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**Journal Title:** Circulation: Heart Failure

**Volume:** Volume 9, Number 1

**Publisher:** American Heart Association | 2016-01-01, Pages e002115-e002115

**Type of Work:** Article | Post-print: After Peer Review

**Publisher DOI:** 10.1161/CIRCHEARTFAILURE.115.002115

**Permanent URL:** <https://pid.emory.edu/ark:/25593/rw30v>

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Final published version:

<http://dx.doi.org/10.1161/CIRCHEARTFAILURE.115.002115>

### **Copyright information:**

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*Accessed November 20, 2019 1:04 PM EST*



Published in final edited form as:

*Circ Heart Fail.* 2016 January ; 9(1): e002115. doi:10.1161/CIRCHEARTFAILURE.115.002115.

## 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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### Abstract

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

**Methods and Results**—We measured plasma PIGF and sFlt-1 in 791 HF patients undergoing elective coronary angiogram. Median (IQR) PIGF and sFlt-1 levels were 24 (20–29) pg/ml and 382 (277–953) pg/ml, respectively. After five years of follow up, and after using receiver operator characteristic curves to determine optimal cutoffs, high levels of sFlt-1 ( $\geq 280$  pg/ml; adjusted hazard ratio (HR) = 1.47, 95% confidence interval (CI) 1.03–2.09,  $p=0.035$ ) but not PIGF ( $\geq 25$  pg/ml; adjusted HR = 1.26, 95% CI 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.

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### DISCLOSURES

Dr. Butler reports research support from the NIH, and European Union; and is a consultant to Amgen, Bayer, Cardiocell, Celladon, Novartis, Ono Pharma, StealthPeptide, Takeda, Trevena, and Zensun. Dr. Georgiopoulou reports research support from NIH. Dr. Kalogeropoulos reports research support from the NIH and AHA.

**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.

### Keywords

heart failure; vascular markers; placental-like growth factor; soluble Fms-like tyrosine kinase 1 receptor

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Placental-like growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, and soluble Fms like tyrosine kinase-1 are vascular biomarkers that were found to be involved in the pre-eclampsia process and peri-partum cardiomyopathy.<sup>1</sup> PlGF stimulates endothelial healing and recruitment of mononuclear bone marrow cells, and thus, it has a role in stimulating microvascular angiogenesis.<sup>2</sup> In contrast, sFlt-1 plays a counter-regulatory role by sequestering and blocking circulating PlGF.<sup>3</sup> Both markers were found to be elevated in heart failure (HF), and higher sFlt-1 has been associated with adverse clinical outcomes.<sup>4</sup> Furthermore in animal models, antagonizing VEGF by bevacizumab has been associated with development of HF.<sup>5</sup> 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, 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 2001–7 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 (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 ejection fraction (EF). Exclusion criteria included congenital heart disease, previous heart transplant, known cardiac infiltrative disease (e.g., 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 prior to enrollment.

Since difference between levels of vascular markers in heart failure 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 (≥ 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 following enrollment. Mortality data were collected through medical records review, information from family members, and Social Security Death Index query.

### Animal models of heart failure

The Cleveland Clinic Institutional Animal Care and Use Committee approved all animal studies. We assessed circulating sFlt-1 levels in two well-established rodent HF models representing cardiac insults caused by acute myocardial infarction (left anterior descending [LAD] coronary ligation) and by pressure overload (transverse aortic constriction [TAC]), as previously described.<sup>6</sup> For the LAD ligation model, the left atrium was retracted for visualization of the proximal LAD using a surgical microscope (Leica M500) and the left anterior descending coronary artery was ligated with 10-0 prolene suture. For the TAC model, mid-sternal 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 sacrifice after 4 weeks post-operatively from LAD mice (n=4) and TAC mice (n=4), and 8 weeks post-operatively 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 Chi square 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 (HR) and 95% confidence intervals (CI) for 5-year survival. In multivariable models, we adjusted for traditional cardiac risk factors, including age, gender, 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, beta blockers) and log transformed ejection fraction. Analyses were also repeated after adjustment for baseline log transformed BNP and cardiac troponin I levels. Receiver operator characteristic (ROC) curve analyses with five-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 five-fold cross validation divides the data into five approximately equally-sized portions. A Cox model is trained on four parts of the data and then estimates the risk of mortality in the fifth part. This is repeated for each of the five parts. We calculated the area under the curve (AUC) with the estimated risk. This process is carried out for a grid of sFlt-1 and PIGF cutoff values, ranging from 105.6 to 21,044 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 AUC values. We also used logistic regression to assess factors associated with increased odds of having high levels of vascular markers ( 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 to the reference group of 312 healthy young subjects assessed by our lab (mean age  $42 \pm 14$  years, male 41%, smoking 7%)(Supplemental Table 1), and after adjustment for age, gender, race, diabetes, hypertension, hyperlipidemia and smoking, patients with HF had significantly higher levels of sFlt-1 [median of 382 (277 – 955) pg/ml vs 249 (226–276) pg/ml, adjusted  $p = 0.005$ ] and PIGF [median of 24 (20–29) pg/ml vs 15.5 (13.5–18) pg/ml, adjusted  $p < 0.001$ ] (Figure 1).

### Demographics based on sFlt-1 levels

Using AUC and 5-fold cross validation as described above, cutoff of 280 pg/ml for sFlt-1 and 25 pg/ml for PIGF were found to maximize AUC 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 CAD was also found to be an independent predictor of high PIGF.

### sFlt-1 and PIGF and long term survival in HF patients

After five 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 HR (95%CI) 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, non-ischemic heart failure with low ejection fraction (CAD 39%,  $EF 26 \pm 14\%$ ) enrolled from the Atlanta cardiomyopathy consortium (TACC) with a mean follow up of  $3.5 \pm 1$  years. In this group of patients, only

sFlt-1 but not PIGF showed significant association with increased mortality risk (Supplemental Figure).

### 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 to control group animals (n=4, mean sFlt-1 = 259.3 pg/ml), the myocardial infarction animal models of heart failure showed significant elevation in sFlt-1 levels after 4 and 8 weeks of LAD 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 to 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, while they still independently predicted poor long-term survival. Utilizing 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 pre-eclampsia population where low/high levels of PIGF/sFlt-1, respectively, were found to be associated with diffuse endothelial dysfunction and vascular rarefaction.<sup>1, 3, 4, 7-9</sup> Subsequently, protein urea, increased blood pressure and eclampsia could develop.<sup>10</sup> High sFlt-1 was also found to be associated with increased risk of peripartum cardiomyopathy.<sup>1, 7</sup> Similarly, administration of sFlt-1 in VEGF receptor depleted animals resulted in profound dilated cardiomyopathy with significant decreased in capillary density.<sup>1</sup> On the other hand, intra-myocardial 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.<sup>11-13</sup> 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 myocardial infarction. High levels of PIGF were more in favor of myocardial recovery, while high sFlt-1 levels were associated with increased risk of myocardial dysfunction.<sup>1, 2, 14-16</sup> The beneficial effect of exogenous PIGF in animal models post-MI was neutralized by administration of sFlt-1.<sup>2</sup> Similarly, use of mimics to sFlt-1 (VEGF antagonists) like bevacizumab were found to be associated with increased risk of heart failure development.<sup>5, 17</sup> The prognostic value of circulating levels of vascular markers has previously been reported in HF with impaired EF (and largely non-ischemic) patient population.<sup>4</sup> In our observations from two separate cohorts we have confirmed the association with adverse outcomes. Although, our sub-group analyses revealed this

association to be mainly driven by those with preserved ejection fraction and CAD, we didn't find significant interaction between sFlt-1 and EF. Furthermore, sFlt-1 was associated with adverse outcomes in TACC population (mainly non-ischemic 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. Similar findings were suggested before in Ky et al study.<sup>4</sup> Nakamura et al<sup>18</sup> 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.<sup>2, 15</sup> This may suggest protective role of PIGF in heart failure 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 levels and other co-morbidities that attenuate neo-vascular response (e.g., advance age, diabetes, increased oxidative stress and hypertension).<sup>19</sup>

Despite we found that levels of sFlt-1 and PIGF are higher in patients with HF compared with a normal healthy population, it's worth noting that the concentrations of vascular markers are much lower than those reported during normal pregnancy and in pre-eclampsia.<sup>20</sup> In comparison to pregnancy where the placenta is the major trigger of the vascular marker production,<sup>21</sup> 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 myocardial infarction.<sup>14–16</sup> 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.<sup>22</sup> These findings together with our observation in animal models suggest any endothelial stressor may trigger increase sFlt-1 production and release.

### Study Limitations

Despite being one of 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 LV 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 2001–2007, yet new heart failure 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

### SOURCES OF FUNDING

This research was supported by grants from the National Institutes of Health and the Office of Dietary Supplements (R01HL103866, P20HL113452). The GeneBank study was supported by NIH grants P01HL076491, P01HL098055, R01HL103931, and the Cleveland Clinic Clinical Research Unit of the Case Western Reserve University CTSA (UL1TR 000439-06).

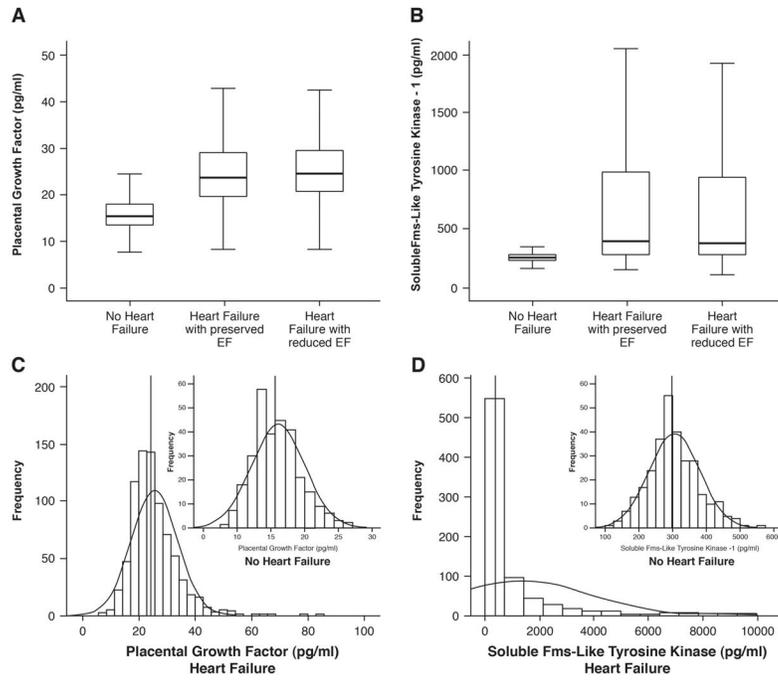
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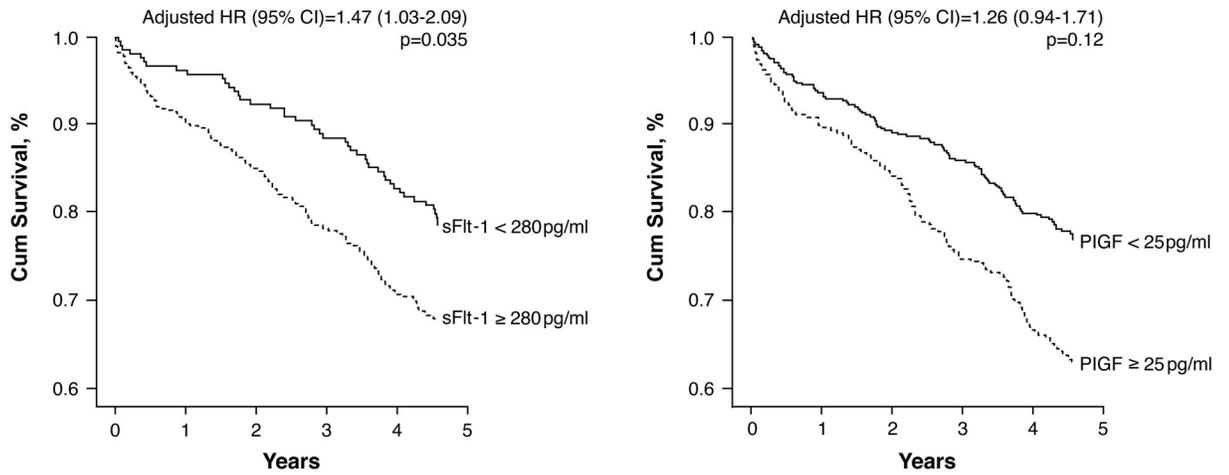
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### CLINICAL PERSPECTIVE

It has been recognized that vascular-endothelial dysfunction plays an important role in the progression of heart failure (HF). Vascular markers were mainly studied in a pre-eclampsia population where low/high levels of soluble Fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor were found to be associated with diffuse endothelial dysfunction and vascular rarefaction. Our data provide direct biochemical evidence demonstrating the distinct clinical relevance of sFlt-1 in the setting of HF, suggesting that vascular-endothelial dysfunction plays an important pathophysiologic role. Utilizing two distinct HF animal models, we further demonstrated that different forms of cardiac insult induces production and release of circulating sFlt-1 levels irrespective of the types of inciting cardiac insults. Taken together, these findings demonstrated the sFlt-1 is generated as a consequence of the development and progression of HF.

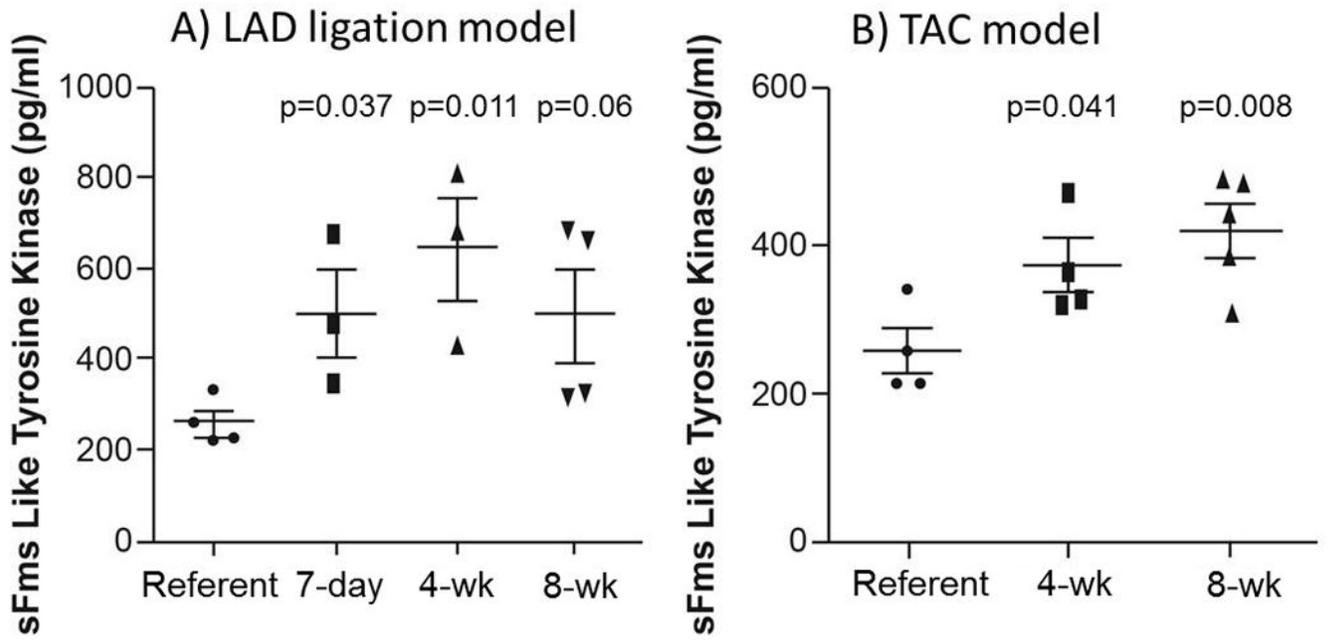


**Figure 1.** Plasma levels of PIGF (A) and sFlt-1 (B) in patients with HF stratified by impaired vs preserved LVEF vs non-HF controls. Distribution of PIGF (C) and sFlt-1 (D) in heart failure and non-heart failure population.



sFlt-1 < 280, pg/ml	210	202	195	190	179	162	PIGF < 25 pg/ml	442	415	397	385	356	326
sFlt-1 ≥ 280, pg/ml	581	530	501	466	427	368	PIGF ≥ 25 pg/ml	349	317	299	271	250	204

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



**Figure 3.** Plasma levels of sFlt-1 in mouse HF models. A) post-myocardial infarction left anterior descending coronary artery ligation model. B) Transverse aortic constriction model.

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**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, years	66±11	67±10	66±11	0.805
Male, %	60	63	58	0.238
African American, %	4	2	5	0.139
Former/Current smokers, %	70	72	69	0.548
Diabetes mellitus, %	41	45	40	0.294
Systolic blood pressure, mmHg	128(115–143)	132(119–148)	127(114–142)	0.011
Body mass index, kg/m <sup>2</sup>	28(25–32)	29(25–32)	28 (25–33)	0.593
Creatinine clearance, ml/min/1.73m <sup>2</sup>	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
Beta 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
LV 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 (277–955)	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

**Table 2**

Long Term Mortality Using Vascular Markers.

	Soluble Fms Like Tyrosine Kinase-1		Placental 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-year Death <sup>*</sup> , %	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, gender, race, logged BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, log-transformed creatinine clearance, diabetes, smoking, coronary artery disease, ACE inhibitors/ARBs, beta-blockers and log-transformed LV ejection fraction.

<sup>\*</sup> Kaplan-Meier percentages.

Model 2: adjusted for model 1 plus BNP

Model 3: adjusted for model 2 plus cardiac troponin I