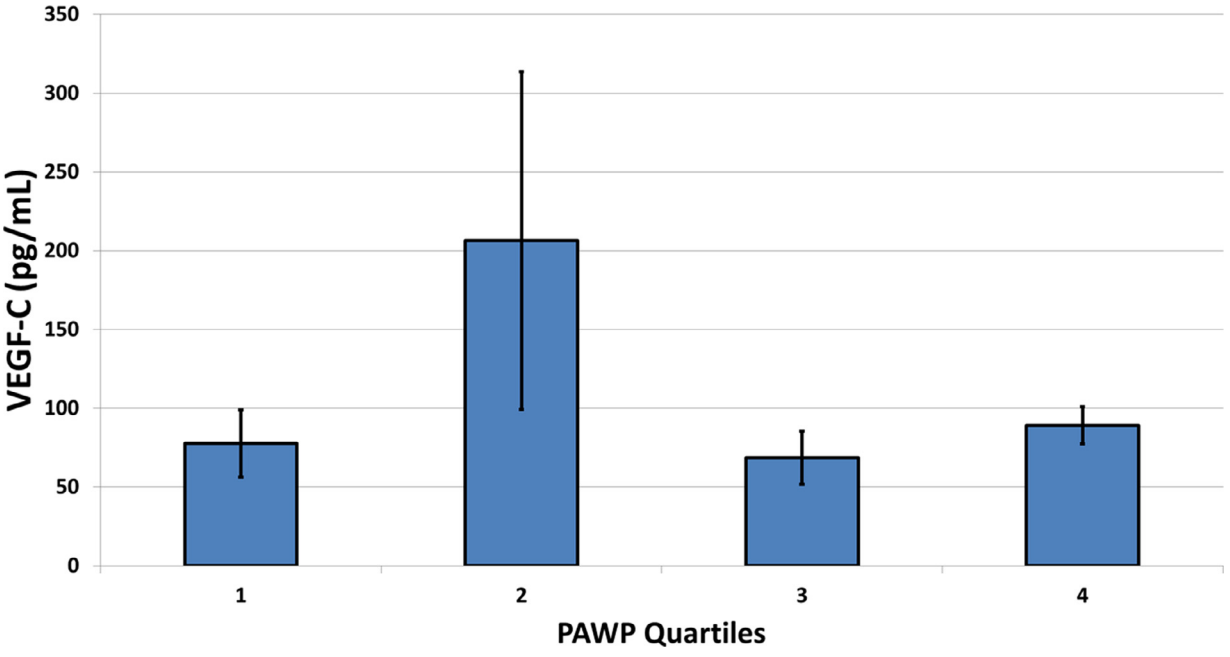

Figure 2. Average VEGF-C levels and SEM stratified by PAWP quartiles. For PAWP quartiles: Q1 = 0 to 10 mm Hg; Q2 = 11 to 14 mm Hg; Q3 = 15 to 19 mm Hg; Q4 = ≥20 mm Hg.

(BNP), lower cardiac output, and a trend toward lower EF ($p = 0.07$). Assessed as a continuous variable, VEGF-D was well correlated with BNP ($R = 0.82$), and modestly correlated with eGFR ($R = 0.39$). Subjects with an elevated VEGF-D level did not have higher right atrial pressures, but did have elevated PAWP and reduced cardiac output. Analyzed as a continuous variable, VEGF-D showed a modest correlation with PAWP ($R = 0.34$; Supplemental Figure 1). Multiple variable regression analysis including VEGF-D, cardiac output, BNP, and eGFR revealed that VEGF-D alone remained significantly associated with PAWP ($p = 0.02$). Despite a worse hemodynamic profile, subjects with VEGF-D > median were not more symptomatic by NYHA class, 6MWT distance, KCCQ overall summary scores, or subjective post-6MWT dyspnea scores on a 0 to 4 Likert scale. Comparison between high and low VEGF-D subjects is shown in Table 2.

We then assessed only the 27 subjects with elevated PAWP (≥15mm Hg), to determine if there was interaction between VEGF-D levels and symptoms in these select hemodynamically congested subjects. Similar to analysis in the entire cohort, subjects with VEGF-D ≥ median in this congested cohort had a greater degree of PAWP elevation and a lower EF. Despite this, there was no difference in 6MWT distance, subjective dyspnea by 1 to 4 Likert scale after 6MWT, or NYHA class comparing subjects with VEGF-D < median versus VEGF-D ≥ median. Subjects with an elevated PAWP and elevated VEGF-D also had similar KCCQ overall summary scores compared with those with lower VEGF-D. Score on the KCCQ question specifically addressing the frequency of orthopnea was also

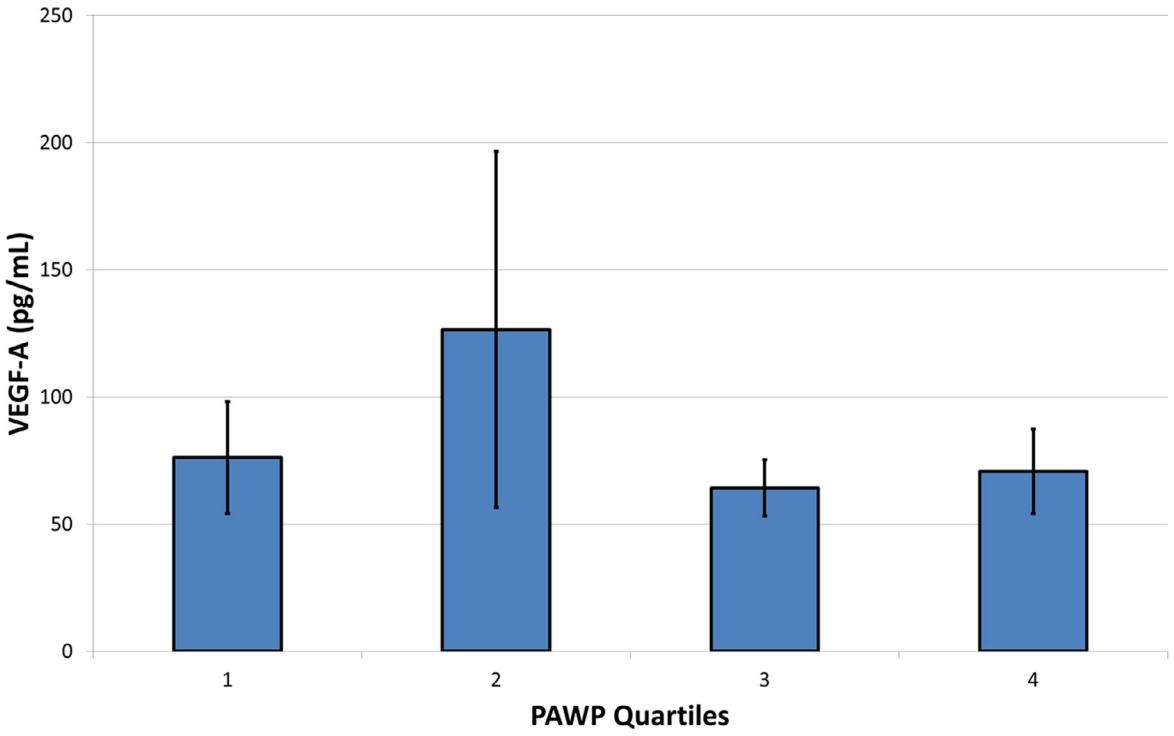

Figure 3. Average VEGF-A levels and SEM stratified by PAWP quartiles. For PAWP quartiles: Q1 = 0 to 10 mm Hg; Q2 = 11 to 14 mm Hg; Q3 = 15 to 19 mm Hg; Q4 = ≥20 mm Hg.

Table 2
Comparison between high and low VEGF-D subjects

Variable	VEFG-D < median (n = 28)	VEGF-D > median (n = 28)	p Value
Age (years)	53.5 ± 13.5	56.9 ± 13.7	0.36
Weight (kg)	99 ± 18.7	95 ± 25.9	0.44
BMI (kg/m^2)	32.7 ± 6	32.3 ± 9.2	0.82
Men	17/28	18/28	NS
EF (%)	47 ± 21%	38 + 21%	0.07
NYHA class (1-4)	2.9 ± 0.6	2.8 ± 0.6	0.5
BNP (pg/ml)	**320 ± 526**	**1349 ± 3222**	**0.05**
eGFR (ml/min/1.73 m^2)	62.9 ± 26	54.4 ± 27	0.23
Right atrial pressure (mm Hg)	7.5 ± 2.5	9.5 ± 5.7	0.12
PAWP (mm Hg)	**12.7 ± 6.3**	**16.8 ± 7.4**	**0.03**
Cardiac output (L/min)	**6.3 ± 2**	**5.1 ± 1.4**	**0.01**
6MWT distance (meters)	269 ± 106	242 ± 116	0.4
Post-6MWT subjective dyspnea (0-4)	3.6 ± 2.3	2.9 ± 2.8	0.35
KCCQ score	46.6 ± 23.6	54.6 ± 25.3	0.25

Bold values indicate p ≤ 0.05 is significant.

BMI = body mass index; BNP = B-type natriuretic peptide; EF = ejection fraction; eGFR = estimated GFR calculated from MDRD equation; KCCQ = Kansas City Cardiomyopathy Questionnaire; 6MWT = 6 minute walk test; NYHA = New York Heart Association; PAWP = pulmonary artery wedge pressure.

similar comparing high versus low VEGF-D groups. Table 3 summarizes both subjective and objective measures of heart failure symptom severity in subjects with elevated PAWP when compared by VEGF-D levels.

Finally, we assessed whether VEGF-D levels were associated with the duration of heart failure diagnosis in those 44 subjects with a prespecified diagnosis of HF before enrollment. In these subjects with a pre-RHC diagnosis of HF, there was a significant association between HF duration category and VEGF-D level by 1-way between-subjects ANOVA for all 5 studied HF duration categories [F (4,39) = 2.6, p = 0.05]. Those with a VEGF-D > median were more likely to have a HF diagnosis longer than 3 years (17/22, 77%) versus those with VEGF-D < median (10/22, 45%; chi-square = 4.7 p = 0.03). Subjects with a PAWP < median and heart failure duration <3 years had a lower mean VEGF-D than those with a PAWP ≥ median and a heart failure duration > 3 years (15 ± 21 pg/ml vs 242 ± 272 pg/ml, p = 0.015) (Figure 4).

Discussion

The mechanism by which the patient with cHF may remain free of pulmonary congestion despite persistently

Table 3
Comparison between high and low VEGF-D subjects in only those with an elevated pulmonary artery wedge pressure

Variable	VEGF-D < median (n = 13)	VEGF-D > median (n = 14)	p Value
Age (years)	51.6 ± 16.6	54.3 ± 14.5	0.7
PAWP (mm Hg)	**18.2 ± 2.8**	**23 ± 4.9**	**0.006**
EF (%)	**49± 22**	**30 ± 18**	**0.03**
NYHA class	2.9 ± 0.3	2.9 ± 0.5	0.9
6MWT distance (meters)	288 ± 82	226 ± 108	0.11
Post-6MWT subjective dyspnea (0-4)	3.5 ± 2	2.3 ± 3	0.25
KCCQ overall summary score	49 ± 17	55 ± 24	0.4
• Orthopnea KCCQ question score (1-7)	3.5 ± 1.9	3.7 ± 2	0.8

Bold values indicate $p \leq 0.05$ is significant.

Comparison between VEGF-D <median versus >median levels in only patients with an elevated pulmonary artery wedge pressure (≥15 mm Hg) at RHC (n = 27).

EF = ejection fraction; *KCCQ* = Kansas City Cardiomyopathy Questionnaire; *6MWT* = 6 minute walk test; *NYHA* = New York Heart Association; *PAWP* = pulmonary artery wedge pressure.

elevated left heart and pulmonary venous filling pressures is unclear, although previous studies implicate an augmentation of lymphatic flow as at least partially responsible. However, the biological mechanism underlying this augmentation in lymphatic flow in cHF is unknown. In this study, we describe for the first time a hemodynamic correlation between elevated left heart filling pressures and VEGF-D levels (but not VEGF-A or VEGF-C). We further found that elevated VEGF-D was not associated with elevated right atrial pressure and remained independently associated with PAWP after multiple regression analysis including cardiac output, BNP, and eGFR as covariates. In patients with elevated PAWP, those with higher VEGF-D were not more symptomatic despite having an even greater degree of PAWP elevation and lower cardiac output compared with those with lower VEGF-D, suggesting that elevated VEGF-D is associated with moderation of HF symptoms in the setting of worsening hemodynamics. Finally, we found a correlation of VEGF-D with HF diagnosis duration in those patients with a pre-RHC diagnosis of heart failure. Together, our findings support the novel hypothesis that VEGF-D is elevated in states of chronic pulmonary venous congestion. Given its known biological activity, this further leads to the hypothesis that elevated VEGF-D may result in augmented lymphatic clearance of interstitial pulmonary fluid, thereby mitigating signs and symptoms of pulmonary edema.

Our understanding of lymphangiogenesis remains relatively nascent, but recent discoveries have implicated the

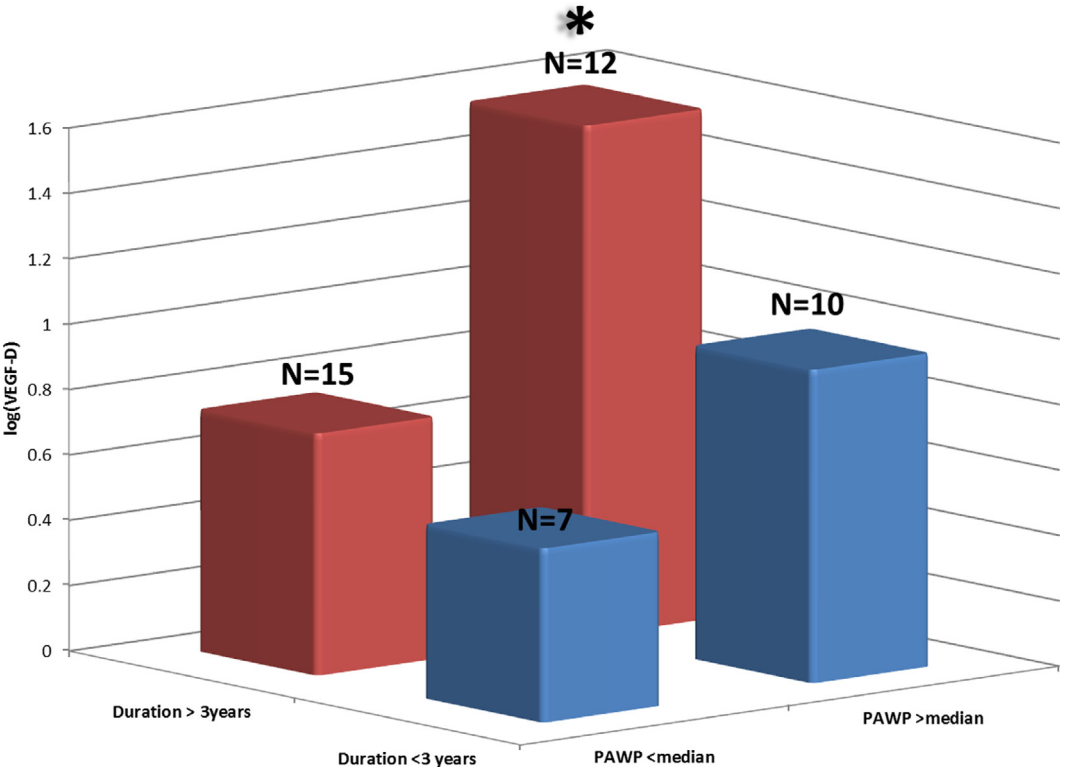

Figure 4. VEGF-D levels (log transformed for scale) in patients with a prespecified diagnosis of heart failure (n = 44) stratified by PAWP (< vs > median) and heart failure diagnosis duration (< vs > 3 years). *= $p < 0.05$ compared with subjects with [PAWP <median and duration <3 years].

family of VEGFs as implicitly involved. VEGF-A largely drives vasculogenesis and angiogenesis,[14] although may also lead to augmented lymphangiogenesis.[15] VEGF-C is necessary for embryologic lymphangiogenesis.[14] VEGF-D has a high degree of homology to VEGF-C and promotes lymphatic growth and cellular differentiation as well as dilation of lymphatic vessels.[14] There are important biological differences in VEGF-C and -D relevant to our findings. Murine studies suggest that VEGF-D expression may be highly localized to pulmonary tissue.[16] VEGF-D is elevated in the human disease lymphangioleiomyomatosis, hallmarked by destructive lymphatic and smooth muscle pulmonary hyperplasia, further implicating VEGF-D in pulmonary-specific processes.[17] And finally, VEGF-D expression may uniquely be driven by mechanical pressure stimulation mediated by the cell adhesion molecule cadherin-11.[18,19] This provides a potential mechanistic link between elevated intravascular pressure and VEGF-D production and lends biological plausibility to our findings.

Given the known biological activity of VEGF-D, one would hypothesize that VEGF-D-driven augmentation of pulmonary lymphatic flow capacity may mitigate symptoms associated with pulmonary congestion in states of left heart failure. Indeed, our findings suggest that, despite having a greater degree of hemodynamic pulmonary congestion, patients with an elevated VEGF-D did not have more severe congestive symptoms and performed equally well on 6MWT. Further, the correlation of VEGF-D with left, but not right, heart filling pressures in our study raises the possibility that VEGF-D may be a marker to differentiate pre- versus postcapillary etiologies of pulmonary hypertension. Notably, only 5 of our 56 patients qualified as having precapillary pulmonary hypertension by the revised sixth World Symposium on Pulmonary Hypertension definition (mean pulmonary pressure >20 mm Hg, pulmonary vascular resistance >3 Wood units, and PAWP < 15 mm Hg),[13] although no patient in the highest VEGF-D quartile had precapillary pulmonary hypertension. This hypothesis warrants further study in an appropriate clinical population.

Importantly, this study describes a correlation between a clinical state (chronic pulmonary venous congestion) and a biological marker (VEGF-D) and thus is mechanistically hypothesis generating. It does not examine whether VEGF-D causes augmented pulmonary lymphangiogenesis and/or lymphatic flow capacity in the heart failure state or whether VEGF-D is associated with reduced interstitial lung water in heart failure. The measurements of pulmonary lymphatic hyperplasia or lymphatic flow in vivo remain logistically challenging. Despite extensive phenotyping of enrolled subjects, it is possible that some had undiagnosed inflammatory diseases associated with elevated VEGF-D levels, although one would expect these diseases to be equally distributed along the PAWP spectrum. Compared with larger heart failure studies, older patients and those with ischemic heart disease were relatively less well represented in our cohort. Finally, our study enrolled all qualifying patients who underwent RHC and did not require a pre specified diagnosis of heart failure. Although 79% of analyzed patients did carry a heart failure diagnosis in our study (and several without a pre specified diagnosis of heart failure had a hemodynamic profile consistent with the diagnosis), the applicability of these findings to a specifically heart failure cohort should be confirmed. Differences in VEGF-D expression between heart failure patients with preserved and reduced ejection fraction should be further examined.

In conclusion, we have identified a correlation between VEGF-D and elevated left heart filling pressures. Despite the above-noted limitations, we believe this study points toward lymphangiogenesis in general and VEGF-D specifically as important new diagnostic, prognostic, and potentially future therapeutic targets in patients with heart failure. Future studies should focus on establishing the mechanistic link between heart failure states, VEGF-D, and pulmonary lymphangiogenesis.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.05.056.

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