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Elevated Soluble Fms-Like Tyrosine Kinase-1 and Placental-Like Growth Factor Levels Are Associated With Development and Mortality Risk in Heart Failure

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Background—Vascular endothelial dysfunction may play an important role in the progression of heart failure (HF). We hypothesize that elevated levels of vascular markers, placental-like growth factor, and soluble Fms-like tyrosine kinase-1 (sFlt-1) are associated with adverse outcomes in patients with HF. We also assessed possible triggers of sFlt-1 elevation in animal HF models.

Methods and Results—We measured plasma placental-like growth factor and sFlt-1 in 791 HF patients undergoing elective coronary angiogram. Median (interquartile range) placental-like growth factor and sFlt-1 levels were 24 (20–29) and 382 (277–953) pg/mL, respectively. After 5 years of follow-up, and after using receiver operator characteristic curves to determine optimal cutoffs, high levels of sFlt-1 (≥ 280 pg/mL; adjusted hazard ratio, 1.47; 95% confidence interval, 1.03–2.09; $P=0.035$) but not placental-like growth factor (≥ 25 pg/mL; adjusted hazard ratio, 1.26; 95% confidence interval, 0.94–1.71, $P=0.12$) were associated with adverse cardiovascular outcomes. In addition, significant elevation of sFlt-1 levels was observed in left anterior descending artery ligation and transverse aortic constriction HF mouse models after 4 and 8 weeks of follow-up, suggesting vascular stress and ischemia as triggers for sFlt-1 elevation in HF.

Conclusions—Circulating sFlt-1 is generated as a result of myocardial injury and subsequent HF development. Elevated levels of sFlt-1 are associated with adverse outcomes in stable patients with HF.

Placental-like growth factor (PLGF), a member of the vascular endothelial growth factor (VEGF) family, and soluble Fms-like tyrosine kinase-1 (sFlt-1) are vascular biomarkers that were found to be involved in the preeclampsia process and peripartum cardiomyopathy.¹ PLGF stimulates endothelial healing and recruitment of mononuclear bone marrow cells, and thus, it has a role in stimulating microvascular angiogenesis.² In contrast, sFlt-1 plays a counter-regulatory role by sequestering and blocking circulating PLGF.³ Both markers were found to be elevated in heart failure (HF), and higher sFlt-1 has been associated with adverse clinical outcomes.⁴ Furthermore, in animal models, antagonizing VEGF by bevacizumab has been associated with development of HF.⁵ These observations may imply that such vascular processes may be mechanistically linked to HF disease progression. Therefore, we aim to test the hypothesis that PLGF and sFlt-1 are associated with adverse outcomes in HF regardless of underlying reduced or preserved ejection fraction (EF), and in distinct but complementary animal HF models, that sFlt-1 generation occurs independent of cardiac insult.

Methods

Study Population

A total of 791 subjects with HF were enrolled from Cleveland Clinic GeneBank study, a large, prospective cohort study conducted between 2001 and 2007 that established a well-characterized clinical repository with clinical and longitudinal outcome data composed of consenting subjects undergoing an elective diagnostic cardiac catheterization procedure. This analysis included 791 subjects with HF, out of 2000 patients, without evidence of myocardial infarction (MI, cardiac troponin I < 0.03 ng/mL) and with plasma samples available for analysis. The inclusion criteria included age older than 18 years, ability to understand and sign written informed consent to participate, and a diagnosis of HF with either reduced or preserved EF. Exclusion criteria included congenital heart disease, previous heart transplant, known cardiac infiltrative disease (eg, amyloidosis), previous other solid organ transplantation, and end-stage HF requiring outpatient continuous inotrope infusion. Institutional review board approval was obtained, and informed consent was signed by all subjects before enrollment.

Because difference between levels of vascular markers in HF population and normal population is unknown, we have also enrolled a group of 312 healthy young subjects to determine reference range of vascular biomarkers.

sFlt-1 and PIGF Measurement and Patient Groupings

After informed consent, all patients had collection of blood samples at baseline. Levels of sFlt-1 and PIGF were measured using investigational immunoassays on the Architect ci8200 platform in a research core laboratory (Abbott Laboratories, Abbott Park, IL). B-type natriuretic peptide (BNP) levels were also measured in the same samples using the sample platform. Patients were grouped into those with high levels of vascular markers (\geq cutoff) and low levels ($<$ cutoff). An estimate of creatinine clearance was calculated using the Cockcroft–Gault equation. The presence of coronary artery disease was confirmed by luminal stenosis of at least 70% in any major coronary artery. Left ventricular EF was determined by the last best available data from clinical records (echocardiography, radionuclide imaging, or ventriculogram during coronary angiography in order of preference). Adjudicated long-term survival was ascertained for all subjects after enrollment. Mortality data were collected through medical records review, information from family members, and Social Security Death Index query.

Animal Models of HF

The Cleveland Clinic Institutional Animal Care and Use Committee approved all animal studies. We assessed circulating sFlt-1 levels in 2 well-established rodent HF models representing cardiac insults caused by acute MI (left anterior descending [LAD] coronary ligation) and by pressure overload (transverse aortic constriction [TAC]), as previously described.⁶ For the LAD ligation model, the left atrium was retracted for visualization of the proximal LAD using a surgical microscope (Leica M500, Prescott's Inc., Monument, CO) and the LAD coronary artery was ligated with 10-0 prolene suture. For the TAC model, midsternal incision was made to expose transverse aorta between truncus anonymus and the left carotid artery. With 6-0 silk suture, a ligature is tied around the transverse aorta against a 26-gauge needle. Both HF models were performed in 12-week male C57BL/6J mice, and plasma samples were collected by ventricular puncture at the time of euthanize after 4 weeks postoperatively from LAD mice ($n=4$) and TAC mice ($n=4$) and 8 weeks postoperatively from LAD mice ($n=3$) and TAC mice ($n=6$). Plasma sFlt-1 was assayed using a mouse assay (Quantikine ELISA, R&D Systems, Minneapolis, MN).

Statistical Analysis

The Student *t* test or Wilcoxon-rank sum test, for continuous variables, and χ^2 test for categorical variables were used to examine differences between the groups. Survival and event rates were described with the Kaplan–Meier method. Cox proportional hazards regression was used to determine hazard ratios and 95% confidence intervals for 5-year survival. In multivariable models, we adjusted for traditional cardiac risk factors, including age, sex, race, log-transformed body mass index, diabetes mellitus, systolic blood pressure, low-density lipoprotein, high-density lipoprotein, calculated glomerular filtration rate, smoking, coronary artery disease and medications (angiotensin converting enzyme inhibitors, β -blockers), and log-transformed EF. Analyses were also repeated after adjustment for baseline log-transformed BNP and cardiac troponin I levels. Receiver operator characteristic curve analyses with 5-fold cross-validation were used to determine the optimal sFlt-1 and PIGF cutoffs. For a given cutoff, we used a Cox model to estimate mortality risk. The 5-fold cross-validation divides the data into 5 approximately equally sized portions. A Cox model is trained on 4 parts of the data and then estimates the risk of mortality in the fifth part. This is repeated for each of the 5 parts. We calculated the area under the curve with the estimated risk. This process is performed for a grid of sFlt-1 and PIGF cutoff values, ranging from 105.6 to 21044 pg/mL with an increment of 1 pg/mL for sFlt-1 and ranging from 8.2 to 84.5 pg/mL with an increment of 0.5 pg/mL for PIGF. The optimal cutoff is chosen to maximize area under the curve values. We also used logistic regression to assess factors associated with increased odds of having high levels of vascular markers (\geq cutoffs).

Results

Baseline Patient Characteristics

Overall, a total of 791 patients with diagnosis of HF were included in this study. The baseline characteristics of the study population are presented in Table 1. Compared with the reference group of 312 healthy young subjects assessed by our laboratory (mean age, 42 ± 14 years; male, 41%; smoking, 7%; Table I in the Data Supplement), and after adjustment for age, sex, race, diabetes mellitus, hypertension, hyperlipidemia and smoking, patients with HF had significantly higher levels of sFlt-1 [median of 382 (277–955) versus 249 (226–276) pg/mL, adjusted $P=0.005$] and PIGF [median of 24 (20–29) versus 15.5 (13.5–18) pg/mL, adjusted $P<0.001$; Figure 1].

Demographics Based on sFlt-1 Levels

Using area under the curve and 5-fold cross-validation as described above, cutoff of 280 pg/mL for sFlt-1 and 25 pg/mL for PIGF was found to maximize area under the curve for association with adverse outcomes. Table 1 showed demographics based on high/low sFlt-1 levels using these cutoffs. Patient with high sFlt-1 had significantly lower creatinine clearance as well as higher BNP levels. Using multivariate logistic regression analysis, we found decreased creatinine clearance as well as increased BNP levels to be independent predictors of high sFlt-1 and PIGF. Presence of coronary artery disease was also found to be an independent predictor of high PIGF.

sFlt-1 and PIGF and Long-Term Survival in HF Patients

After 5 years of follow-up, 228 (28.8%) of the patients reached the primary outcome (all-cause mortality). High levels of sFlt-1 and PIGF were significantly associated with higher rate of adverse outcomes (Table 2). After adjustment for coronary artery disease risk factors, medications, creatinine clearance, EF, BNP, and cardiac troponin I, high sFlt-1 but not PIGF remained an independent predictor of all-cause mortality with hazard ratio (95% confidence interval) of 1.47 (1.03–2.09); $P=0.035$ and 1.26 (0.94–1.71); $P=0.12$, respectively (Figure 2). Subgroup analysis showed sFlt-1 to be associated with adverse outcomes mainly in patients with EF $>40\%$ and patients with coronary artery disease. However, there was no significant interaction between sFlt-1 and EF. Similarly, no interaction was found between PIGF and EF. We validated these results on a group of 175 patients with predominantly, nonischemic HF with low EF (coronary artery disease, 39%; EF, $26\pm 14\%$) enrolled from the Atlanta cardiomyopathy consortium (TACC) with a mean follow-up of 3.5 ± 1 years. In this group of patients, only sFlt-1 but not PIGF showed significant association with increased mortality risk (Figure I in the Data Supplement).

sFlt-1 in Animal Models of HF

Giving the results of subgroup analyses, where sFlt-1 was mainly associated with adverse outcomes in patients with preserved EF and coronary artery disease, we tried to further investigate possible triggers of sFlt-1 release using animal models. In comparison with control group animals ($n=4$; mean sFlt-1= 259.3 pg/mL), the MI animal models of HF showed significant elevation in sFlt-1 levels after 4 and 8 weeks of LAD

Table 1. Baseline Characteristics Stratified By sFlt-1 Levels

	Total (n=791)	sFlt-1<280 pg/mL (n=210)	sFlt-1≥280 pg/mL (n=581)	P Value
Age, y	66±11	67±10	66±11	0.805
Male, %	60	63	58	0.238
Black, %	4	2	5	0.139
Former/current smokers, %	70	72	69	0.548
Diabetes mellitus, %	41	45	40	0.294
Systolic blood pressure, mm Hg	128 (115–143)	132 (119–148)	127 (114–142)	0.011
Body mass index, kg/m ²	28 (25–32)	29 (25–32)	28 (25–33)	0.593
Creatinine clearance, mL/min/1.73 m ²	82.1 (60–109)	85 (66–113)	81 (58–109)	0.03
LDL cholesterol, mg/dL	92 (73–113)	92 (78–116)	91 (71–112)	0.156
HDL cholesterol, mg/dL	31 (26–39)	32 (27–39)	31 (26–40)	0.197
Coronary artery disease, %	76	81	75	0.117
Myocardial infarction, %	58	63	56	0.098
Atrial fibrillation, %	51	42	54	0.004
β-Blocker, %	67	71	66	0.145
ACE inhibitors or ARBs, %	68	73	66	0.039
Nitrates, %	39	41	39	0.542
ICD, %	10	8	11	0.258
CRT, %	1	0	1	0.087
B-type natriuretic peptide, pg/mL	298 (119–647)	183 (84–424)	342 (147–792)	0.001
Left ventricular ejection fraction, %	40 (25–55)	40 (30–55)	40 (25–55)	0.937
PIGF, pg/mL	24 (20–29)	23 (19–26)	25 (21–31)	<0.001
sFlt-1, pg/mL	382 (277–953)	241 (223–262)	588 (359–1459)	<0.001
Cardiac troponin I, ng/mL	0.009 (0.001–0.029)	0.007 (0–0.018)	0.011 (0.001–0.038)	0.003

ACE indicates angiotensin converting enzyme; ARB, angiotensin receptor blockers; CRT, cardiac resynchronization therapy; HDL, high-density lipoprotein; ICD, implantable cardioverter defibrillator; LDL, low-density lipoprotein; PIGF, placental-like growth factor; and sFlt-1, soluble Fms-like tyrosine kinase-1.

artery ligation (Figure 3). Similar findings were found as well in TAC models on 4 and 8 weeks after model design (Figure 3).

Discussion

There are several key observations. First, we demonstrate that patients with HF have significantly higher circulating sFlt-1 and PIGF levels in comparison with normal healthy population and regardless of impaired or preserved left ventricular EF. Increased levels of sFlt-1 were found to be associated with worse renal function, although they still independently predicted poor long-term survival. Using different forms of cardiac insult in animal models, we further demonstrated that elevated sFlt-1 levels occur irrespective of the types of inciting cardiac insults. Taken together, these observations imply that in patients with stable HF, high sFlt-1 may be generated as a consequence of the development and progression of HF. This may, in turn, reflect the critical role of imbalance in myocardial angiogenesis and the ventricular-arterial relationship in patients with HF.

Vascular markers were mainly studied in a preeclampsia population where low/high levels of PIGF/sFlt-1, respectively, were found to be associated with diffuse endothelial dysfunction and vascular rarefaction.^{1,3,4,7–9} Subsequently, protein urea, increased blood pressure, and eclampsia could develop.¹⁰ High sFlt-1 was also found to be associated with increased risk of peripartum cardiomyopathy.^{1,7} Similarly, administration of sFlt-1 in VEGF receptor depleted animals resulted in profound dilated cardiomyopathy with significant decreased in capillary

density.¹ However, intramyocardial provision of exogenous VEGF in animal models for pressure overload (aortic banding) has been shown to preserve coronary blood flow reserve and left ventricular performance.^{11–13} However, there are limited reports regarding the role of vascular markers in the pathogenesis and disease progression of HF. Both PIGF and sFlt-1 were found to be elevated after MI. High levels of PIGF were more in favor of myocardial recovery, whereas high sFlt-1 levels were associated with increased risk of myocardial dysfunction.^{1,2,14–16} The beneficial effect of exogenous PIGF in animal models post-MI was neutralized by administration of sFlt-1.² Similarly, use of mimics to sFlt-1 (VEGF antagonists)-like bevacizumab was found to be associated with increased risk of HF development.^{5,17} The prognostic value of circulating levels of vascular markers has previously been reported in HF with impaired EF (and largely nonischemic) patient population.⁴ In our observations from 2 separate cohorts, we have confirmed the association with adverse outcomes. Although, our subgroup analyses revealed this association to be mainly driven by those with preserved EF and coronary artery disease, we did not find significant interaction between sFlt-1 and EF. Furthermore, sFlt-1 was associated with adverse outcomes in TACC population (mainly nonischemic HF with low EF). Taken together, with our animal model findings, these results suggest the adverse association of sFlt-1 and HF is independent of the insulting factor.

Interestingly, high levels of PIGF in our population also showed unfavorable association with adverse outcomes.

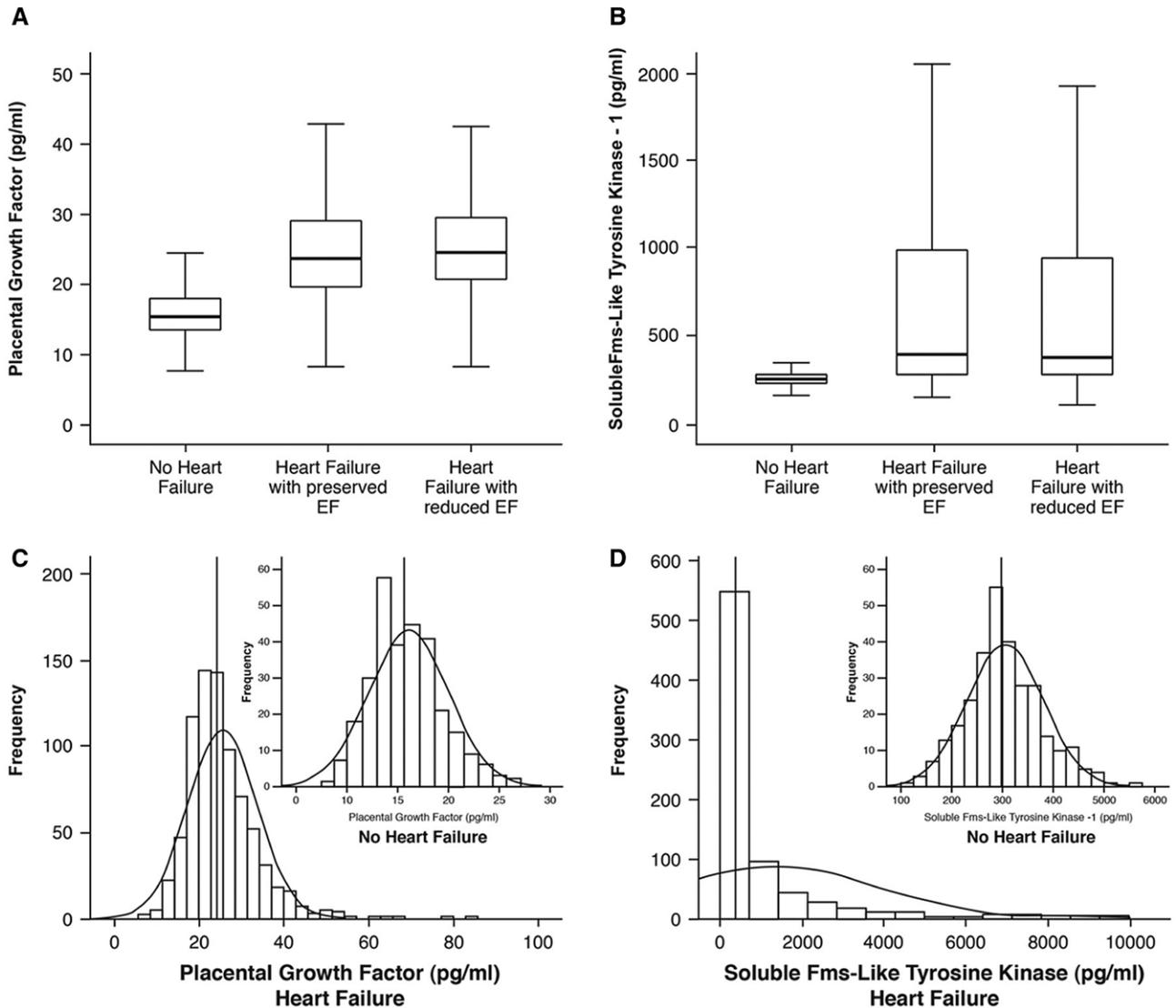


Figure 1. Plasma levels of placental-like growth factor (PIGF, **A**) and soluble Fms-like tyrosine kinase-1 (sFlt-1, **B**) in patients with heart failure (HF) stratified by impaired versus preserved left ventricular ejection fraction (LVEF) versus non-HF controls. Distribution of PIGF (**C**) and sFlt-1 (**D**) in HF and non-HF population.

Similar findings were suggested before in Ky et al⁴ study. Nakamura et al¹⁸ also found increased PIGF levels in patients with increased HF severity in ischemic cardiomyopathy. In contrary, reports from post-MI survivors showed favorable cardiac recovery in those with high PIGF.^{2,15} This may suggest

protective role of PIGF in HF setting, and association with adverse outcome might be related to increase cardiac severity with a compensatory effort of the hypoxic tissue to overcome the on-going microvascular ischemia. The beneficial effect of PIGF/VEGF may be blocked and attenuated by high sFlt-1

Table 2. Long-Term Mortality Using Vascular Markers

	Soluble Fms-Like Tyrosine Kinase-1			Placental-Like Growth Factor		
	<280 pg/mL (n=210)	≥280 pg/mL (n=581)	P Value	<25 pg/mL (n=442)	≥25 pg/mL (n=349)	P Value
5-y death,* %	44/210=21.0%	184/581=31.7%	0.003	100/442=22.6%	128/349=36.7%	<0.001
Unadjusted HR	1	1.67 (1.21–2.31)	0.002	1	1.77 (1.36–2.30)	<0.001
Adjusted HR (model 1)	1	1.70 (1.20–2.41)	0.003	1	1.35 (1.00–1.81)	0.049
Adjusted HR (model 2)	1	1.48 (1.04–2.11)	0.029	1	1.29 (0.96–1.73)	0.093
Adjusted HR (model 3)	1	1.47 (1.03–2.09)	0.035	1	1.26 (0.94–1.71)	0.124

Model 1: adjusted for age, sex, race, logged body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log-transformed creatinine clearance, diabetes mellitus, smoking, coronary artery disease, ACE inhibitors/ARBs, β -blockers, and log-transformed left ventricular ejection fraction. Model 2: adjusted for model 1 plus B-type natriuretic peptide. Model 3: adjusted for model 2 plus cardiac troponin I. HR indicates hazard ratio.

*Kaplan–Meier percentages.

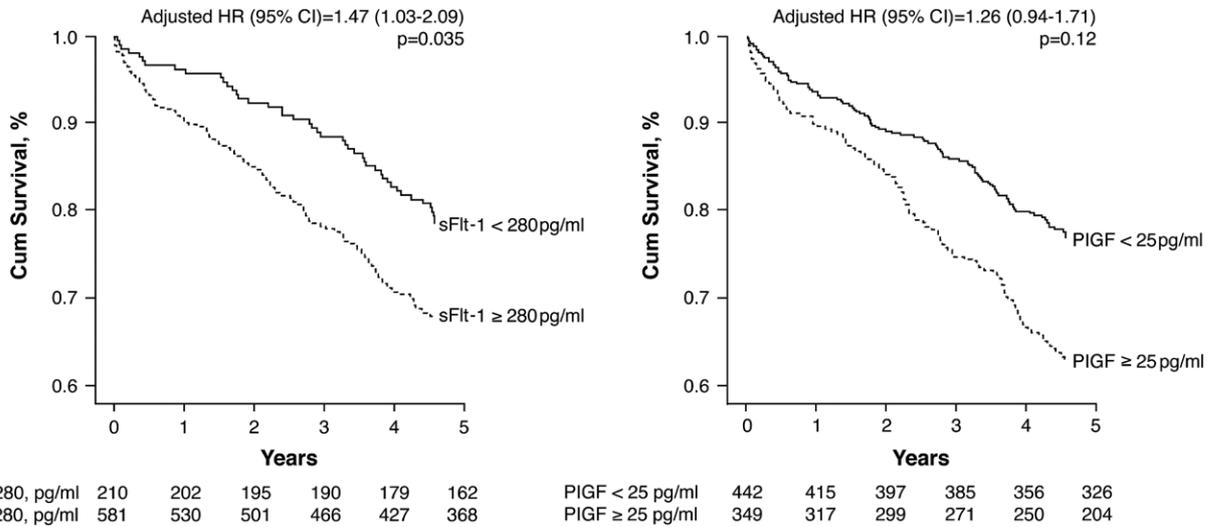


Figure 2. Kaplan–Meier curves and adjusted hazard ratio for association between vascular markers and all-cause mortality.

levels and other comorbidities that attenuate neovascular response (eg, advance age, diabetes mellitus, increased oxidative stress, and hypertension).¹⁹

Despite we found that levels of sFlt-1 and PIGF are higher in patients with HF compared with a normal healthy population, it is worth noting that the concentrations of vascular markers are much lower than those reported during normal pregnancy and in preeclampsia.²⁰ In comparison with pregnancy where the placenta is the major trigger of the vascular marker production,²¹ it is still unclear what the trigger is for circulating sFlt-1 and PIGF production in the setting of HF. Flt-1 is normally expressed on endothelial cells, and whether cleavage of this receptor is done by an enzyme, ischemia, or other stressors remains unclear. In our animal HF models, significant elevation in sFlt-1 was observed after both the LAD ligation and TAC models, which suggests vascular stress, myocardial ischemia, or even sympathetic drive may be possible triggers of sFlt-1 elevation. Indeed, previous reports found increased levels of sFlt-1 after MI.^{14–16} Furthermore, increased levels of sFlt-1 were found in patients with resistant hypertension, and they were found to be associated with responders to renal denervation.²² These findings together with our observation in animal models suggest that any endothelial stressor may trigger increase sFlt-1 production and release.

Study Limitations

Despite being one of the largest HF cohorts reported with vascular marker levels and long-term outcomes, our study has some limitations. First, we do not have complete data about cause of death, hospitalization, or consistent echocardiographic indices (like left ventricular hypertrophy or diastolic indices) in all subjects. Second, we studied vascular markers at a single time point, and changes of vascular markers or treatment responses need to be further explored. Third, the GeneBank study enrolled patients between 2001 and 2007, yet new HF treatment modalities, like biventricular pacing and aldosterone antagonist, were not commonly used. Whether these treatment modalities have any effect on the association between vascular markers and HF outcomes need to be further investigated.

Conclusion

sFlt-1 may be released as a result of myocardial injury and subsequent HF development. In high levels, sFlt-1 is associated with adverse outcomes in stable HF patients.

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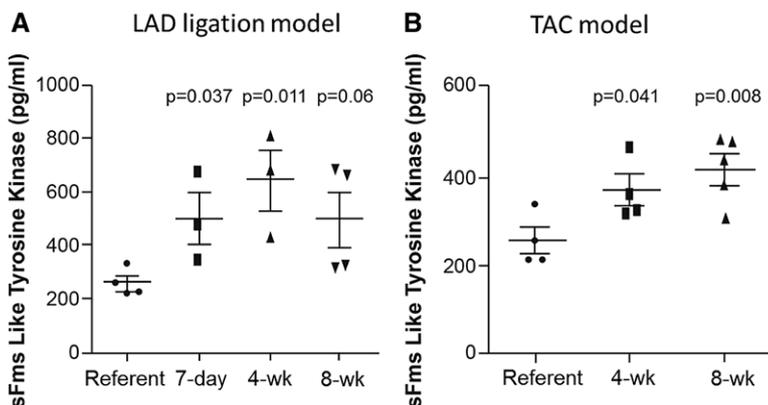


Figure 3. Plasma levels of sFlt-1 in mouse heart failure (HF) models. **A**, Postmyocardial infarction (left anterior descending coronary artery ligation model); **B**, transverse aortic constriction model.

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Disclosures

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