Placental Growth Factor as a Predictor of Cardiovascular Events in Patients with CKD from the NARA-CKD Study

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ABSTRACT

Placental growth factor (PIGF) contributes to atherogenesis through vascular inflammation and plaque destabilization. High levels of PIGF may be associated with mortality and cardiovascular disease, but the relationship between PIGF level and adverse outcomes in patients with CKD is unclear. We conducted a prospective cohort study of 1351 consecutive participants with CKD enrolled in the Novel Assessment of Risk management for Atherosclerotic diseases in CKD (NARA-CKD) study between April 1, 2004, and December 31, 2011. During a median follow-up of 3 years, 199 participants died and 383 had cardiovascular events, defined as atherosclerotic disease or heart failure requiring hospitalization. In adjusted analyses, mortality and cardiovascular risk increased in each successive quartile of serum PIGF level; hazard ratios (HRs) (95% confidence intervals [95% CIs]) for mortality and cardiovascular risk, respectively, were 1.59 (0.83 to 3.16) and 1.55 (0.92 to 2.66) for the second quartile, 2.97 (1.67 to 5.59) and 3.39 (2.20 to 5.41) for the third quartile, and 3.87 (2.24 to 7.08) and 8.42 (5.54 to 13.3) for the fourth quartile. The composite end point of mortality and cardiovascular events occurred during the study period in 76.4% of patients in both the highest PIGF quartile (≥19.6 pg/ml) and the lowest eGFR tertile (<30 ml/min per 1.73 m²). The association between PIGF and mortality or cardiovascular events was not attenuated when participants were stratified by age, sex, traditional risk factors, and eGFR. These data suggest elevated PIGF is an independent risk factor for all-cause mortality and cardiovascular events in patients with CKD.


CKD is increasingly recognized as a major public health problem that is linked to poor clinical outcomes. Cardiovascular disease is the leading cause of death in patients with CKD, at a much higher rate than in the general population.1–4 Despite this growing recognition of the cardio-renal connection, conventional treatment strategies, such as modification of traditional risk factors, are unlikely to contribute substantially to cardiovascular disease risk reduction in this population.5 Little is known about nontraditional risk factors and biomarkers identifying patients with CKD at a higher risk of cardiovascular disease, but we have recently

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proposed that placental growth factor (PIGF) signaling is involved in the aggravation of atherosclerotic disease associated with decreased renal function.6,7

PIGF, with a similar sequence to vascular endothelial growth factor (VEGF), is a prototypical pleiotropic cytokine capable of stimulating angiogenesis and inducing atherosclerosis by binding and activating its membrane-bound receptor, fms-like tyrosine kinase-1 (Flt-1).10–13 PIGF expression in atherosclerotic lesions regulates and activates monocytes and macrophages, which subsequently produces inflammatory and angiogenic mediators, resulting in an increased risk of plaque rupture.14,15 Conversely, inhibition of PIGF through genetic approaches and use of anti-PIGF antibodies reduces the size of atherosclerotic plaques.16

In clarifying the role of PIGF signaling in humans, accumulating evidence suggests that elevated PIGF is an independent predictor of cardiovascular risk and mortality among women in the general population and in patients with diabetes and acute coronary syndrome.17–21 However, because other investigators have not found an independent association between PIGF and mortality in patients with heart failure and suspected myocardial infarction,22,23 the effect of PIGF on mortality and cardiovascular disease remains unclear. We recently found a significant inverse relationship between PIGF production and renal function and that the PIGF/Flt-1 signaling pathway is partly involved in the pathogenesis of CKD-associated atherosclerosis. However, the association between circulating levels of PIGF and cardiovascular events in patients with CKD is less clear.

Therefore, we conducted a longitudinal study to test the hypothesis that PIGF is a strong and useful predictor of all-cause mortality and cardiovascular events in patients with CKD who participated in the Novel Assessment of Risk management for Atherosclerotic diseases in Chronic Kidney Disease (NARA-CKD) study.

RESULTS

Baseline Characteristics
A total of 1351 patients met the inclusion criteria (Figure 1). The median age (interquartile range) of all participants was 65 years (52–74 years), and 825 (61%) of the participants were men. The distribution of serum PIGF levels was skewed to the right, with a median level of 14.5 pg/ml (interquartile range, 10.1–19.6 pg/ml). Baseline characteristics are presented in Table 1. Factors associated with PIGF in the univariate analysis are described in Supplemental Table 1. Multivariate linear regression revealed that high levels of PIGF were independently associated with more advanced CKD stage, high levels of C-reactive protein, low levels of serum albumin and HDL, and use of antiplatelet agents (Supplemental Table 2).

PIGF and Risk of All-Cause Mortality
During a median follow-up of 3.3 years, 199 participants died (44.5/1000 person years), including 62 from sudden death (31%), 32 from heart failure (16%), 31 from malignancy (16%), 30 from infection (15%), 13 from stroke (6.5%), and six from acute myocardial infarction (3.0%). Median PIGF levels were significantly higher in those who died (19.1 pg/ml; interquartile range, 14.6–24.6 pg/ml) than those who survived (13.8 pg/ml; interquartile range, 9.6–18.9 pg/ml) (P<0.001). Higher serum PIGF levels were associated with a greater risk of all-cause mortality in the crude Kaplan–Meier analysis (Supplemental Figure 1A) (P<0.001 by the log-rank test for trend). Adjusted hazard ratios (HRs) are presented in Table 2 for each PIGF quartile. Participants in the higher quartiles had a greater risk of all-cause mortality. Compared with patients in the lowest quartile, patients in the highest quartile had a 3.87-fold greater mortality risk in models adjusting for clinical demographics, risk factors, current medications, and laboratory results. The HR for all-cause mortality for each 10 pg/ml increase in PIGF was attenuated by the addition of more covariates; however, PIGF remained independently associated with all-cause mortality in model 4 (HR, 1.61; 95% confidence interval [95% CI], 1.37 to 1.89; P<0.001). The graph of HRs for different levels of PIGF is an upward sloping curve, with higher PIGF levels showing a consistently increased risk of mortality in the fully adjusted model (Figure 2). This relationship was similar within predefined subgroups stratified by age, sex, diabetes, hypertension, dyslipidemia and smoking, but not obesity (Figure 3).

PIGF and Risks of Cardiovascular Events
During the median follow-up of 2.93 years, a total of 383 cardiovascular events (96.8/1000 person years) occurred, defined as atherosclerotic disease or heart failure requiring hospitalization. In detail, 255 and 176 participants developed new atherosclerotic disease and congestive heart failure during the study period, respectively, and of these, 48 patients had both new atherosclerotic disease and congestive heart failure. Atherosclerotic disease consisted of new development of ischemic heart disease and acute coronary syndrome in 115 patients (30%), cardiac sudden death in 58 patients (15%), stroke in 49 patients (13%), and peripheral artery disease in 29 patients (8%). Median PIGF levels were significantly higher in patients who experienced cardiovascular events (20.0 pg/ml; interquartile range, 15.8–24.6 pg/ml) than those who remained event-free (12.8 pg/ml; interquartile range, 9.1–16.5 pg/ml) (P<0.001). Crude Kaplan–Meier analysis revealed that higher PIGF levels were significantly associated with a greater risk of cardiovascular events, as shown in Supplemental Figure 1B (P<0.001). Participants in the two lowest PIGF quartiles had a similar risk of cardiovascular events, whereas those in the two highest quartiles had a 3.39- and 8.42-fold greater risk of cardiovascular events, respectively, compared with those in the first quartile (Table 2). When analyzing atherosclerotic disease and heart failure separately, unadjusted and adjusted multivariate analysis showed that being in the two highest PIGF quartiles was a strong risk factor of atherosclerotic disease and heart failure (Supplemental Figure 2, Supplemental Table 3). In the fully adjusted model, each 10 pg/ml increase in PIGF was independently associated with a greater risk of...
cardiovascular events (HR, 2.03; 95% CI, 1.82 to 2.26, \( P < 0.001 \)). Furthermore, the risk of cardiovascular events increased with increasing PlGF levels (Figure 2), and this association was observed in all of the subgroups analyzed (Figure 3). 

Stratified by eGFR
A significant inverse relationship between serum levels of PlGF and eGFR was observed \( (P < 0.001) \); the median (interquartile range) PlGF concentrations were 11.9 pg/ml (8.2–15.6 pg/ml), 14.8 pg/ml (10.3–19.3 pg/ml), and 18.1 pg/ml (13.0–23.1 pg/ml) in patients with eGFR of \( \geq 60 \), 30–59, and < 30 ml/min per 1.73 m², respectively. Patients in the highest PlGF quartile and the lowest eGFR tertile comprised 76.4% of the composite end points of all-cause mortality and cardiovascular events, whereas patients in the lowest quartile of PlGF and the highest tertile of eGFR accounted for 5.1%. The combination of reduced eGFR and elevated PlGF was associated with an increased risk of the composite end point in an additive manner (Figure 4A). In the multivariable-adjusted model, each 10 pg/ml increase in PlGF was independently associated with all-cause mortality and cardiovascular events when patients were stratified by eGFR (Figure 4, B and C); this relationship was observed even in patients with severe renal dysfunction.

Sensitivity Analysis
Results of sensitivity analyses were similar to those of our main analysis when we substituted eGFR using the Modification of Diet in Renal Disease (MDRD) equation and CKD-EPI equation instead of CKD stage; when we excluded very elderly patients; when we excluded patients on maintenance dialysis and patients who began dialysis during the follow-up period; when we restricted the analysis to participants who were followed for at least 2 years after enrollment; when proteinuria was included in the fully adjusted model; when plasma levels of brain natriuretic peptide (BNP) were included in the fully adjusted model; and when serum levels of VEGF were included in the fully adjusted model (Table 3).

VEGF and Risks of All-Cause Mortality and Cardiovascular Events
In addition to PlGF, we measured the serum level of VEGF, which is another ligand against Flt-1, in 1327 participants. The median levels of VEGF were 290 pg/ml (interquartile range, 160–508 pg/ml), and serum levels of VEGF were not significantly correlated with eGFR \( (r = 0.01, P = 0.84) \) unlike PlGF \( (r = -0.34, P < 0.001) \). VEGF levels at baseline were significantly associated with a greater risk of mortality in the unadjusted model, but the association was attenuated in the fully adjusted model (Supplemental Figure 3, Table 4).

Combination Use of PlGF and BNP
As shown in Table 2, although HR in all-cause mortality and cardiovascular events became lower when adjusted by other factors, including BNP, PlGF levels remained a strong predictor of both mortality and cardiovascular events. We compared predictive values for all-cause mortality and cardiovascular events among PlGF alone, BNP alone, and combination use of PlGF and BNP. For all-cause mortality, combination use of BNP and PlGF did not significantly improve the c statistics compared with BNP alone (from 0.70 to 0.69, \( P = 0.87 \)) but did improve integrated discrimination improvement (IDI) \( (0.04; 95\% \text{ CI}, 0.02 \text{ to } 0.06; P < 0.001) \) and category-free net reclassification improvement (NRI) \( (0.49; 95\% \text{ CI}, 0.31 \text{ to } 0.67; P < 0.001) \) (Supplemental Figure 4, Table 5). For cardiovascular events, the combination use significantly improved the c statistics \( (0.76 \text{ to } 0.80, P = 0.011) \), IDI \( (0.14; 95\% \text{ CI}, 0.12 \text{ to } 0.17; P < 0.001) \), and category-free NRI \( (0.84; 95\% \text{ CI}, 0.71 \text{ to } 0.96; P < 0.001) \) compared with BNP alone.

DISCUSSION
In this study we first evaluated the effect of serum PlGF concentrations on all-cause mortality and cardiovascular events in patients with CKD. We demonstrated that higher PlGF levels were associated with both all-cause mortality and
cardiovascular events, independent of clinical demographic parameters and traditional risk factors. This trend remained after adjustment for current medications for preventing atherosclerosis and heart failure and laboratory data related to cardiovascular disease. Of note, this association of PIGF with adverse outcomes was stronger than traditional and CKD-specific risk factors. Subgroup and sensitivity analysis further strengthen the robustness of our findings.
Table 2. Risk of various outcomes by baseline PlGF level

<table>
<thead>
<tr>
<th>Models</th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>2.01 (1.12 to 3.77)</td>
<td>1.55 (0.86 to 2.90)</td>
<td>1.54 (0.84 to 2.91)</td>
<td>1.52 (0.82 to 2.90)</td>
<td>1.59 (0.83 to 3.16)</td>
<td>1.72 (0.84 to 3.68)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>4.08 (2.41 to 7.32)</td>
<td>2.73 (1.61 to 4.92)</td>
<td>2.74 (1.59 to 4.99)</td>
<td>2.85 (1.65 to 5.19)</td>
<td>2.97 (1.67 to 5.59)</td>
<td>2.55 (1.39 to 4.92)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>6.97 (4.22 to 12.3)</td>
<td>4.76 (2.87 to 8.42)</td>
<td>4.25 (2.53 to 7.61)</td>
<td>4.47 (2.65 to 8.03)</td>
<td>3.87 (2.24 to 7.08)</td>
<td>3.37 (1.90 to 6.34)</td>
</tr>
<tr>
<td>For each 10 pg/ml increase</td>
<td>1.87 (1.65 to 2.09)</td>
<td>1.90 (1.65 to 2.16)</td>
<td>1.76 (1.51 to 2.02)</td>
<td>1.80 (1.55 to 2.09)</td>
<td>1.61 (1.37 to 1.89)</td>
<td>1.53 (1.30 to 1.81)</td>
</tr>
</tbody>
</table>

 Cardiovascular events

<table>
<thead>
<tr>
<th>Models</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
<td>1.0 (Ref)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.71 (1.06 to 2.82)</td>
<td>1.37 (0.85 to 2.26)</td>
<td>1.31 (0.80 to 2.18)</td>
<td>1.37 (0.83 to 2.29)</td>
<td>1.55 (0.92 to 2.66)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>4.82 (3.20 to 7.55)</td>
<td>3.63 (2.40 to 5.71)</td>
<td>3.48 (2.29 to 5.50)</td>
<td>3.64 (2.38 to 5.77)</td>
<td>3.39 (2.20 to 5.41)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>11.6 (7.87 to 17.9)</td>
<td>9.17 (6.19 to 14.2)</td>
<td>7.41 (4.95 to 11.6)</td>
<td>7.97 (5.31 to 12.5)</td>
<td>8.42 (5.54 to 13.3)</td>
</tr>
<tr>
<td>For each 10 pg/ml increase</td>
<td>2.22 (2.03 to 2.42)</td>
<td>2.24 (2.04 to 2.46)</td>
<td>2.09 (1.88 to 2.31)</td>
<td>2.08 (1.87 to 2.31)</td>
<td>2.03 (1.82 to 2.26)</td>
</tr>
</tbody>
</table>

Values are HRs (95% CIs). Model 1 adjusted for age and sex. Model 2 adjusted for covariates in model 1 plus risk factors, including diabetes, hypertension, dyslipidemia, obesity, smoking, previous coronary artery disease, previous stroke, and CKD stage. Model 3 adjusted for covariates in model 2 plus current medications, including RAS blockers, calcium channel blockers, β-blockers, mineralocorticoid receptor antagonists, lipid-lowering agents, diuretics, and antiplatelet agents. Model 4 adjusted for covariates in model 3 plus laboratory data, including hemoglobin, serum albumin, serum calcium, phosphorus, C-reactive protein, HbA1c, LDL cholesterol, HDL cholesterol, and triglycerides. Model 5 adjusted for covariates in model 4 plus BNP. Ref, reference.

Figure 2. Multivariable-adjusted HRs for all-cause mortality and for cardiovascular events over the distribution of serum PlGF concentrations. The median of the lowest quartile of PlGF concentration served as the reference group (7.4 pg/ml). The Cox model was adjusted for age, sex, diabetes, hypertension, dyslipidemia, obesity, smoking, previous coronary artery disease, previous stroke, and CKD stage. Model 2 adjusted for covariates in model 1 plus risk factors, including diabetes, hypertension, dyslipidemia, obesity, smoking, previous coronary artery disease, previous stroke, and CKD stage. Model 3 adjusted for covariates in model 2 plus current medications, including RAS blockers, calcium channel blockers, β-blockers, mineralocorticoid receptor antagonists, lipid-lowering agents, diuretics, and antiplatelet agents. Model 4 adjusted for covariates in model 3 plus laboratory data, including hemoglobin, serum albumin, serum calcium, phosphorus, C-reactive protein, HbA1c, LDL cholesterol, HDL cholesterol, and triglycerides. Model 5 adjusted for covariates in model 4 plus BNP. Ref, reference.

Recently, a growing body of clinical evidence suggests that PlGF alone or in combination with other biomarkers is a powerful predictor of survival or cardiovascular events in patients with stable and unstable coronary artery disease; however, some studies have not demonstrated that PlGF is responsible for promoting angiogenesis and destabilizing plaques through mediating macrophage accumulation in atherosclerotic lesions via Flt-1. In contrast, in one animal study, neutralization of PlGF reduced plaque size and the severity of macrophage infiltration. The combination of vascular inflammation and malnutrition, known as the malnutrition-inflammation-atherosclerosis syndrome, worsens outcomes in patients with CKD through aggravation of heart failure and accelerated atherosclerosis.

PIGF, originally discovered in the placenta, has also been found in the heart, lung, thyroid, and endothelial cells. As an intrinsic mediator of vascular inflammation, PIGF is responsible for promoting angiogenesis and destabilizing plaques through mediating macrophage accumulation in atherosclerotic lesions via Flt-1. In contrast, in one animal study, neutralization of PIGF reduced plaque size and the severity of macrophage infiltration. The combination of vascular inflammation and malnutrition, known as the malnutrition-inflammation-atherosclerosis syndrome, worsens outcomes in patients with CKD through aggravation of heart failure and accelerated atherosclerosis.

CKD not yet on dialysis. Therefore, the relationship between circulating PIGF and outcomes remains unclear. However, in our setting of CKD, we found for the first time, to our knowledge, an increased risk of both mortality and cardiovascular events with increasing PIGF levels, with patients in the highest quartile having a 3.87- and 8.42-fold increased risk compared with those in the lowest quartile, respectively. On the basis of these results, we suggest that PIGF is a useful and practical tool for cardiovascular risk stratification in patients with CKD, more so than in patients with heart failure or acute coronary syndrome. We hypothesize that PIGF signaling is involved in the pathogenesis of CKD-associated atherosclerosis. An inverse relationship between PIGF and eGFR in this and other studies support this hypothesis.

PIGF, originally discovered in the placenta, has also been found in the heart, lung, thyroid, and endothelial cells. As an intrinsic mediator of vascular inflammation, PIGF is responsible for promoting angiogenesis and destabilizing plaques through mediating macrophage accumulation in atherosclerotic lesions via Flt-1. In contrast, in one animal study, neutralization of PIGF reduced plaque size and the severity of macrophage infiltration. The combination of vascular inflammation and malnutrition, known as the malnutrition-inflammation-atherosclerosis syndrome, worsens outcomes in patients with CKD through aggravation of heart failure and accelerated atherosclerosis.

Indeed, in our patient population, PIGF was independently correlated with CKD severity, increased levels...
of C-reactive protein, and decreased levels of albumin and HDL. Taken together, PlGF may play a pivotal role in the pathogenesis of malnutrition-inflammation-atherosclerosis syndrome, which helps to explain the high mortality rates among patients with CKD.

In the VEGF family system, VEGF is another powerful ligand for Flt-1. However, in this study, VEGF was not correlated with CKD severity and was less associated with all-cause mortality and cardiovascular events, suggesting that PlGF could be a more important predictor of mortality and cardiovascular events in patients with CKD than VEGF and thereby clinically relevant to the pathophysiology of CKD-associated atherosclerosis.

The mechanisms underlying PlGF elevation in patients with CKD are largely unclear, but we have three hypotheses. First, our recent work demonstrated that exposure to serum from patients with advanced CKD increases PlGF production in human endothelial cells and is associated with increased endothelial dysfunction and oxidative stress. Circulating humoral factors in CKD, such as uremic toxins, may contribute to the upregulation of PlGF in CKD. Second, in addition to a circulating form in the peripheral blood, soluble Flt-1 is stored in the vascular wall and antagonizes PlGF. A marked decrease in sum of soluble Flt-1 is associated with lower eGFR, which may relatively increase circulating levels of PI GF and augment its biologic effects in CKD. Third, because the expression of PlGF in vascular cells is enhanced by angiotensin II and aldosterone, PlGF is thought to be a downstream target of the renin-angiotensin system (RAS) and mineralocorticoid receptor. In CKD, there is isolated or synergistic RAS and mineralocorticoid receptor hyperactivity, which is possibly mediated by upregulation of PI GF. Further clinical research is needed to investigate the association between PI GF levels and RAS.

Serum levels of PI GF in our patients were considerably lower than those in pregnant women, suggesting that abundant PI GF might be needed to develop and maintain the placental vascular system compared with the adult cardiovascular system. We think that the circulating level of PI GF is generally much lower than the tissue level of PI GF, but it is a surrogate marker of the tissue level of PI GF in patients with CKD, whereas PI GF itself may act as a paracrine factor and cause the development of atherosclerotic lesions.

Of note, patients with CKD with higher levels of PI GF had a greater risk of heart failure requiring hospitalization. Participants in the highest versus the lowest PI GF quartile appeared to have a higher HR for heart failure than for atherosclerotic disease. The use of PI GF alone and BNP alone had similar diagnostic accuracy for prediction of cardiovascular events, and the combination use of PI GF and BNP had higher diagnostic accuracy than either biomarker alone. A recent study has shown that PI GF is a strong predictor of an elevated left ventricular mass index in patients with CKD, suggesting that PI GF may be involved in the development of cardiac hypertrophy.

The present results suggest that PI GF can potentially serve as a novel therapeutic target for the treatment of CKD-associated cardiovascular disease. Indeed, we have observed that administration of soluble Flt-1 reduces atherosclerotic plaque areas in remnant kidneys in mice. In humans, aflibercept (VEGF Trap) and the anti-PI GF antibody TB-403, can be new antiangiogenic and antithrombotic drugs that antagonize PI GF signaling with already well established safety and tolerability. Further interventional trials of these agents are required to determine whether reducing serum PI GF levels provides cardioprotective effects in patients with CKD.

Several limitations of this study should be noted. First, it is an observational study; therefore, the cause-effect relationship between serum PI GF levels and adverse outcomes is not evident. Second, because the study participants are residents of a single Japanese prefecture, the results may not be generalizable to other populations. Third, the population enrolled in this study consists of patients with CKD who underwent renal biopsy or coronary angiography and may not be representative of all patients with CKD. Fourth, only baseline samples were analyzed and changes of circulating levels of PI GF over time were not addressed. Fifth, we

![Figure 3](https://www.jasn.org)
did not measure other biomarkers associated with mortality and cardiovascular events in CKD, such as troponin T and fibroblast growth factor-23. However, adjusting for levels of BNP, another biomarker of mortality and cardiovascular events, did not decrease the magnitude of the association between PlGF and mortality and cardiovascular events, respectively.

In conclusion, PlGF is strongly and independently associated with not only all-cause mortality but also cardiovascular events in patients with CKD, suggesting that PlGF is a novel prognostic biomarker in CKD. Further research is required to investigate the mechanisms underlying the effect of higher serum PlGF levels on adverse outcomes and determine whether therapeutic strategies that lower PlGF levels can improve outcomes in patients with CKD.

**CONCISE METHODS**

**Patients**

NARA-CKD is a multicenter prospective cohort study designed to investigate the prognostic value of PlGF as a risk factor of all-cause mortality and cardiovascular disease in patients with CKD. The study population consisted of consecutive patients who underwent renal biopsy or coronary angiography in our department at Nara Medical University Hospital and four referral hospitals between April 1, 2004, and December 31, 2011. We screened 1270 patients who underwent renal biopsy and 3003 patients who underwent the first examination of coronary angiography.

The exclusion criteria consisted of (1) previous or current acute coronary syndrome and heart failure within 3 months; (2) previous or current malignancy requiring therapy within

**Table 3. Sensitivity analysis**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>No. of Patients</th>
<th>No. of Events</th>
<th>HR (95% CI)*</th>
<th>No. of Events</th>
<th>HR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>1351</td>
<td>199</td>
<td>1.61 (1.29 to 1.62)</td>
<td>383</td>
<td>2.04 (1.82 to 2.27)</td>
</tr>
<tr>
<td>eGFR using the MDRD equation*</td>
<td>1191</td>
<td>156</td>
<td>1.74 (1.43 to 2.10)</td>
<td>287</td>
<td>2.04 (1.80 to 2.31)</td>
</tr>
<tr>
<td>eGFR using the CKD-EPI equation*</td>
<td>1191</td>
<td>156</td>
<td>1.74 (1.43 to 2.10)</td>
<td>287</td>
<td>2.04 (1.80 to 2.31)</td>
</tr>
<tr>
<td>Excluding very elderly participants</td>
<td>1210</td>
<td>172</td>
<td>1.71 (1.44 to 2.01)</td>
<td>332</td>
<td>2.11 (1.88 to 2.37)</td>
</tr>
<tr>
<td>Excluding dialysis and predialysis patients</td>
<td>1144</td>
<td>143</td>
<td>1.93 (1.56 to 2.36)</td>
<td>258</td>
<td>2.06 (1.80 to 2.36)</td>
</tr>
<tr>
<td>Follow-up of at least 2 yr</td>
<td>1159</td>
<td>172</td>
<td>1.65 (1.36 to 1.99)</td>
<td>325</td>
<td>2.08 (1.85 to 2.34)</td>
</tr>
<tr>
<td>Fully adjusted model including proteinuria*b</td>
<td>1209</td>
<td>160</td>
<td>1.80 (1.48 to 2.17)</td>
<td>296</td>
<td>2.09 (1.84 to 2.36)</td>
</tr>
<tr>
<td>Fully adjusted model including BNP*c</td>
<td>1175</td>
<td>189</td>
<td>1.53 (1.30 to 1.81)</td>
<td>372</td>
<td>1.97 (1.76 to 2.20)</td>
</tr>
<tr>
<td>Fully adjusted model including VEGF*d</td>
<td>1327</td>
<td>195</td>
<td>1.63 (1.38 to 1.91)</td>
<td>377</td>
<td>2.02 (1.80 to 2.25)</td>
</tr>
</tbody>
</table>

*a eGFR was substituted to CKD stage as an adjusted covariate.
*b Also adjusted for proteinuria when data were available.
*c Also adjusted for BNP when data were available.
*d Also adjusted for VEGF when data were available.

*Adjusted HRs for 10 pg/ml increase are shown. All categories were adjusted for age, sex, diabetes, hypertension, dyslipidemia, obesity, smoking, previous coronary artery disease, previous stroke, CKD stage, RAS blocker, calcium channel blocker, β-blocker, mineralocorticoid receptor antagonist, lipid-lowering agent, diuretic, antiplatelet agent, hemoglobin, serum albumin, serum calcium, serum phosphorus, serum C-reactive protein, HbA1c, LDL cholesterol, HDL cholesterol, and triglycerides.
Table 4. Risk of various outcomes by baseline VEGF level

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>c Statistics</th>
<th>P Value*</th>
<th>IDI</th>
<th>P Value</th>
<th>NRI</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Quartile 1 (&lt;160 pg/ml)</td>
<td>0.70 (0.65–0.74)</td>
<td>0.001</td>
<td>0.38 (0.29–0.47)</td>
<td>0.02</td>
<td>0.64 (0.54–0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quartile 2 (160–290 pg/ml)</td>
<td>0.78 (0.73–0.82)</td>
<td>0.001</td>
<td>0.43 (0.34–0.52)</td>
<td>0.02</td>
<td>0.70 (0.61–0.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quartile 3 (291–507 pg/ml)</td>
<td>0.70 (0.65–0.74)</td>
<td>0.001</td>
<td>0.41 (0.32–0.50)</td>
<td>0.02</td>
<td>0.69 (0.59–0.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quartile 4 (&gt;508 pg/ml)</td>
<td>0.73 (0.68–0.79)</td>
<td>0.001</td>
<td>0.40 (0.31–0.50)</td>
<td>0.02</td>
<td>0.66 (0.56–0.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>For each 100 pg/ml increase</td>
<td>0.72 (0.65–0.79)</td>
<td>0.001</td>
<td>0.39 (0.31–0.49)</td>
<td>0.02</td>
<td>0.63 (0.53–0.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (&lt;160 pg/ml)</td>
<td>0.70 (0.65–0.74)</td>
<td>0.001</td>
<td>0.40 (0.31–0.50)</td>
<td>0.02</td>
<td>0.64 (0.54–0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quartile 2 (160–290 pg/ml)</td>
<td>0.77 (0.74–0.81)</td>
<td>0.001</td>
<td>0.43 (0.34–0.52)</td>
<td>0.02</td>
<td>0.70 (0.61–0.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quartile 3 (291–507 pg/ml)</td>
<td>0.78 (0.73–0.79)</td>
<td>0.001</td>
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</tr>
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<td>0.001</td>
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<td>0.02</td>
<td>0.63 (0.53–0.74)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P value for c statistics.

the last 5 years; (3) AKI; (4) pregnancy; (5) age <18 years; and (6) unwillingness to participate. Among the 1270 patients who underwent renal biopsy, there were 476 patients excluded, including 247 because of AKI, 213 because of missing data, 10 because of malignancy, five because of pregnancy, and one because they were aged <18 years. There were 3003 patients who underwent coronary angiography, of which 2420 were excluded. These included 1014 patients with acute coronary syndrome, 959 with acute heart failure, and 447 who did not meet CKD criteria, defined as an eGFR <60 ml/min per 1.73 m² of body surface area or continuous proteinuria over 3 months (Figure 1).

For all eligible participants, we collected data on demographic characteristics, medical and family history, and current medications. A physical examination was performed at baseline, during which blood samples were collected for evaluation of laboratory parameters, including serum levels of PlGF. In total, 1377 enrolled participants completed the baseline examination, of whom 14 were lost to follow-up and 12 did not have sufficient sample volume for measurement, leaving 1351 patients for the analysis. Participants were followed until death or withdrawal from the study.

The institutional review board or ethics committee at each site approved the protocol, and all patients provided written informed consent.

Measurement

Serum samples were immediately centrifuged and were kept frozen at −80°C until assayed. Serum levels of PlGF and VEGF were measured with a commercially available sandwich ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions.

Clinical Definitions

The following clinical definitions were adopted. (1) eGFR was calculated using the MDRD equation and the CKD Epidemiology Collaboration (CKD-EPI) equation modified for Japan as previously described.42,43 (2) CKD stage was categorized according to a modified National Kidney Foundation classification system as follows: stage 1 (eGFR ≥90 ml/min per 1.73 m²); stage 2 (eGFR, 60–89 ml/min per 1.73 m²); stage 3a (eGFR, 45–59 ml/min per 1.73 m²); stage 3b (eGFR, 30–44 ml/min per 1.73 m²); stage 4 (eGFR, 15–29 ml/min per 1.73 m²); and stage 5 (eGFR <15 ml/min per 1.73 m²); and stage 5D
(on dialysis). (3) Hypertension was defined as systolic BP \( \geq 140 \) mmHg, diastolic BP \( \geq 90 \) mmHg, or use of oral antihypertensive medications. (4) Diabetes was defined as fasting glucose \( \geq 126 \) mg/dl or use of oral hypoglycemic agents or insulin. (5) Dyslipidemia was defined as LDL cholesterol \( \geq 140 \) mg/dl or use of lipid-lowering medications. (6) Obesity was defined as body mass index of \( \geq 25 \) kg/m\(^2\). (7) Smoking was defined as former or current smoking of more than one cigarette per day. (8) Previous coronary artery disease was defined as a history of myocardial infarction, angina pectoris, or coronary artery bypass grafting. (9) Previous stroke was defined as a history of cerebral infarction or hemorrhage not caused by trauma or infection. (10) Patients were diagnosed with proteinuria if urine dipstick test results were \( \geq 1+ \) for protein.

**Clinical Outcomes**

The primary outcomes were time to death from any cause and time to a cardiovascular event, defined as any atherosclerotic disease or heart failure requiring hospitalization. Atherosclerotic disease was a composite of cardiovascular death and nonfatal ischemic heart disease, stroke, aortic disease, and peripheral artery disease.

Cardiovascular death was defined as death because of due to myocardial infarction or stroke, or documented sudden cardiac death. Ischemic heart disease was defined as myocardial infarction or coronary artery disease with \( >75\% \) luminal narrowing in one of the three major coronary arteries or major branches requiring percutaneous coronary intervention or coronary artery bypass surgery. Stroke was defined as a new fixed neurologic deficit caused by cerebral infarction or hemorrhage not known to be caused by brain trauma, tumor, infection, or other identifiable causes. Cerebrovascular disease requiring surgical or percutaneous revascularization in the cerebrovascular circulation was also included. Aortic disease included aortic dissection or rupture not secondary to trauma or collagen disease. Peripheral artery disease was defined as amputation caused by vascular disease or peripheral surgical or percutaneous revascularization. Heart failure was defined according to Framingham criteria.44

All participants were followed for at least 1 year after enrollment. All events were confirmed through medical records and self-reporting. Participants who visited the hospital every 1–3 months reported on their general health and conditions, including cardiovascular events, to attending physicians blinded to serum PlGF levels. Any event of participants without regular hospital visits was obtained by blinded clinicians through an in-person or telephone interview.

**Statistical Analyses**

Baseline characteristics, risk factors for cardiovascular disease, current medications, and laboratory findings were compared across quartiles of serum PlGF levels using the chi-squared test for categorical data and the Kruskal–Wallis test for skewed continuous variables. Spearman’s rank correlation coefficient was used to assess the correlation between eGFR and PlGF and VEGF. Univariate and multivariate linear regression were used to identify clinical characteristics with an independent association with PlGF.

The effect of PlGF on all-cause mortality and cardiovascular events was estimated using crude Kaplan–Meier analysis according to PlGF quartiles, and differences were assessed with the log-rank test. A Cox hazard regression model was used to assess for unadjusted and adjusted associations between serum PlGF levels and clinical outcomes. The lowest quartile of PlGF levels was defined as the reference group.

Candidate variables for adjustment were chosen because of their close association with all-cause mortality or cardiovascular events. We initially adjusted for patient demographics, including age and sex, in model 1. Model 2 consisted of model 1 plus traditional risk factors, including diabetes, hypertension, dyslipidemia, smoking, obesity, previous coronary artery disease, previous stroke, and CKD stage. Model 3 consisted of model 2 plus current medications for preventing atherosclerosis and heart failure, including RAS blockers (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and direct renin inhibitors), calcium channel blockers, \( \beta \)-blockers, mineralocorticoid receptor antagonists, lipid-lowering agents, diuretics (loop and thiazide diuretics), and antiplatelet agents. Model 4 consisted of model 3 plus laboratory findings associated with cardiovascular events, including hemoglobin, serum albumin, serum C-reactive protein, serum calcium, serum phosphorus, glycated hemoglobin, LDL cholesterol, and HDL cholesterol. Model 5 consisted of model 4 plus plasma levels of BNP.

Because clinical demographics, traditional coronary risk factors, and reduced renal function are independent risk factors for death and atherosclerotic disease, we examined whether the association between PlGF levels and each outcome was similar in subgroups stratified by age, sex, diabetes, hypertension, dyslipidemia, smoking, obesity, and baseline renal function. Baseline renal function was categorized into three groups according to eGFR. Dialysis patients were included in the subgroup with eGFR<30 ml/min per 1.73 m\(^2\).

We further assessed the robustness of our results using sensitivity analysis. Multivariate-adjusted hazard analysis for all-cause mortality and cardiovascular events was repeated with the following five sensitivity analyses. The first sensitivity analysis used alternative measurements of renal function as adjusted covariates. The CKD-EPI equation more accurately categorizes mortality risk than the MDRD equation; therefore, CKD stage was replaced by eGFR estimated using both the MDRD equation and CKD-EPI equation. The second analysis did not include very elderly participants aged \( \geq 80 \), and the third analysis did not include participants who were receiving maintenance dialysis at baseline or began dialysis during the study period.

The fourth analysis was restricted to participants with at least 2 years of follow-up, and the fifth analysis included proteinuria and BNP when available, in the fully adjusted model as risk factors for death and cardiovascular events because both are established biomarkers of cardiovascular events in patients with CKD. The sixth analysis included VEGF, which is another angiogenic factor relevant with PlGF, when available, in the fully adjusted model.

To evaluate the discriminatory ability of joint effects of PlGF and BNP, we calculated the c statistic, IDI, and category-free NRI at 2-year follow-up in the subset of participants in whom plasma levels of BNP were measured. The differences between the c statistics were tested by Delong methods to examine whether combination use of PlGF and BNP improved the discrimination ability for study outcomes compared with either biomarker alone. Bootstrap CIs were reported for the area under the receiver–operating characteristics curves, IDI, and NRI. A two-sided \( P<0.05 \) was considered statistically significant.
JMP10.0.2 (SAS Institute Inc., Cary, NC) and SAS version 9.4 (SAS Institute Inc.) were used to perform all statistical analyses.

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DISCLOSURES

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