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The Association of Angiogenesis Markers With Acute Kidney Injury and Mortality After Cardiac Surgery

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Abstract

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TRIBE-AKI Consortium: For complete list of members of the consortium please refer to Parikh *et al*.[16]

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Rationale & Objective: The process of angiogenesis after kidney injury may determine recovery and long-term outcomes. We evaluated the association of angiogenesis markers with acute kidney injury (AKI) and mortality after cardiac surgery.

Study Design: Prospective cohort.

Setting & Participants: 1,444 adults undergoing cardiac surgery in the TRIBE-AKI (Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury) cohort.

Exposure(s): Plasma concentrations of two pro-angiogenic markers (vascular endothelial growth factor A [VEGF] and placental growth factor [PGF]) and one anti-angiogenic marker (soluble VEGF receptor 1 [VEGFR1]), measured pre- and postoperatively within six hours after surgery.

Outcome(s): AKI, long AKI duration (≥ 7 days), and one-year all-cause mortality Analytical Approach: Multivariable logistic regression.

Results: Following cardiac surgery, plasma VEGF concentrations decreased 2-fold, and PGF and VEGFR1 concentrations increased 1.5-fold and 8-fold, respectively. There were no meaningful associations of preoperative concentrations of angiogenic markers with outcomes of AKI and mortality. Higher postoperative VEGF and PGF concentrations were independently associated with lower odds of AKI (adjusted ORs of 0.89 [95% CI, 0.82–0.98] and 0.69 [95% CI, 0.55–0.87], respectively), long AKI duration (0.65 [95% CI, 0.49–0.87] and 0.48 [95% CI, 0.28–0.82], respectively), and mortality (0.74 [95% CI, 0.62–0.89] and 0.46 [95% CI, 0.31–0.68], respectively). In contrast, higher postoperative VEGFR1 concentrations were independently associated with higher odds of AKI (1.56; 95% CI, 1.31–1.87), longer AKI duration (1.75; 95% CI, 1.09–2.82), and mortality (2.28; 95% CI, 1.61–3.22).

Limitations: Angiogenesis markers were not measured after hospital discharge so we are unable to determine long-term trajectories of angiogenesis marker levels during recovery and follow-up.

Conclusions: Higher postoperative pro-angiogenic markers, VEGF and PGF, were associated with lower AKI and mortality risk, whereas higher postoperative anti-angiogenic VEGFR1 was associated with higher risk of AKI and mortality.

Keywords

acute kidney injury (AKI); AKI duration; mortality; cardiac surgery; angiogenesis; biomarker; vascular endothelial growth factor A (VEGF); VEGF-A; placental growth factor (PGF); soluble VEGF receptor 1 (VEGFR1); cytokine; pro-angiogenic growth factor

Introduction

Acute kidney injury (AKI) complicates about 5–10% of all hospital admissions and up to 30% of hospitalizations for cardiac surgery.[1,2] Longer AKI duration and greater AKI severity are independently associated with increased mortality.[3,4] Following the initial insult of AKI, biological processes either guide recovery or progression of disease: some lead to adaptive repair and regeneration of tubules, while others lead to maladaptive repair and fibrosis, which result in replacement of the kidney interstitium with connective tissue.[5] The severity and duration of AKI are likely a consequence of the severity of the initial injury and the consequential opposing biological processes of adaptive and maladaptive repair.

Understanding the dominant biological pathways that guide kidney recovery and repair may open new avenues for diagnostic assessment and potential therapeutic interventions. As the kidney is a highly vascularized organ, angiogenesis, the process of formation of new blood vessels, is believed to be a critical mechanism that determines kidney recovery after AKI.[6] In fact, preclinical models of AKI have demonstrated that enhanced angiogenesis preserves peritubular capillaries, attenuates tubulointerstitial fibrosis, and restores kidney function following injury.[7–10] There is also evidence of peritubular capillary loss in progressive kidney disease in humans, and the degree of peritubular capillary loss correlates with severity of interstitial fibrosis.[11] However, the process of angiogenesis has not been well-characterized in human AKI.

We conducted an ancillary study of TRIBE-AKI (Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury) to understand the association of angiogenic growth factors with the development and duration of AKI and one-year mortality after cardiac surgery. We measured plasma levels of three angiogenic markers: vascular endothelial growth factor A (VEGF, also known as VEGF-A), placental growth factor (PGF), and soluble VEGF receptor 1 (VEGFR1, also known as sFlt-1), before and after cardiac surgery. VEGF is a prototypical pro-angiogenic mediator that plays a critical role in maintenance of peritubular capillaries and promotion of endothelial survival.[12,13] Sharing a similar genetic sequence, PGF is another central proangiogenic cytokine that stimulates neovascularization.[14] Both VEGF and PGF potentiate their effects via the membrane-bound receptor VEGFR1, and, accordingly, the soluble form of VEGFR1 serves as an anti-angiogenic modulator, sequestering and reducing the bioavailability of both VEGF and PGF.[15] To confirm that the perioperative plasma changes of these markers correspond to kidney-specific changes, we also evaluated angiogenic marker expression in human AKI kidney biopsy samples and non-AKI controls. We hypothesized that pro-angiogenic growth factors (VEGF and PGF) would be associated with adaptive repair and favorable outcomes, whereas the anti-angiogenic growth factor (VEGFR1) would be associated with maladaptive repair and adverse outcomes in the perioperative setting.

Methods

Study Design and Participants of TRIBE-AKI

The TRIBE-AKI cardiac surgery cohort has been described in detail previously (Figure S1).[16–23] We prospectively enrolled 1444 adults undergoing cardiac surgery (coronary artery bypass grafting [CABG] or valve surgery), who were at high-risk for AKI at six academic medical centers in North America between July 2007 and December 2010. Plasma samples were collected at three time points on all participants: preoperative, postoperative day 1 (within 6 hours after surgery), and postoperative day 2 (corresponding to the first day after surgery). We collected preoperative characteristics, operative details, and postoperative complications using definitions of the Society of Thoracic Surgeons.[24,25] We estimated preoperative GFR using the CKD Epidemiology Collaboration (CKD-EPI) equation.[26] Ethical approval for our study and protocols was obtained from each of the Institutional Review Boards at the respective participating sites. All participants provided written informed consent.

Angiogenic Marker Measurement

After collection, plasma samples were centrifuged at 2000×g for 10 minutes at 4°C, separated into 1 ml aliquots, and immediately stored at −80°C until markers were measured. VEGF (VEGF-A isoform), PGF, and VEGFR1 were measured using the Meso Scale Discovery platform (Meso Scale Diagnostics, Gaithersburg, MD), which uses electrochemiluminescence detection combined with patterned arrays.

Twenty percent of samples were measured in duplicate. The inter-assay coefficient of variation (CV) for VEGF was 4%–5% with a detection range of 0.96 – 3940 pg/mL, and 9 (2%) samples were below the lower limit of detection. The inter-assay CV for PGF was 6%, with a detection range of 1.7 – 6920 pg/mL, and no samples were above or below the limits of detection. The inter-assay CV for VEGFR1 was 5%–6%, with a detection range of 3.9 – 16,340 pg/mL, and 1 (0.2%) sample was above the upper limit of detection. There were weak correlations between PGF, VEGF, and VEGFR1 levels and duration of storage with Pearson's correlation coefficients of −0.05 (p=0.08), −0.12 (p <0.01), and −0.03 (p=0.2), respectively. Two preoperative marker samples were missing due to inadequate sample volume for measurement.

Outcome Definitions

There were three primary outcomes for our study. The first was the development of AKI, defined as ≥0.3 mg/dL increase or ≥50% increase in serum creatinine from baseline preoperative level to postoperative level at any time point during the hospital stay. Preoperative creatinine values were measured within 2 months before surgery (median: 3 [IQR, 0–14] days). Postoperative creatinine was measured as part of clinical care, soon after surgery and at least once every day during hospitalization.

The second outcome was duration of AKI, defined by the number of days participants maintained a ≥0.3 mg/dL increase or ≥50% increase in serum creatinine during hospitalization. Patients without AKI were excluded from the analysis of AKI duration; hence, the sample size was only 492 participants. AKI duration was dichotomized into short duration of <7 days or long duration of ≥7 days based on prior literature showing a strong association between ≥7 days of AKI and long-term mortality.[3,27]

The third outcome was all-cause mortality within the first year following surgery. We obtained vital status after discharge through various mechanisms (and cross-referenced when possible). For those living in the United States, we performed phone calls to patients' homes, searched the National Death Index, reviewed hospital records, and examined linkages with Center for Medicare and Medicaid Services (CMS) databases. For Canadian participants (those enrolled into the TRIBE-AKI study in London, Ontario), we also performed phone calls and analyzed data from the Registered Persons Database held at the Institute for Clinical Evaluative Sciences (ICES) to acquire vital status. These datasets were linked using unique, encoded identifiers and analyzed at ICES. There were 20 participants who died in the hospital and were included in the analysis, however, there were 36 participants with missing data for mortality and were excluded from the analysis.

Statistical Analyses

Descriptive statistics for continuous variables were reported as mean (standard deviation) or median (interquartile range) and for categorical variables as frequencies (%). The primary analyses used baseline and postoperative day 1 marker levels because they capture the extremes of marker levels, and, most importantly, have utility in early detection of AKI.

As no acceptable clinical cut-offs are available, we evaluated the association between angiogenic markers and outcomes both as continuous (natural log (ln)-transformed marker levels) and categorical (tertiles) variables. To evaluate the association between each angiogenic marker and all dichotomous outcomes, we used logistic regression models. For the outcome of AKI duration, we also considered it as a continuous outcome and used Poisson regression to analyze the association between angiogenesis markers and AKI duration. Furthermore, sensitivity analysis using stages of AKI was performed to highlight the association between angiogenesis markers and different severities of AKI. Angiogenic marker values below and above the limit of detection (<1%) were imputed with the lower or upper limit of detection, respectively.[28] Pearson correlations were performed among the three angiogenic markers at the preoperative and postoperative day 1 time points.

Both univariable and multivariable analyses were evaluated. Multivariable analyses were adjusted for the following variables: age, gender, race, cardiopulmonary bypass (CPB) time >120 minutes, non-elective surgery, type of surgery (Coronary Artery Bypass Graft [CABG] or valve surgery), preoperative eGFR, diabetes, hypertension, heart failure, myocardial infarction, preoperative urine albumin-creatinine ratio, center, and corresponding preoperative marker when evaluating the associations between postoperative markers and outcomes. We adjusted for change in serum creatinine from preoperative baseline level to the corresponding angiogenic marker measurement when evaluating the association between postoperative markers and the outcome of mortality. We did not adjust for change in serum creatinine with the outcome of AKI, as change in serum creatinine is used to define AKI outcomes. To assess storage bias, we performed Pearson correlations between angiogenesis markers levels and duration of storage.

The performance of the individual and combined angiogenesis panel as well as combined panel plus clinical model was evaluated with the use of AUC. To assess statistically significant changes between AUCs, we performed a logistic regression model adjusting for all clinical covariates in the association between markers of angiogenesis and outcomes. Net reclassification improvement (NRI) was also calculated for the addition of the three angiogenesis markers to the clinical panel.

All analyses were two-tailed, and p-values less than 0.05 were considered significant. Small cell counts are only presented for data collected by TRIBE-AKI and not from ICES data holdings. For the latter, counts of 5 or fewer participants are suppressed to minimize the risk of participant re-identification. Analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC) and R 2.15.0 (R Foundation for Statistical Computing, Vienna Austria).

Immunofluorescence Staining and Quantification

Paraffin-embedded tissue was obtained from human tissue without AKI following right radical nephrectomy, and AKI tissue was obtained at time of kidney procurement from brain-dead deceased donors. Kidney biopsy tissue from non-AKI samples was deparaffinized by xylene following hydration by ethanol. Kidney sections were kept in citrate buffer for antigen retrieval in a 600-watt microwave for 10 minutes, and kidney sections were blocked with 3% bovine serum albumin in phosphate-buffered saline. Immunostaining was performed with primary antibodies [anti-mouse megalin (Millipore)], anti-rabbit VEGF (Santa Cruz Biotechnology, sc-152), and anti-rabbit VEGFR1 (Novus Biologicals, NB100–57643) overnight at 4°C, followed by appropriate Alexa Fluor 488– and Alexa Fluor 594–conjugated secondary antibodies, washed with PBS, and mounted with Slowfade (Invitrogen). We used the anti-megalin 1:200 dilution, anti-VEGF 1:100 dilution, anti-VEGFR1 1:100 dilution, Alex Fluor 488 1:200 dilution, and Alex Fluor 595 1:200 dilution. Images were acquired by Andor CSU-WDi spinning disc confocal microscope equipped with Nikon Ti-E Cfi Plan Apo Lambda 60x oil immersion objective for immunofluorescence analysis, and images were processed using NIH Image J software (version 1.51H) or Adobe Photoshop CS 2014. We measured fluorescence intensity of VEGF, VEGFR1, and proximal tubular area (megalin-positive tubular area) using Image J on four AKI tissue samples and two samples of non-AKI tissue. We then calculated fluorescence intensity of VEGF and VEGFR1 divided by the tubular area. Prior to immunostaining, quantitative PCR analysis was performed on non-AKI tissue to assess the expression of angiogenic markers. Compared to VEGF levels, quantitative PCR analysis showed greater than 250-fold reduction of PGF expression in non-AKI tissue (data not shown), so PGF staining in non-AKI and AKI tissue was not performed as the levels would be extremely low in tissue for staining.

Results

Perioperative trends of angiogenic markers in TRIBE-AKI

Among the 1,444 adults who underwent cardiac surgery in the TRIBE-AKI cohort, the mean age was 72 years, and 69% were men (Table 1). Most surgeries were elective (84%) with a mean preoperative estimated GFR (eGFR) of 68 ml/min/1.73 m^2. Serum creatinine-based AKI developed in 492 (34%) participants. Participants reached the definition of AKI at a median of 2 (IQR, 1–3) days post-cardiac surgery. Of those with AKI, 41 (8%) had long duration of AKI (≥7 days). After one-year of follow-up, 81 (6%) participants died.

Each angiogenic marker had a distinct trajectory after cardiac surgery (Figures 1a and 1b). VEGF concentrations decreased 2-fold on postoperative day 1 but approached preoperative levels on day 2. PGF concentrations increased 1.5-fold on day 1 and remained elevated to a similar extent on day 2. Lastly, VEGFR1 concentrations increased 8-fold on day 1 and decreased to a 2-fold increase relative to preoperative levels on day 2.

We next compared marker concentrations from the first postoperative sample in patients with and without AKI, long AKI duration, and mortality, respectively (Table 2). Participants who developed adverse outcomes had *lower* postoperative concentrations of *pro*-angiogenic

markers VEGF and PGF, compared with those who did not develop adverse outcomes for all three primary outcomes of AKI, long duration of AKI, and mortality. While VEGF concentrations on average decreased following surgery, VEGF declined to a larger extent among participants who developed adverse outcomes (AKI, longer duration of AKI, and death), compared with those who did not. In contrast, participants who developed adverse outcomes had *higher* postoperative concentrations of the *anti*-angiogenic marker VEGFR1, compared with those who did not develop adverse outcomes.

Correlations between pre- and postoperative angiogenic markers were modest (Table S1), with the exception of a moderate positive correlation between postoperative VEGF and PGF ($r=0.70$; $p<0.05$) and a moderate negative correlation between postoperative VEGF and VEGFR1 ($r=-0.53$; $p<0.05$).

Associations of angiogenic markers with outcomes

After multivariable regression, higher postoperative VEGF concentrations were independently associated with lower risk of adverse outcomes (Figure 2 and Table 3**)**. Similarly, postoperative PGF concentrations were independently associated with lower risk of adverse outcomes with 31% lower odds of AKI, 52% lower odds of long duration of AKI, and 54% lower odds of mortality (Table 3). In contrast, higher postoperative VEGFR1 concentrations were independently associated with higher risk for each outcome. Preoperatively, VEGFR1 and PGF concentrations were not significantly associated with any of the outcomes (Table S2). Only preoperative VEGF concentrations were weakly associated with development of postoperative AKI. The results were similar when marker levels were examined in tertiles for all outcomes (Tables S3, S4, and S5), when restricting the outcome of AKI to stages 2 and 3 (Table 4), and when evaluating the outcome of AKI duration as a continuous outcome, without a pre-specified cut-off of 7 days (Table S6). There were no significant interactions between levels of angiogenic markers and clinical AKI for the outcome of mortality. There were no strong associations of preoperative cardio-protective medications with AKI and angiogenesis markers as shown in Tables S7, and S8.

Performance of Combined Postoperative Angiogenesis Panel

The AUCs of the combination of the three postoperative angiogenic markers outperformed those of the individual postoperative markers for the outcomes of AKI, long duration of AKI, and all-cause mortality (Table S9**)**. When added to the clinical model, the combined postoperative angiogenesis marker panel significantly improved the AUCs for AKI to 0.72 (95% CI, 0.69–0.75), for long duration of AKI to 0.88 (95% CI, 0.84–0.92), and for all-cause one-year mortality to 0.74 (95% CI, 0.69–0.80) (Figure 3). The changes in AUCs between the clinical model and the clinical plus combined angiogenesis panel were statistically significant for all outcomes. Furthermore, the NRIs for the addition of angiogenesis markers to the clinical model were 0.24 (95% CI, 0.13–0.34), 0.55 (95% CI, 0.27–0.84), and 0.38 (95% CI, 0.15–0.61) for AKI, long duration of AKI and mortality, respectively.

Angiogenic marker staining in human kidney tissue

To evaluate the kidney specificity of the observed changes in the angiogenic markers, we examined human AKI kidney tissue and human non-AKI tissue for these markers with immunofluorescence staining. Preliminary quantitative polymerase chain reaction analysis showed very low expression of PGF in non-AKI tissue, and thus, we only stained for VEGF and VEGFR1 in human kidney tissue samples. There was a significant difference in immunoreactivity between non-AKI and AKI kidney tissues for both VEGF and VEGFR1. Proximal tubular staining of VEGF was absent in the setting of AKI compared with non-AKI controls, whereas substantial tubular VEGFR1 staining in AKI tissue samples compared with non-AKI controls was observed (Figure S2a). Quantification of immunofluorescence staining of four samples of AKI tissue and two samples of non-AKI tissues showed significantly increased VEGF expression in non-AKI compared with AKI tissue; in contrast, VEGFR1 expression was significantly increased in AKI tissue compared with non-AKI tissue (Figure S2b).

Discussion

In this study, we explored the association of plasma angiogenic markers VEGF, PGF, and VEGFR1 with the development and duration of AKI as well as one-year mortality after cardiac surgery. Consistent with our hypothesis and the underlying physiology of angiogenesis in repair following acute ischemic insults, we found that higher pro-angiogenic marker concentrations measured within 6 hours after cardiac surgery were associated with favorable outcomes, whereas elevated anti-angiogenic marker concentrations were associated with adverse outcomes. The impact of the respective adaptive and maladaptive repair potential captured by these markers was observed during perioperative follow-up of participants by associations with the development and duration of AKI and with long-term implications demonstrated by association with mortality at 1 year. Furthermore, the addition of all three angiogenesis markers to the clinical model substantially improved the prediction of long duration of AKI and mortality. While a growing number of injury markers have been studied in the setting of AKI, our study demonstrates the potential prognostic ability of repair angiogenic growth factors in a large-scale clinical setting.

Recovery from injury is highly dependent on the repair pathways that are activated at the time of injury. Our group has described the role of several injury proteins in the setting of cardiac surgery that contribute to in-hospital outcomes, as well as long-term mortality.[29–31] However, these proteins do not fully explain the prognosis and severity of patient outcomes after cardiac surgery. Hence, further understanding of angiogenesis after injury via the measurement of pro- and anti-angiogenic markers may help elucidate prognosis and patient outcomes after cardiac surgery. Furthermore, the perioperative plasma changes in these markers corresponded to changes in kidney tissue following AKI, in which VEGF protein expression decreased, and VEGFR1 expression substantially increased. This suggests that VEGF and VEGFR1 may play roles as kidney-specific markers following injury, given that the distinct trajectory patterns were validated through quantification of immunostaining in four independent AKI tissue samples.

The angiogenic response post-injury, as captured by these angiogenic markers, constitutes early crucial steps in the complex multiple biological pathways involved in progressive kidney injury.[32,33] Identifying the role of angiogenesis in this post-injury phase may help delineate the boundaries between reversible and irreversible kidney injury and offer a window for therapeutic interventions when kidney function may still be salvageable. Interestingly, the reduction in VEGF levels post-injury is in stark contrast to the trajectory of injury and inflammatory markers, which routinely increase in the setting of injury. Yet, despite these reductions following surgery, participants with higher postoperative VEGF concentrations had lower risk of AKI, long duration of AKI, and mortality.

VEGF is an endothelial specific growth factor that promotes angiogenesis and participates in interstitial matrix modeling.[12,13] It has been shown to increase significantly in cells exposed to hypoxia but decrease once the hypoxic insult is prolonged, suggesting biphasic regulation of VEGF dependent on the duration of the insult.[34,35] To our knowledge, our study is first to assess the association of VEGF in adults with AKI after cardiac surgery. Curiously, plasma VEGF levels decreased in all groups after surgery; however, this observation is consistent with a recent study reporting that VEGF decreases in response to hypoxia *in vivo* and that *in vivo* VEGF activity may be distinct from those *in vitro*.[36]

Higher VEGF concentrations have been shown to be associated with higher risk of progression to ESRD in patients with diabetic nephropathy.[37] Similarly, higher PGF concentrations were found to be present in CKD patients and to be independently associated with cardiovascular events and all-cause mortality in this population.[38] In contrast to these findings, we found higher VEGF and PGF after cardiac surgery to be associated with lower odds of development and long duration of AKI as well as lower odds of mortality. We postulate that in the setting of acute injury, pro-angiogenic factors such as VEGF and PGF are necessary for reparative processes of angiogenesis; however, in the setting of chronic disease such as diabetic nephropathy and CKD, long-term, unimpeded angiogenesis may lead to fibrosis and irreversible damage.[37] Perhaps this may be the underlying mechanism explaining why higher preoperative VEGF levels in the quiescent, pre-injury state were associated with higher risk of AKI, while higher postoperative levels were associated with decreased risk of AKI. It is also possible that VEGF plays different roles in angiogenesis based on the activation of other biological markers, and further studies evaluating the role of VEGF in the setting of other novel markers are needed.[39]

Although not previously identified as a reparative mediator in response to kidney injury, PGF has been shown to play a crucial role in angiogenesis and has specifically been studied in preeclampsia.[40,41] We found that PGF, similar to VEGF, is associated with favorable renal outcomes and increased survival post-cardiac surgery and is positively correlated with VEGF. VEGFR1 has also been implicated as an inhibitor of angiogenesis and has also been studied in the setting of preeclampsia.[41] Consistent with the underlying biology of VEGFR1 as a competitive inhibitor of VEGF and PGF, we found that higher VEGFR1 was associated with increased odds of AKI, longer AKI duration, and mortality post-cardiac surgery. Notably, preoperative VEGF, PGF and VEGFR1 concentrations significantly differed between participants with and without AKI at baseline. Although this is likely attributed to the large sample size detecting small non-clinically significant differences, we have taken

caution to adjust for preoperative levels when evaluating the associations between the postoperative marker levels and outcomes. The findings from our study may help highlight biological pathways that can be targets for future therapies aiming to treat AKI post-cardiac surgery. Additionally, patients at high risk for adverse outcomes post-cardiac surgery may be prioritized for inclusion in future intervention trials of AKI to improve efficiency and reduce cost. Furthermore, if our results are validated, angiogenesis markers can be used to risk stratify sicker patients for closer clinical follow up post-cardiac surgery.

This study also has limitations that should be considered. The strength of inference testing is limited due to lack of adjustment for multiple comparisons in the context of several completed and planned analyses from the TRIBE cohort. However, this study is an ancillary study from the TRIBE cohort, with an *a priori* hypothesis of the three angiogenesis markers presented. The generalizability of our data is limited to cardiac surgery patients and further research is needed to assess if these associations between angiogenesis markers and outcomes exist outside of the cardiac surgery setting. Furthermore, as in our previous studies, our cohort is predominantly of European ancestry and at high-risk for AKI following cardiac surgery, and thus these results may not be readily generalizable to diverse populations. We acknowledge that our definition of AKI is limited, as it does not include the 48-hour time period or urine output criteria as defined by the Acute Kidney Injury Network (AKIN) classification.[42] This may have led to the inclusion of less severe AKI cases with normal urine output; however, we conducted a sensitivity analysis showing outcomes with different stages of AKI (Table 4). In addition, the baseline serum creatinine used to define AKI was up to 2 months before surgery. Although there is no standard definition of optimal baseline serum creatinine, an ideal measurement would be most reflective of participant's baseline, which may require clinical adjudication.[43] However, the mean serum creatinine value assessed within a year before hospital admission approximated clinical adjudication of baseline serum creatinine.[44]

There may be unmeasured confounding related to inflammatory and post-surgical responses, which may further affect the association between angiogenic markers and the development of AKI and mortality. Because our study protocol did not capture angiogenesis marker measurements after discharge, we could not determine the long-term trajectories of angiogenesis marker levels during follow-up. Logistically, plasma samples were stored for several years prior to measurement for this ancillary study, which may introduce storage bias due to degradation. However, this bias is likely minimal as we found only weak correlations between angiogenesis marker levels and duration of storage.[45,46] Furthermore, these specific markers and assays have been measured in plasma stored for a decade in another population at risk for cardiac injury, thus suggesting clinical utility and mitigating stability issues.[47] As few of the TRIBE participants were off-pump (9%), we could not evaluate whether the use of cardiopulmonary bypass modified the association of angiogenic markers and our specified outcomes. Prior literature has shown an increase in VEGF levels for on-pump versus off-pump cardiac surgery patients.[48] Lastly, the AKI kidney tissue for immunofluorescence staining was obtained from deceased donors who may have distinct clinical events leading up to AKI that differ from cardiac surgery patients. The staining of these biomarkers does not necessarily confirm kidney specificity, as filtration and back leakage into tubular cells may also explain the presence of these markers in kidney tissue. This phenomenon is less

likely with VEGFR1, however, given its higher molecular weight of about 180 kDa versus VEGF, which is about 46 kDa.[49,50]

In conclusion, we have demonstrated that growth factors involved in angiogenesis are associated with AKI, long AKI duration, and mortality post-cardiac surgery. We found higher postoperative levels of the pro-angiogenic growth factors VEGF and PGF are independently associated with shorter duration of kidney injury and decreased mortality, while higher postoperative levels of the anti-angiogenic mediator VEGFR1 are independently associated with longer kidney injury and increased mortality. Further studies are needed to both validate our findings as well as assess generalizability in non-cardiac surgery settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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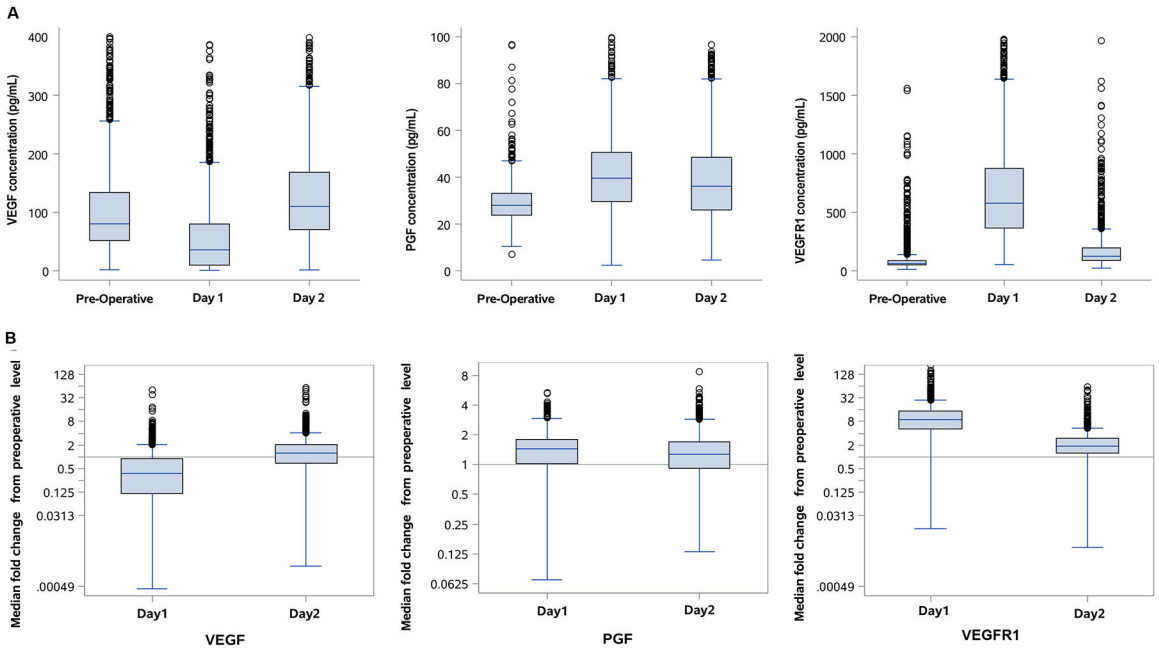

Figure 1.

A. Boxplots of vascular endothelial growth factor (VEGF), plasma placental growth factor (PGF), and VEGF receptor 1(VEGFR1) concentrations are presented at preoperative time point and on postoperative days 1 and 2. Each angiogenic marker had a distinct trajectory after cardiac surgery. VEGF initially decreased postoperatively on day 1 and approached baseline on day 2. Both PGF and VEGFR1 continued to increase postoperatively on day 1 and day 2. Each box represents the interquartile range (IQR), the horizontal lines represent the median, and the lower and upper whiskers represent the 5th and 95th percentile, respectively. For the VEGF boxplot, 123 values (400–2545) were excluded from visual presentation. For the PGF boxplot, 25 values (100–158) were excluded from visual presentation. For the VEGFR1 boxplot, 98 values (2000–16340) were excluded from visual presentation. All comparisons had a p-value <0.001

B. Boxplots of median fold change in angiogenic marker levels from preoperative baseline levels. VEGF concentrations decreased 2-fold on postoperative day 1, but approached preoperative levels on day 2. PGF concentrations increased 1.5-fold on day 1 and remained elevated to a similar extent on day 2. VEGFR1 concentrations increased 8-fold on day 1 and decreased to a 2-fold increase relative to preoperative levels on day 2. All comparisons had a p-value <0.001.

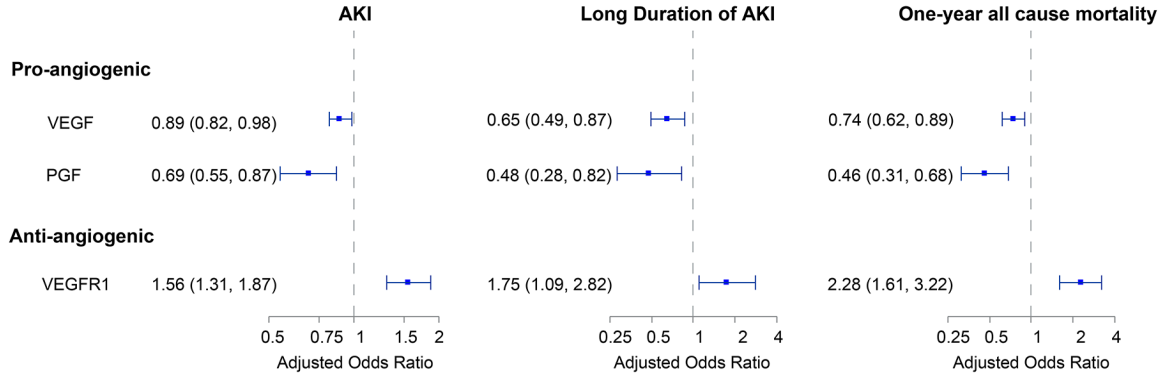

Figure 2.

Forest plots summarizing the associations of plasma vascular endothelial growth factor (VEGF), placental growth factor (PGF), and VEGF receptor 1 (VEGFR1) with the outcomes of AKI, long AKI duration, and one-year all-cause mortality after cardiac surgery. For the outcomes of AKI and AKI duration ≥ 7 day, odds ratios (ORs) are adjusted for age (years), gender, white race, CPB time >120 minutes, non-elective surgery, surgery type, preoperative eGFR, diabetes, hypertension, congestive heart failure, myocardial infarction, preoperative urine albumin-creatinine ratio, site, and corresponding preoperative marker. For the outcome of mortality, ORs are adjusted for the above covariates, as well as change in serum creatinine from the preoperative level to postoperative day 1 level. Postoperative VEGF concentrations were independently associated with lower risk of adverse outcomes with 11% lower odds of AKI, 35% lower odds of long duration of AKI, and 26% lower odds of mortality. Postoperative PGF concentrations were independently associated with lower risk of adverse outcomes with 31% lower odds of AKI, 52% lower odds of long duration of AKI, and 54% lower odds of mortality. In contrast, higher postoperative VEGFR1 concentrations were independently associated with higher risk for each outcome with 56% higher odds of AKI, 75% higher odds of long duration of AKI, and about 2 times higher odds of mortality.

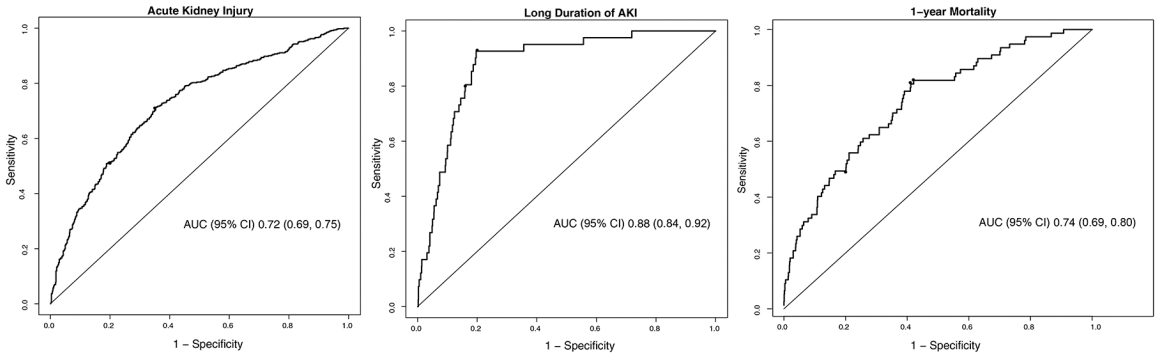

Figure 3.

Receiver operating characteristic (ROC) curves showing the performance of the combined angiogenesis panel plus the clinical model for the outcomes of acute kidney injury (AKI), long duration of AKI and one-year mortality. When added to the clinical model, the combined postoperative angiogenesis marker panel significantly improved the area under the curve (AUCs) for AKI to 0.72 (95% CI, 0.69–0.75), for long duration of AKI to 0.88 (95% CI, 0.84–0.92) and for all-cause mortality to 0.74 (95% CI, 0.69–0.80). The changes in AUCs between the clinical model and the clinical plus combined angiogenesis panel were statistically significant for all outcomes. The clinical model included age, gender, race, cardiopulmonary bypass time >120 minutes, non-elective surgery, type of surgery (coronary artery bypass graft or valve surgery), preoperative eGFR, diabetes, hypertension, heart failure, myocardial infarction, preoperative urine albumin to creatinine ratio, and change in serum creatinine.

Table 1.

Patient characteristics by acute kidney injury status

Characteristic	Acute Kidney Injury**	
	No (n=952)	Yes (n=492)
Age, years	72 (10)	72 (9)
Sex, female	310 (33%)	137 (28%)
White race	895 (94%)	463 (94%)
Diabetes	340 (36%)	214 (43%)
Hypertension	739 (78%)	412 (84%)
EF <35% or grade 3 or 4 LV function	86 (9%)	55 (11%)
Myocardial infarction*	246 (26%)	126 (26%)
Congestive heart failure	178 (19%)	147 (30%)
Preoperative serum creatinine, mg/dl	1.0 (0.3)	1.1 (0.4)
Preoperative Egfr	70 (19)	65 (20)
CKD stage 3 or higher	285 (30%)	201 (41%)
Albuminuria (UACR >30 mg/g)*	40 (4%)	44 (9%)
Surgery type*		
CABG and valve	201 (21%)	138 (28%)
CABG or valve	750 (79%)	354 (72%)
Perfusion time, minutes*	106 (51)	129 (65)
Cardiopulmonary bypass (on-pump)	852 (90%)	452 (92%)
CPB duration >120 minutes*	285 (30%)	242 (49%)
Cross-clamp time, minutes*	73 (39)	89 (47)
One-year all-cause mortality	32 (4%)	49 (10%)

Above values are represented as mean (standard deviation) or n(%).

AKI, acute kidney injury; EF, ejection fraction; LV, left ventricle; eGFR, estimated glomerular filtration rate calculated using CKD-EPI equation; CKD, chronic kidney disease; UACR, urinary albumin-creatinine ratio; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; ICU, intensive care unit.

The standardized mean differences for each row are Age 0, Female sex 11, White Race 0, Diabetes −14, Hypertension −15, EF <35% or grade 3 or 4 LV function −7, Myocardial Infarction 0, Congestive heart failure −26, Preoperative serum creatinine −29, Preoperative eGFR 26,CKD stage 3 or higher −23, Albuminuria −20, CABG and valve −16, CABG or valve 16, Perfusion time −40, Cardiopulmonary bypass (on-pump) −7, CPB duration >120 −40, Cross clamp −37, and One year all-cause mortality −24, respectively.

* Indicates missing values for the specified variable; myocardial infarction has 19 missing values, surgery type has 1 missing value, albuminuria has 16 missing values, perfusion time has 60 missing values, cardiopulmonary bypass time has 3 missing values, cross-clamp time has 62 missing values, and length of ICU stay has 1 missing value.

** 0.3 mg/dl or 50% rise in serum creatinine

Table 2.

Angiogenic marker concentrations by AKI, long AKI duration, and one-year all-cause mortality

Outcome	Pro-angiogenic								Anti-angiogenic: VEGFR1 (pg/mL)			
	VEGF (pg/mL)				PGF (pg/mL)							
	Pre-op		Post-op		Pre-op		Post-op		Pre-op		Post-op	
AKI												
AKI (n=492)	89 (55, 156)	*	28 (5, 77)	*	29 (25, 34)	*	38 (26, 50)	*	70 (54, 95)	*	725 (454, 1200)	*
No AKI (n=952)	82 (52, 139)		40 (13, 83)		28 (24, 33)		41 (32, 51)		62 (50, 81)		560 (349, 854)	
AKI Duration												
≥7 days (n=41)	89 (60, 146)		9 (3, 23)	*	28 (24, 33)		26 (17, 38)	**	72 (52, 105)		1120 (754, 1693)	**
<7 days (n=451)	89 (55, 160)		33 (6, 82)		29 (25, 34)		38 (27, 51)		69 (54, 94)		696 (447, 1184)	
Mortality												
Non-survivors (n=81)	91 (52, 156)		13 (4, 45)	*	30 (24, 34)		31 (18, 43)	**	71 (57, 107)	**	820 (521, 1400)	**
Survivors (n=1327)	83 (53, 142)		37 (10, 82)		28 (24,33)		40 (30, 51)		63 (51, 86)		601 (371, 954)	

Data are presented as median (interquartile range).

All postoperative marker values are significantly different from preoperative values (Wilcoxon sign rank test).

*Denotes markers that are significantly different between those with and without the outcome at a given time point (Wilcoxon rank sum test).

Table 3.

Association of postoperative angiogenic markers with AKI, AKI duration ≥ 7 days, and one-year all-cause mortality

Outcome			
	Pro-angiogenic		**Anti-angiogenic**
	VEGF (pg/mL)	**PGF (pg/mL)**	**VEGFR1 (pg/mL)**
AKI			
Unadjusted	0.84 (0.78–0.90)	0.62 (0.51–0.75)	1.64 (1.42–1.91)
Adjusted[1]	0.89 (0.82–0.98)	0.69 (0.55–0.87)	1.56 (1.31, 1.87)
AKI duration ≥ 7 days			
Unadjusted	0.65 (0.52–0.81)	0.51 (0.34–0.78)	1.72 (1.18–2.50)
Adjusted[1]	0.65 (0.49–0.87)	0.48 (0.28–0.82)	1.75 (1.09–2.82)
Mortality			
Unadjusted	0.72 (0.62–0.84)	0.44 (0.32–0.60)	1.90 (1.45–2.48)
Adjusted[1,2]	0.74 (0.62–0.89)	0.46 (0.31–0.68)	2.28 (1.61–3.22)

Note: values expressed as Odds Ratio (95% CI) per 1-unit greater of natural log-transformed marker concentration.

[1]Adjusted for clinical covariates including: age (years), gender, white race, CPB time >120 minutes, non-elective surgery, surgery type, preoperative eGFR, diabetes, hypertension, congestive heart failure, myocardial infarction, preoperative urine albumin to creatinine ratio, site, and corresponding preoperative marker.

[2]Mortality adjusted model also adjusted for change in serum creatinine from preoperative level to day 1 postoperative level.

Table 4.

Association of angiogenesis markers with mild AKI and severe AKI

Outcome			
	Pro-angiogenic		Anti-angiogenic
	VEGF (pg/mL)	PGF (pg/mL)	VEGFR1 (pg/mL)
Stage 1 AKI (n=432) Unadjusted Adjusted[1]	0.87 (0.8, 0.94) 0.93 (0.85, 1.02)	0.69 (0.56, 0.85) 0.77 (0.61, 0.99)	1.56 (1.33, 1.82) 1.49 (1.24, 1.8)
Stage 2 and 3 AKI (n=60) Unadjusted Adjusted[1]	0.61 (0.51, 0.74) 0.65 (0.52, 0.8)	0.32 (0.22, 0.46) 0.36 (0.23, 0.57)	2.47 (1.79, 3.4) 2.26 (1.52, 3.37)

Note: values expressed as Odds Ratio (95% CI) per 1-unit greater of natural log-transformed marker concentration. no AKI group (n=952) was the reference group.

[1]Adjusted for clinical covariates including: age (years), gender, white race, CPB time >120 minutes, non-elective surgery, surgery type, preoperative eGFR, diabetes, hypertension, congestive heart failure, myocardial infarction, preoperative urine albumin to creatinine ratio, site, and corresponding preoperative marker