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RESEARCH ARTICLE

Plasma soluble fms-like tyrosine kinase-1, placental growth factor, and vascular endothelial growth factor system gene variants as predictors of survival in heart failure

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Aims Soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), components of the vascular endothelial growth factor (VEGF) system, play key roles in angiogenesis. Reports of elevated plasma levels of sFlt-1 and PlGF in coronary heart disease and heart failure (HF) led us to investigate their utility, and VEGF system gene single nucleotide polymorphisms (SNPs), as prognostic biomarkers in HF.

Methods and results ELISA assays for sFlt-1, PlGF and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were performed on baseline plasma samples from the PEOPLE cohort ($n = 890$), a study of outcomes among patients after an episode of acute decompensated HF. Eight SNPs potentially associated with sFlt-1 or PlGF levels were genotyped. sFlt-1 and PlGF were assayed in 201 subjects from the Canterbury Healthy Volunteers Study (CHVS) matched to PEOPLE participants. All-cause death was the major endpoint for clinical outcome considered. In PEOPLE participants, mean plasma levels for both sFlt-1 (125 ± 2.01 pg/ml) and PlGF (17.5 ± 0.21 pg/ml) were higher (both $p < 0.044$) than in the CHVS cohort (81.2 ± 1.31 pg/ml and 15.5 ± 0.32 pg/ml, respectively). sFlt-1 was higher in HF with reduced ejection fraction compared to HF with preserved ejection fraction ($p = 0.005$). The *PGF* gene SNP rs2268616 was univariately associated with death ($p = 0.016$), and was also associated with PlGF levels, as was rs2268614 genotype. Cox proportional hazards modelling ($n = 695$, 246 deaths) showed plasma sFlt-1, but not PlGF, predicted survival (hazard ratio 6.44, 95% confidence interval 2.57–16.1; $p < 0.001$) in PEOPLE, independent of age, NT-proBNP, ischaemic aetiology, diabetic status and beta-blocker therapy.

Conclusions Plasma sFlt-1 concentrations have potential as an independent predictor of survival and may be complementary to established prognostic biomarkers in HF.

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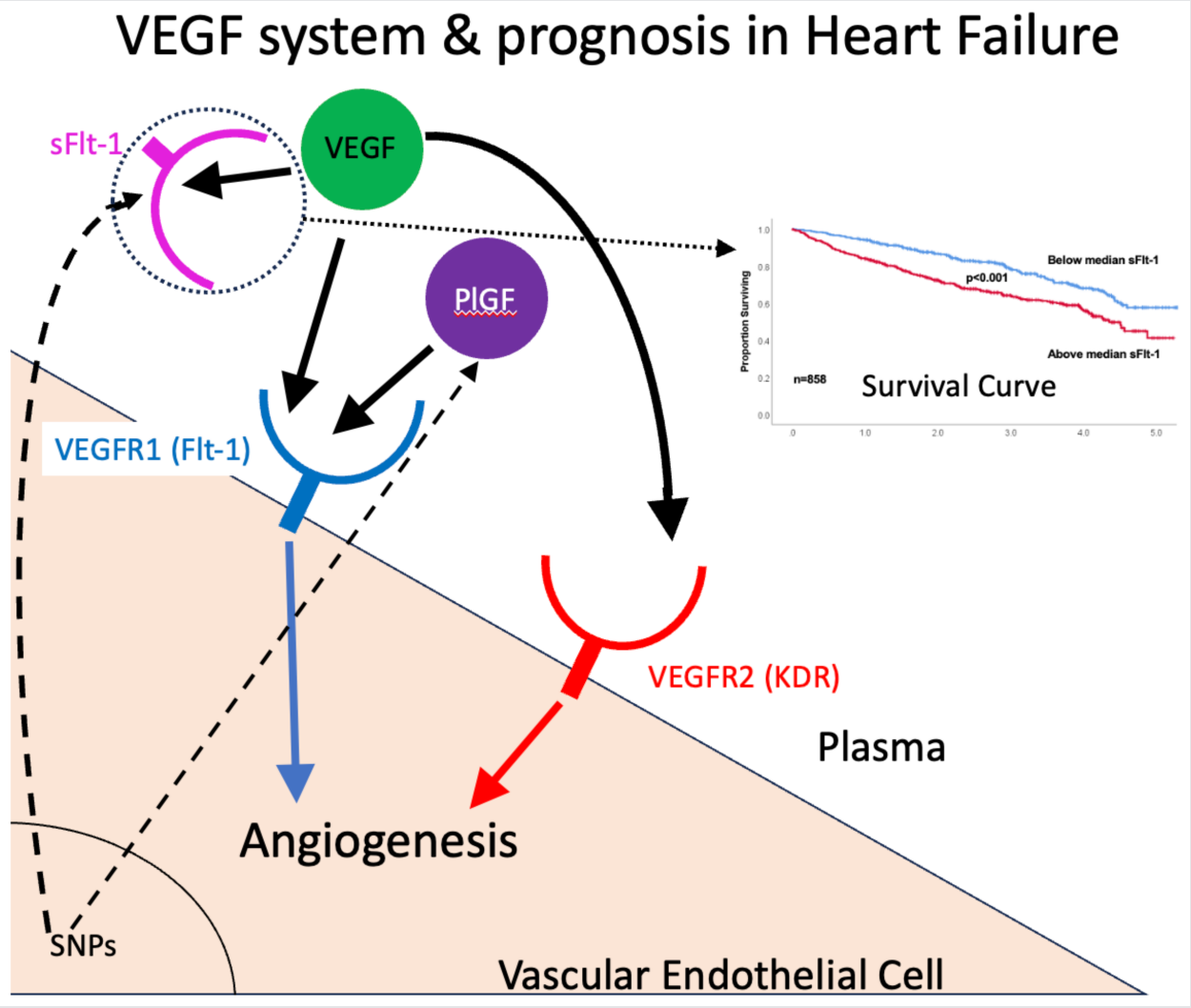
Graphical Abstract



Vascular endothelial growth factor (VEGF) system and prognosis in heart failure. Flt-1, fms-like tyrosine kinase-1; KDR, kinase insert domain receptor; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; VEGFR1, vascular endothelial growth factor receptor 1; VEGFR2, vascular endothelial growth factor receptor 2.

Keywords Heart failure • Single nucleotide polymorphism • Prognostic biomarkers • sFlt-1 • Placental growth factor • Mortality

Introduction

Soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), members of the vascular endothelial growth factor (VEGF) family, play key roles in angiogenesis and therefore have great potential to influence the progression of coronary heart disease.[1] sFlt-1, a mRNA splice variant of Flt-1 (VEGF receptor 1, a receptor for VEGF-A which circulates in plasma), is an inhibitor of VEGF activity.[2] High levels of sFlt-1 may antagonize the pro-vascularization effects of VEGF-A and PlGF. The ratio of PlGF:sFlt-1 may be an indicator of angiogenic potential.[3] VEGF-A levels in the myocardium have been reported to rise in the early stages of heart failure (HF), but drop off of VEGF-A release during sustained hypoxia may contribute to decompensated HF.[4] PlGF levels in plasma have been associated with the severity of HF of ischaemic aetiology in patients from a Japanese cohort.[5]

Heart failure is a continuing public health problem with over 64 million patients affected worldwide.[6,7] In New Zealand, HF prevalence was estimated at a rate >1300 per 100 000 in 2020 and HF mortality was 5.2 (3.9–6.9) per 100 000 from 2010 to 2012.[8] While there is evidence that the overall incidence of HF has decreased since 2000 in many developed countries, HF diagnosis at younger ages is now more common, as the incidence in age groups <55 years has increased.[9] There is increasingly recognition that HF can occur despite the presence of normal left ventricular ejection fraction (EF), resulting in HF with preserved EF (HFpEF).[10] Cohorts such as that recruited for the PEOPLE study, investigated here, have characterized the prevalence and clinical details of HF in Asian-Pacific populations.[11]

Established guideline-endorsed biomarkers with proven prognostic utility in HF include circulating B-type natriuretic peptide (BNP) and its co-secreted congener, N-terminal pro-BNP (NT-proBNP).[12] Endothelial dysfunction is a feature of HF with factors including neurohormonal activation and systemic inflammation contributing to this pathophysiology.[13] Biomarkers that inform on the state of the vascular endothelium would be useful in the management of established HF.

Plasma levels of sFlt-1 and PlGF are reportedly elevated in coronary heart disease[3,14] and HF.[15,16] We aimed to investigate their potential as prognostic biomarkers in HF. Variants of the *FLT1* and *PGF* genes may influence plasma concentrations of their gene products and potentially be markers of angiogenic activity and prognosis in HF. We investigated several gene variants in both genes, and one in the *KDR* gene, which encodes VEGF receptor 2,[14] for their possible association with analyte levels and clinical endpoints.

Methods

Study populations

PEOPLE study

The Prospective Evaluation of Outcome in Patients with Heart Failure with Preserved Left Ventricular Ejection Fraction (PEOPLE) study recruited 941 patients from four centres in New Zealand, between March 2010 and August 2014.[11] The PEOPLE study design and the outcomes for patients with HFpEF compared with those with reduced EF (HFrEF) have been reported previously.[11,17] Recruitment occurred either when the patient was in hospital (70%), having been admitted with a primary diagnosis of acute HF (study assessment followed stabilization), or as an outpatient (30%) within 6 months of an episode. All patients were over 21 years of age and provided informed consent. Exclusion criteria included severe valve disease, transient acute pulmonary oedema in the context of primary acute coronary syndrome, end-stage renal failure, specific HF subgroups (including constrictive pericarditis, congenital heart disease, hypertrophic cardiomyopathy, cardiac amyloid, and chemotherapy-associated cardiomyopathy), isolated right HF, life-threatening comorbidity with life expectancy <1 year, and inability to provide consent. The study was approved by the New Zealand Multi-Region Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000374066). All participants provided written informed consent. Patients were assessed at recruitment (baseline visit), with clinic visits at 6 weeks and 6 months later, and with telephone contacts at 1 and 2 years. All baseline measurements, including blood sampling, were performed with patients assessed as stable and compensated and considered fit for discharge or undergoing outpatient management rather than in the acute decompensated state.

Healthy volunteers

Control subjects were 201 individuals from the Canterbury Healthy Volunteers Study (CHVS)[18] age- and gender-matched to the PEOPLE cohort. CHVS participants were randomly selected from the electoral rolls for the region of Canterbury, New Zealand. Participants at the time of recruitment had no personal history of overt cardiovascular disease, including HF, angina, coronary artery disease, myocardial infarction, or history of peripheral vascular disease. Participants completed a study questionnaire on their medical history, smoking status, alcohol consumption, and self-reported physical activity. Height, weight, waist, and hip measurements were taken. Blood pressure was recorded, and a blood sample taken for blood biomarker and genetic analyses. Transthoracic echocardiography was performed using a Philips iE33 ultrasound system (Royal Philips Electronics, Amsterdam, The Netherlands). All participants consented to the research team accessing their medical records for ongoing follow-up. The study was approved by the Upper South A Ethics Committee (reference no. CTY/01/05/062), and each participant provided written informed consent.

Clinical events

Clinical events were determined from recruitment questionnaires, planned follow-up clinic visits, consultation of patient notes, the New Zealand Ministry of Health and Hospital Patient Management System databases, linked through the National Health Index number for each patient. Survival times were calculated from the date of index admission. The investigation conforms to the principles outlined in the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46.

Analyte measurements

Plasma samples were collected and stored in sealed tubes at −80°C as previously described.[14] sFlt-1 and PlGF were analysed using chemiluminescent quantitative sandwich enzyme immunoassays (R&D Systems, Inc. Minneapolis, MN, USA). Inter-assay coefficients of variation (CV) were 6.3% and 11.0%, and limits of detection were 4.17 and 7.0 pg/ml, for the sFlt-1 and PlGF assays, respectively. Circulating levels of natriuretic peptides were assayed as previously described.[19]

DNA extraction and single nucleotide polymorphism genotyping

Extraction of genomic DNA for genotyping was performed using a Kingfisher Flex Purification System (ThermoFisher Scientific, Auckland, New Zealand) as specified by the manufacturer. DNA samples were genotyped for the rs748252 (C8764T, assay ID C_1919152_10), rs35832528 (Glu982Ala, assay ID C__62951453_10) and rs9513070 (A189427G, assay ID C_30362252_10) polymorphisms in the *FLT1* gene and rs2359192 (A1800C, C_15773563_10), rs2268614 (T7277C, C_2195634_1), rs2268615 (C9694A, C_2195635_1) and rs2268616 (A10721G, C_15874530_10) in the *PGF* gene in 5 µl reaction volumes in 384-well plates using allele-specific TaqMan genotyping

probes (ThermoFisher Scientific), including 1x Roche LightCycler 480 Probes Master mix and 100 ng of genomic DNA in a Roche LC480 (Roche Diagnostics, Auckland, New Zealand) as described elsewhere.[14] As we had shown previously that plasma sFlt-1 levels were associated with the *KDR* gene polymorphism rs1870377 (T1719A, C_11895315_20) in a cohort of acute coronary syndrome patients,[14] this single nucleotide polymorphism (SNP) was also genotyped in DNA samples from the PEOPLE cohort.

Statistical analysis

Univariate analyses were performed to test for associations between SNP genotype and demographics, analyte levels and echocardiographic measurements using χ^2 and ANOVA tests. The Shapiro–Wilk test of normality was utilized to ascertain if data were normally distributed and where applicable log-transformed before analysis and geometric means with 95% confidence intervals (CI) reported and adjusted for age. Normally distributed data are reported as arithmetic means ± the standard error. The survival of stratified groups was compared using Kaplan–Meier analysis and the log-rank test. Independent associations between genotype and survival were tested using Cox proportional hazards multivariate analysis. The study had 90% power to detect a hazard ratio (HR) >1.5 as statistically significant (two tailed $\alpha < 0.05$) in the PEOPLE cohort for multivariate analysis of survival. Ethnicity was self-declared and categorized as Māori/Pacific Islander, European, other or unknown, or in some analyses European versus non-European. All analyses were performed using SPSS version 28 (IBM, Armonk, NY, USA). Statistical significance was set at the 5% level ($p < 0.05$).

Results

Baseline characteristics and genotyping

Baseline characteristics of PEOPLE participants are summarized in *Table 1* Mean age of patients was 69.1 ± 0.5 years and 70% were male. Ischaemic aetiology for HF pathology was the most common (44.0%), and 64.3% of patients were categorized as having HFrEF. The mean left ventricular EF for the cohort was 42.2 ± 0.62%.

Genotypes were obtained for rs748252, rs35832528 and rs9513070 in the *FLT1* gene, for rs2359192, rs2268614, rs2268615 and rs2268616 in the *PGF* gene, and for rs1870377 in the *KDR* gene for PEOPLE participants. Data on the frequencies of these genotypes and their association with baseline characteristics are shown in online supplementary *Tables S1–S4* (*FLT1* rs748252, and rs9513070; *PGF* rs2268615, rs2268616 and rs2359192; *KDR* rs1870377, respectively) and *Table 2* (*PGF* rs2268614 genotype). Due to the very low minor allele frequency for the *FLT1* SNP rs35832528 (0.2%), we did not attempt to test associations with this SNP. No genotyping was performed for the CHVS cohort.

Analyte measurements in the CHVS cohort

To establish appropriate reference ranges for both sFlt-1 and PlGF, these analytes were assayed in plasma samples taken at recruitment from 201 participants in the CHVS cohort,[18] matched for age, gender and ethnicity with the PEOPLE cohort (*Table 3*). sFlt-1 had a median level of 79.1 (interquartile range [IQR] 55.8–102) pg/ml and PlGF had a median level of 15.5 (IQR 9.53–21.4) pg/ml in this heart-healthy cohort. Both sFlt-1 and PlGF levels were significantly lower in the CHVS cohort than those measured in PEOPLE ($p < 0.044$) (*Figure 1*). PlGF levels were positively correlated with age ($n = 201$, $r = 0.377$, $p < 0.001$), waist-to-hip ratio ($n = 200$, $r = 0.153$, $p = 0.031$), systolic blood pressure ($n = 199$, $r = 0.243$, $p = 0.001$) and (NT-proBNP, $p = 0.035$). sFlt-1 levels were higher in current or ex-drinkers of alcohol than in non-drinkers ($n = 200$, current or ex-drinkers 82.9 ± 1.45 pg/ml; non-drinkers 75.0 ± 3.00 pg/ml, $p = 0.021$).

Table 1 Baseline characteristics of the PEOPLE cohort

Baseline characteristics	*n*	
Male sex	890	621 (69.8%)
Age (years)	890	69.1 ± 0.46
Ethnicity		
European	632	71.0%
Māori	150	16.9%
Pasifika	78	8.8%
Other	30	3.3%
Previous MI	610	284 (46.6%)
Previous stroke	882	133 (15.1%)
COPD	884	239 (27.0%)
Antecedent hypertension	881	567 (63.7%)
Type 2 diabetes	887	285 (32.0%)
Chronic kidney disease	888	508 (57.2%)
BMI (kg/m^2)	874	30.4 ± 0.26
Tobacco use (never smoked)	889	333 (37.4%)
Alcohol use (non-drinker)	885	157 (17.6%)
Ischaemic aetiology	890	392 (44.0%)
LVEF	736	42.2% ± 0.62
NYHA class I/II/III/IV (%)	882	18.3/45.9/29.4/6.6
HFrEF	890	572 (64.3%)
Atrial fibrillation	890	522 (58.7%)
NT-proBNP[a] (pg/ml)	852	1690 (5–59 900)
Plasma creatinine[a] (mmol/L)	888	106 (42–340)
Medications		
ACE inhibitor/ARB	890	758 (85.2%)
Beta-blocker	890	729 (81.9%)
Loop diuretic	890	832 (93.5%)
Anticoagulant	890	377 (42.4%)
Antiplatelet	890	486 (54.6%)
Statin	890	528 (59.3%)
Spironolactone	890	271 (30.4%)

Data are given as mean ± standard error of the mean, or median (interquartile range), unless indicated otherwise.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.
[a]Median (range).

Analyte measurements in the PEOPLE cohort

Levels of sFlt-1 were measured from baseline plasma samples of 858 PEOPLE patients (mean 117 [115–120] pg/ml, CV = 6.50%).

Table 2 Baseline characteristics, drug treatment and neurohormonal data for PEOPLE patients stratified by *PGF* rs2268614 genotype group

	n	TT	*n*	TC	*n*	CC	*p*-value
Age (years)	281	70.1 ± 0.76	430	68.7 ± 0.66	179	68.5 ± 1.12	0.315
Male sex	281	198 (70.5%)	430	304 (70.7%)	179	119 (66.5%)	0.561
BMI (kg/m^2)	275	30.0 ± 0.47	423	30.6 ± 0.38	176	30.7 ± 0.57	0.490
History							
Previous MI	208	112 (53.8%)	276	122 (44.2%)	126	50 (39.7%%)	0.024
Hypertension	280	193 (68.9%)	424	255 (60.1%)	177	119 (67.2%)	0.039
Diabetes	281	84 (29.9%)	430	128 (29.8%)	179	52 (29.1%)	0.933
Renal artery stenosis	276	2 (0.7%)	420	9 (2.1%)	175	4 (2.3%)	0.303
Alcohol (current drinker)	280	172 (61.4%)	428	249 (58.2%)	177	96 (54.2%)	0.073
Ischaemic aetiology of HF	281	140 (49.8%)	430	174 (40.5%)	17 978	78 (43.6%)	0.048
Analytes							
Total cholesterol (mmol/L)	251	4.36 ± 0.08	367	4.25 ± 0.06	251	4.21 ± 0.09	0.381
Plasma creatinine[a] (mmol/L)	281	108 (104–113)	428	105 (102–109)	179	109 (104–115)	0.444
eGFR (CKD-EPI) (ml/min/1.73 m^2)	281	56.2 ± 1.21	428	57.6 ± 0.98	179	55.3 ± 1.47	0.373
NT-proBNP[a] (pg/ml)	268	1520 (1300–1760)	413	1420 (1260–1610)	171	1620 (1350–1940)	0.496
PlGF (pg/ml)	249	15.6 (14.6–16.8)	384	16.4 (15.8–17.0)	161	17.2 (16.3–18.1)	0.043
sFlt-1 (pg/ml)	270	114 (109–119)	417	121 (117–126)	171	116 (110–122)	0.079
PlGF:sFlt-1 ratio	249	0.156 ± 0.005	384	0.153 ± 0.004	161	0.167 ± 0.006	0.038
Discharge medications							
ACE inhibitor	281	197 (70.1%)	430	297 (69.1%)	179	129 (72.1%)	0.762
Beta-blocker	281	232 (82.6%)	430	359 (83.5%)	179	138 (77.1%)	0.165
Loop diuretic	281	268 (95.4%)	430	400 (93.0%)	179	164 (91.6%)	0.244
Statin	281	176 (62.6%)	430	241 (56.0%)	179	111 (62.0%)	0.155
Functional measures							
LVEF	230	43.0 ± 1.15	361	44.4 ± 0.90	145	43.7 ± 1.37	0.617
HFrEF	281	180 (64.1%)	430	280 (65.1%)	179	112 (62.6%)	0.833
Follow-up (years)	281	2.79 (0.11–5.28)	430	2.52 (0.01–5.45)	179	2.69 (0.0–5.34)	

Data are given as mean ± standard error of the mean, or median (interquartile range), unless indicated otherwise.
ACE, angiotensin-converting enzyme; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.
[a]Geometric mean (95% confidence interval).

sFlt-1 levels were correlated with NT-proBNP levels ($n = 852$, $r = 0.176$, $p < 0.001$), high-sensitivity troponin T (hsTnT) ($n = 852$, $r = 0.125$, $p < 0.001$), creatinine ($n = 856$, $r = 0.080$, $p = 0.020$), alcohol consumption ($n = 647$, $r = 0.132$, $p = 0.001$) and negatively correlated with systolic blood pressure ($n = 848$, $r = -0.139$, $p < 0.001$). Median sFlt-1 levels were associated with HF severity, as determined by New York Heart Association class at baseline (class I: $n = 159$, 117 [112–123] pg/ml; class II: $n = 378$, 113 [110–117] pg/ml; class III: $n = 257$, 124 [118–130] pg/ml; class IV: $n = 57$ 111 [101–112] pg/ml; $p = 0.006$). As well as being correlated with absolute level of alcohol consumption, sFlt-1 levels were associated with alcohol drinking status ($n = 853$, current drinkers 114 [110–117] pg/ml; ex-drinkers, 126 [121–133] pg/ml; non-drinkers, 117 [110–124] pg/ml, $p = 0.001$). Raw sFlt-1 levels at baseline were associated with *KDR* SNP rs1870377 genotype ($n = 851$, rs1870377 genotype AA 124 [117–132] pg/ml, TA 119 [115–124] pg/ml, TT 114 [111–118] pg/ml, $p = 0.047$), but not when corrected for age ($p = 0.225$) (online supplementary *Table S6*).

PlGF was assayed in 810 PEOPLE plasma samples. PlGF levels (mean 17.5 ± 0.21 pg/ml) were weakly correlated with age ($n = 810$, $r = 0.185$, $p < 0.001$), NT-proBNP levels ($n = 807$, $r = 0.096$, $p = 0.006$), hsTnT ($n = 807$, $r = 0.142$, $p < 0.001$), creatinine ($n = 809$, $r = 0.070$, $p = 0.047$) and negatively correlated with sFlt-1 ($n = 810$, $r = -0.104$, $p = 0.003$) and baseline diastolic blood pressure ($n = 800$, $r = -0.092$, $p = 0.009$).

sFlt-1 was higher in patients with HFrEF (130 ± 2.62 pg/ml, $n = 553$) compared to those with HFpEF (117 ± 3.59 pg/ml, $n = 305$; $p = 0.005$). The ratio of PlGF:sFlt-1 was also associated with HFrEF/HFpEF status (HFrEF $n = 506$, mean ratio = 0.577 [0.568–0.586]; HFpEF $n = 286$, mean ratio = 0.600 [0.590–609], $p = 0.049$). sFlt-1 ($p = 0.052$) and PlGF ($p = 0.355$) plasma levels were not significantly associated with HF aetiology.

For variants of the *PGF* gene, SNP rs2268614 genotype was associated with PlGF levels ($n = 794$, TT, mean 15.6 [14.6–16.8] pg/ml; TC, mean 16.4 [15.8–17.0] pg/ml; CC, mean 17.2 [16.3–18.1] pg/ml; $p = 0.043$). A recessive model revealed association between baseline PlGF levels and rs2268616 genotype ($n = 794$, AA, mean

Table 3 Baseline characteristics of the CHVS cohort

Baseline characteristics	n	
Male sex	201	141 (70.1%)
Age (years)	201	69.1 ± 0.94
Ethnicity	201	
European		171 (85.1%)
Māori/Pasifika		17 (8.5%)
Asian		8 (4.0%)
Other		5 (2.5%)
Antecedent hypertension	201	67 (33.3%)
Type 2 diabetes	201	14 (7.0%)
BMI (kg/m^2)	201	26.5 ± 0.30
Tobacco use (never smoked)	200	99 (49.3%)
Alcohol use (non-drinker)	200	40 (20%)
LVEF	91	64.5 ± 0.47
Medications		
ACE inhibitor/ARB	201	11 (5.5%)
Beta-blocker	201	7 (3.5%)
Diuretic	201	11 (5.5%)
Anticoagulant	201	1 (0.5%)
Antiplatelet	201	20 (10%)
Statin	201	13 (6.5%)
Spironolactone	201	1 (0.5%)

Data are given as mean ± standard error of the mean, unless indicated otherwise. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; LVEF, left ventricular ejection fraction.

16.2 [15.7–16.8] pg/ml; AG/GG, mean 16.6 [15.4–18.0] pg/ml, $p < 0.001$). rs2268616 genotype was associated with HFrEF/HFpEF status (online supplementary *Table S4*). Neither of the *FLT1* SNPs analysed, rs748252 and rs9513070, were associated with analyte levels.

Clinical outcome in the PEOPLE cohort

All-cause mortality (over a median follow-up of 2.70 years) was greater in those with above, compared to below, median sFlt-1 levels ($n = 858$, above median 39.2%, below median 25.7%, $p < 0.001$, total of 278 deaths) (*Figure 2*). sFlt-1 considered as a continuous variable predicted survival in a Cox proportional hazards model which included established risk factors (HR for sFlt-1 6.44, 95% CI 2.57–16.1) (*Table 4*). PlGF levels were not predictive of survival over a median follow-up of 2.67 years ($n = 794$, above median 34.6%, vs. below median 31.9%, $p = 0.314$, 264 events). The ratio of PlGF:sFlt-1 was similarly predictive for survival as sFlt-1 levels alone (HR 15.2, 95% CI 2.38–100, $p = 0.004$). Receiver operating characteristic curve analysis showed that baseline plasma NT-proBNP and sFtl-1, but not PlGF, levels were significant predictors of survival at 2 years (*Figure 3*).

One of the gene variants analysed was associated with survival; the *PGF* gene SNP rs2268616 ($n = 890$, mortality: AA genotype group 33.3%, GA/GG genotype group 17.4%, $p = 0.016$, 283 events) (*Figure 4*). However this SNP was not an independent predictor of survival on Cox regression analysis incorporating the same covariates as presented in *Table 4* ($p = 0.437$).

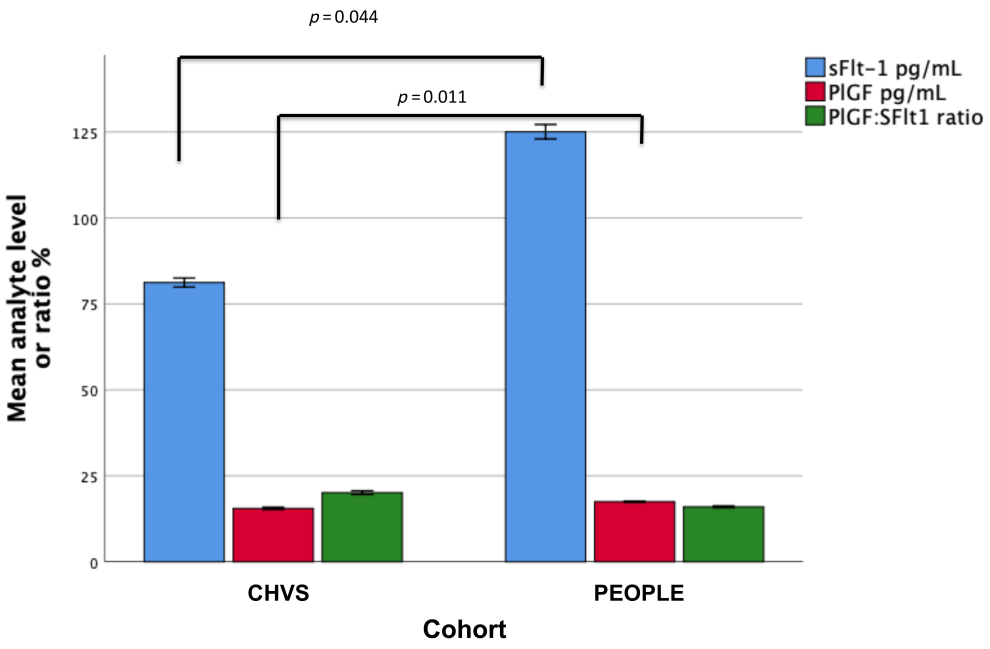
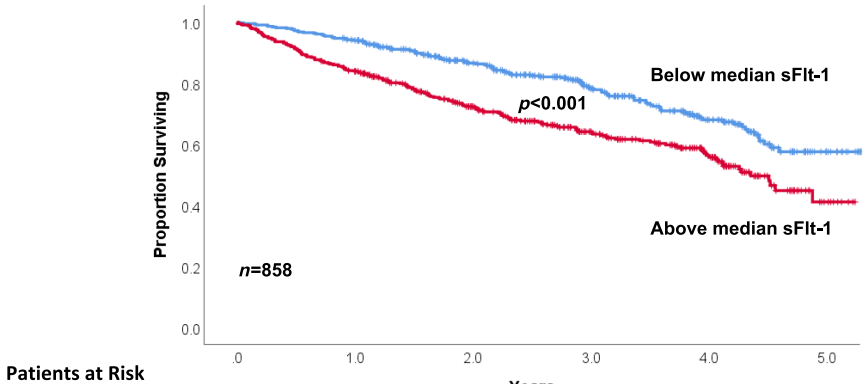


Figure 1 Comparison of baseline levels of soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF) and PlGF:SFlt-1 ratio between the CHVS and PEOPLE cohorts.



Patients at Risk							Events
sFlt-1 below median	435	404	301	193	102	18	112 (25.7%)
sFlt-1 above median	423	349	253	161	89	8	166 (39.2%)

Figure 2 Patient survival in the PEOPLE cohort stratified on above and below median levels of soluble fms-like tyrosine kinase-1 (sFlt-1).

Table 4 Cox proportional hazards regression model for mortality in the PEOPLE cohort ($n = 695$, 246 deaths)

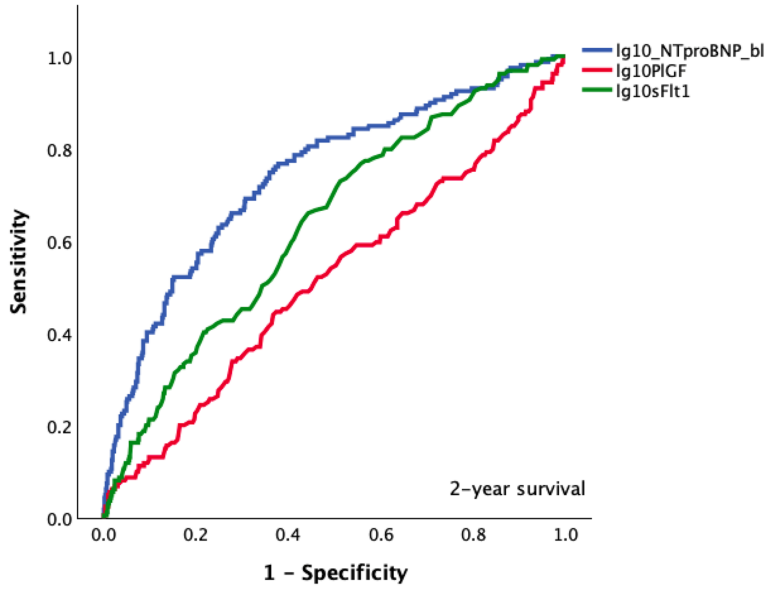
	Coefficient	SE	Wald	df	Significance	Hazard ratio (95% CI)
Age at index admission	0.02	0.01	7.52	1	0.006	1.02 (1.01–1.03)
Male sex	0.36	0.15	5.58	1	0.018	1.43 (1.06–1.93)
Log_{10} NT-proBNP[a]	1.08	0.18	34.5	1	<0.001	2.93 (2.05–4.20)
Log_{10} sFlt-1[a]	1.86	0.47	15.8	1	<0.001	6.44 (2.57–16.1)
Beta-blocker at baseline	−0.54	0.15	12.5	1	<0.001	0.59 (0.44–0.79)
ACE inhibitor or ARB at baseline	0.09	0.17	0.28	1	0.598	1.09 (0.79–1.51)
Spironolactone at baseline	0.14	0.16	0.76	1	0.38	1.15 (0.84–1.58)
Ischaemic aetiology	0.27	0.14	3.75	1	0.053	1.31 (0.99–1.72)
Diabetes	0.36	0.15	5.86	1	0.016	1.43 (1.07–1.90)
eGFR (CKD-EPI)	−0.02	0.04	29.0	1	<0.001	0.98 (0.97–0.99)
LVEF	0.01	0.01	1.82	1	0.18	1.01 (0.99–1.02)
NYHA class at baseline			10.2	3	0.017	
I vs. IV	−0.68	0.30	5.25	1	0.022	0.51 (0.28–0.91)
II vs. IV	−0.46	0.24	3.87	1	0.049	0.63 (0.40–0.99)
III vs. IV	−0.13	0.23	0.32	1	0.570	0.88 (0.55–1.39)

ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; df, degree of freedom; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, standard error; NYHA, New York Heart Association; sFlt-1, soluble fms-like tyrosine kinase-1.
[a]HR represents the change in risk for every 10-fold increase in analyte level.

Discussion

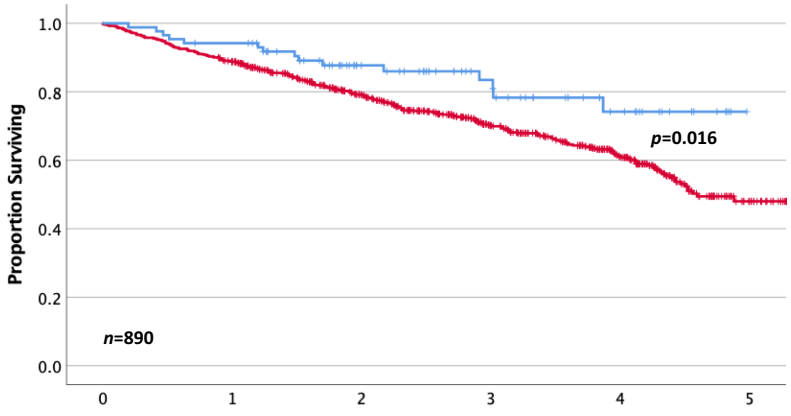
Plasma concentrations of both sFlt-1 and PlGF in samples from HF patients participating in the PEOPLE study were higher than those from age- and gender-matched heart-healthy individuals from the CHVS cohort. It has been suggested that high sFlt-1 may be generated as a consequence of established HF.[15] sFlt-1 levels taken at baseline were found to be a significant predictor of survival in the PEOPLE cohort, independent of established predictors. The ratio of PlGF:sFlt-1 behaved similarly to sFlt-1 levels in multivariate models of survival, but as expected sFlt-1 and this ratio were not independent. While the ratio may have some predictive value, it does not appear to be superior to sFlt-1 levels alone (*Graphical Abstract*). Elevated PlGF:sFlt-1 has been found to predict adverse outcomes in patients with stable coronary artery disease,[3] but was not evaluated in a study of HF patients, where PlGF and sFlt-1 were predictors of adverse outcome.[15]

Levels of sFlt-1 were correlated with levels of biomarkers of cardiovascular stress/cardiac injury (NT-proBNP and hsTnT), creatinine, alcohol consumption, and were negatively correlated with systolic blood pressure. We previously found a correlation between sFlt-1 and alcohol consumption, NT-proBNP and BNP in a cohort of post-acute coronary syndrome patients.[14] Association between sFl-t levels and creatinine clearance and plasma BNP



Analyte	AUC	95% CI for AUC	*p*-value
Baseline NTproBNP	0.743	0.698–0.787	*p*<0.001
Baseline sFlt-1	0.638	0.591–0.685	*p*<0.001
Baseline PlGF	0.515	0.463–0.568	*p*=0.571

Figure 3 Receiver operating characteristic curve analysis of placental growth factor (PlGF), fms-like tyrosine kinase-1 (sFlt-1) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) as predictors of survival at 2 years in the PEOPLE cohort. AUC, area under the curve; CI, confidence interval.



Patients at Risk							Events
rs2268616 AA	804	704	514	322	174	26	268 (33.3%)
rs2268616 GA/GG	86	80	50	33	17	0	15 (17.4%)

Figure 4 Patient survival in the PEOPLE cohort stratified by rs2268616 genotype.

has also been previously reported in HF patients.[15] A difference in sFlt-1 levels between patients with, compared to those without, HF was found in the same study,[15] but no difference in sFlt-1 levels between HFrEF versus HFpEF phenotypes, as we and others[20] found. Our finding that patients with HFrEF had significantly higher levels of sFlt-1 at baseline suggests potential for sFlt-1 as a biomarker for differentiating HFrEF and HFpEF. Others have suggested the use of the biomarker ST2 for distinguishing between these two classes of HF.[21,22] At least one other study has also found elevated sFlt-1 levels in human HF compared to healthy controls.[23]

The positive association of sFlt-1 and level of alcohol consumption has been reported elsewhere,[24] and we observed this in both

the PEOPLE and CHVS cohorts. The U-shaped association of alcohol with cardiovascular disease[25] may in part be explained by its association with sFlt-1 levels in the plasma of individuals, perhaps linking the consumption of alcohol with angiogenesis via alcohol interaction with the VEGF receptor, KDR.[24]

Two *PGF* gene variants were associated with one of the analytes measured in the PEOPLE cohort. We found rs2268614 genotype was associated with plasma PlGF levels, as have others in individuals from healthy general populations,[26] and psoriasis patients.[27] rs2268616 genotype was also associated with baseline PlGF levels and HFrEF/HFpEF status. rs2268616 genotype has a positive association with coronary artery disease in the Type 2 Diabetes Knowledge Portal (https://t2d.hugeamp.org/). Its association with HFrEF/HFpEF status could make this SNP useful for aiding classification of the trajectory of HF in the early stages of its development.

Limitations of this study include that patients in the PEOPLE cohort are predominantly of European ancestry and therefore these results should not be extrapolated to other ethnic groups. sFlt-1 and PlGF were measured at a single time point, and the dynamics of these analytes and their change in response to treatment should be investigated. Missing data for some characteristics limited the power of the study to investigate associations with analyte levels and genotypes.

The interplay of the VEGF system components, particularly VEGF-A, sFlt-1 and PlGF, are central to the formation and maintenance of blood vessels in the myocardium and elsewhere.[28] In this study, we showed both sFlt-1 and PlGF plasma levels were elevated in HF patients compared to heart-healthy volunteers, and sFlt-1 was associated with the severity and clinical outcome of HF. Still there is much we do not know about the factors that drive the derangement of the VEGF system in HF, the influence of this on endothelial dysfunction, and the relative importance of genetic and environmental factors in promoting these changes. Further research on the VEGF system in HF would help clarify the value of these factors as biomarkers and predictors of outcome.

Conclusion

Plasma sFlt-1 levels have potential as a predictor of survival in HF patients, complementary to established prognostic biomarkers in HF. sFlt-1 levels may also have value as a marker to aid differentiation between HFrEF and HFpEF. The *PGF* gene variants rs2268614 and rs2268616 were associated with baseline plasma PlGF levels in a HF cohort.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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