Systemic Profile of Cytokines in Arteriovenous Fistula Patients and Their Associations with Maturation Failure

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Key Points
- Seven of 74 cytokines profiled were elevated in patients with AVF maturation failure compared with participants with successful maturation.
- G-CSF, MDC, RANTES, SDF-1, and TGFα demonstrated significant incremental associations with AVF maturation failure by logistic regression.
- This investigation may open the doors for future therapeutics and markers for risk stratification.

Abstract
Background Systemic cytokines are elevated in patients with chronic kidney disease (CKD) and on hemodialysis compared with the general population. However, whether cytokine levels interfere with vascular remodeling, increasing the risk of arteriovenous fistula (AVF) failure, remains unknown.

Methods This is a case-control study of 64 patients who underwent surgery for AVF creation (32 with AVF maturation failure and 32 matching controls with successful maturation). A total of 74 cytokines, including chemokines, interferons, interleukins, and growth factors, were measured in preoperative plasma samples using multiplex assays. Sixty-two patients were included in the statistical analyses. Associations with AVF failure were assessed using paired comparisons and conditional logistic regressions accounting for paired strata.

Results Seven cytokines were significantly higher in patients with AVF maturation failure than in matching controls (G-CSF, IL-6, MDC, RANTES, SDF-1α/β, TGFα, and TPO). Of these, G-CSF (odds ratio [OR]=1.71; 95% confidence interval [95% CI], 1.05 to 2.79 per 10 pg/ml), MDC (OR=1.60, 95% CI, 1.08 to 2.38 per 100 pg/ml), RANTES (OR=1.55, 95% CI, 1.10 to 2.17 per 100 pg/ml), SDF-1α/β (OR=1.18, 95% CI, 1.04 to 1.33 per 1000 pg/ml), and TGFα (OR=1.39, 95% CI 1.003, 1.92 per 1 pg/ml) showed an incremental association by logistic regression.

Conclusions This study identified a profile of plasma cytokines associated with adverse maturation outcomes in AVFs. These findings may open the doors for future therapeutics and markers for risk stratification.

Introduction
CKD and hemodialysis treatments are associated with a heightened state of systemic inflammation (1,2). Plasma levels of various cytokines and growth factors are known to influence vascular function and postoperative remodeling (3–8). However, whether these components of the systemic inflammatory milieu modify venous remodeling after arteriovenous fistula (AVF) creation and contribute to access failure remains largely unknown.

There are multiple sources of inflammation that lead to elevated cytokines in patients with CKD and on hemodialysis. These include central venous catheters and dialysis itself (2,9,10), underlying comorbidities and autonomic imbalance (11,12), increased gut permeability and dysbiosis (13–15), and symptomatic or subclinical infections of various etiologies (16,17). Additional triggers include cellular senescence, oxidative stress, insulin resistance, fluid and sodium overload, hypoxia, metabolic acidosis, and bone mineral disorders (18–24). This proinflammatory state is further aggravated by insufficient clearance of uremic toxins and cytokines by both the kidneys and hemodialysis treatments (25–28). The relationship between CKD and systemic inflammation was further demonstrated by the association between lower eGFR and higher cystatin C and albuminuria.

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with increasing circulating levels of IL-6, TNFα, high-sensitivity C-reactive protein (hs-CRP), serum albumin, and fibrinogen (29,30). A high proinflammatory cytokine profile (high in IL-1, IL-6, and TNFα; low in IL-2, IL-4, IL-5, IL-12, total complement, and T-cell counts) was also associated with lower survival in patients on hemodialysis (31).

The vasculature is a direct target of systemic inflammation. Systemic cytokines and other inflammatory elements underlie endothelial dysfunction (32), synthetic transformation of smooth-muscle cells (SMCs) (33–36), fibroblast activation (37,38), and vascular immune cell infiltration (39,40). Clinical studies have demonstrated significant associations between low albumin and cholesterol levels, and high CRP and fibrinogen with AVF failure (41–44), but these associations have not remained significant in meta-analyses (45). Recently, elevated panel reactive antibodies, as a measure of immune system reactivity, have been found associated with AVF nonmaturation (46). Animal AVF models also support a role for IL-6, monocyte chemoattractant protein-1, and regulated on activation, normal T cell expressed and secreted (RANTES) protein in intimal thickening and reduced outward remodeling (47–50). However, whether specific cytokines and growth factors are associated with human AVF outcomes remains unknown.

In this study, we evaluate the relationship between systemic cytokines and AVF maturation failure. Our findings may open the doors to new therapeutic approaches, personalized medicine, and improved risk stratification and vascular access planning in patients on hemodialysis.

**Materials and Methods**

**Study Design**

This was a case-control study to analyze the associations between plasma cytokines and AVF maturation outcomes. Patients with CKD aged ≥21 years and scheduled for AVF creation surgery at Jackson Memorial Hospital and University of Miami (UM) Hospital from February 2018 to March 2020 were invited to participate in the study. Following our surgical protocol, we used the most peripheral vein with an internal luminal diameter ≥3.5 mm, the brachial artery if ≥4 mm, or the radial artery if ≥2.5 mm (51). Blood was collected from 96 consented individuals at the time of AVF creation using EDTA Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and centrifuged at 2000 g for 15 minutes at 4°C for plasma isolation (Figure 1). Plasma samples were stored at −80°C. All patients with anatomic AVF maturation failure were retrospectively selected (N=32) from the cohort. Anatomic maturation failure was defined as an AVF that never achieved an internal luminal diameter ≥6 mm as determined during postsurgical follow-up and/or transposition surgeries (51). Blood was collected from 96 consented individuals at the time of AVF creation using EDTA Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and centrifuged at 2000 g for 15 minutes at 4°C for plasma isolation (Figure 1). Plasma samples were stored at −80°C. All patients with anatomic AVF maturation failure were retrospectively selected (N=32) from the cohort. Anatomic maturation failure was defined as an AVF that never achieved an internal luminal diameter ≥6 mm as determined during postsurgical follow-up and/or transposition surgeries (51). All AVF that failed required endovascular and/or surgical salvage procedures or ligation. An equal number of patients with successful maturation was retrospectively selected from the cohort using propensity score matching on the basis of patient age, sex, ethnicity, diabetes, and predialysis status. One-to-one matches were generated by applying a greedy algorithm and Mahalanobis distance. The study was performed according to the ethical principles of the Declaration of Helsinki and regulatory requirements at Jackson Memorial Hospital and UM. The ethics committee and UM Institutional Review Board approved the study.

**Multiplex Cytokine/Chemokine and hs-CRP Assays**

Undiluted plasma samples were profiled using the 71-plex Human Cytokine/Chemokine Discovery Assay (catalog no. HD71) and the 3-plex TGFβ Array (catalog no. TGFβ1–3) by Eve Technologies Corporation (Calgary, Canada). For the purposes of statistical analysis, values below the quantification limit (QL) were assigned the minimum
Study Population
Demographics, Clinical, and AVF Characteristics of the Results

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The preoperative plasma levels of 74 cytokines were quantified in a case-control study of 64 patients who underwent surgery for AVF creation (32 with AVF maturation failure and 32 matching controls with successful maturation; Figure 1). One pair of patients was excluded from the statistical analyses due to undetectable cytokine levels in 64 out of 74 tests in one of the samples. The baseline characteristics of the remaining 31 pairs are included in Table 1. Patients in both the failed and matured subgroups had a mean age of 58–59 years, 35%–45% were women, and they were predominantly of Hispanic (45%–48%) and Black ancestry (35%–39%). Comorbidities were similarly represented in both subgroups, including hypertension (87%–100%), diabetes (58%–65%), congestive heart failure (19%–26%), atherosclerotic cardiovascular disease (42%–45%), hyperlipidemia (45%–61%), and history of smoking (32%–45%). Both study arms also had similar distributions of body mass index (P=0.82), fasting plasma glucose (P=0.59), white blood cell count (P=0.27), and hs-CRP (P=0.35).

Thirteen percent of the patients were still in predialysis, whereas 13%–16% had a history of a previous AVF. Dialysis vintage was similar between the groups (P=0.76). In addition, at least 89%–93% of those already on hemodialysis and followed by our team used either the Optiflux

Table 1. Baseline characteristics of the study cohort

| Characteristics | Matured (N=31) | Failed (N=31) |
|-----------------|---------------|--------------|
| Demographics   |               |              |
| Age, yr, mean±SD | 58.16±12.37  | 59.06±13.30  |
| Women, n (%)    | 14 (45)       | 14 (45)      |
| Hispanic, n (%) | 15 (48)       | 14 (45)      |
| Black, n (%)    | 12 (39)       | 12 (39)      |
| White, n (%)    | 5 (16)        | 5 (16)       |
| Comorbidities and laboratory values |       |              |
| Hypertension, n (%) | 31 (100)   | 27 (87)      |
| Diabetes, n (%)  | 20 (65)       | 18 (58)      |
| CHF, %          | 6 (19)        | 6 (19)       |
| ASCVD, n (%)     | 14 (45)       | 13 (42)      |
| Hyperlipidemia, n (%) | 19 (61) | 14 (45) |
| History of smoking, n (%) | 10 (32) | 14 (45) |
| BMI, kg/m², mean±SD | 27.64±5.21  | 27.35±5.36  |
| WBC count, 10³/μl, mean±SD | 7.62±2.63 | 6.92±2.58  |
| Fasting plasma glucose, mg/dl, median (IQR) | 109 (89.8–175.5) | 129.5 (88.3–173) |
| hs-CRP, mg/L, median (IQR) | 6.82 (1.49–13.55) | 15.68 (4.29–31.42) |
| Vascular access and HD history |       |              |
| Predialysis, n (%) | 4 (13)       | 4 (13)       |
| Previous AVF, n (%) | 5 (16)       | 4 (13)       |
| HD vintage, mo, median (IQR) | 4.27 (2.40–6.46) | 4.60 (2.83–7.82) |
| High-flux dialyzer use, n (%)a | 25/27 (93) | 24/27 (89) |
| AVF features |               |              |
| BC, n (%)       | 10 (32)       | 11 (35)      |
| BB, n (%)       | 13 (42)       | 13 (42)      |
| BBr, n (%)      | 2 (6)         | 3 (10)       |
| Other anastomosis, n (%)b | 6 (19) | 4 (13) |
| Left arm, n (%) | 27 (87)       | 23 (74)      |
| Preaccess vein diameter, mm, median (IQR) | 4 (4–4) | 4 (4–4) |

ASCVD, atherosclerotic cardiovascular disease; AVF, arteriovenous fistula; BB, brachiobasilic; BBr, brachiobrachial; BC, brachiocephalic; BMI, body mass index; CHF, congestive heart failure; HD, hemodialysis; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; WBC, white blood cell. ASCVD was defined as having a history of coronary artery disease, peripheral arterial disease, transient ischemic attack, or stroke.

Patients with confirmed use of high-flux (Optiflux F160NR/F180NR) dialyzer out of 27.

Other types of AVF include aberrant radiobasilic (n=2), aberrant radiocephalic (n=1), aberrant ulnar-cephalic (n=1), brachio-cephalic (n=1), proximal antecubital radiocephalic (n=1), radiocephalic (n=2), and radio-median cubital (n=1).

Statistical Analyses
Statistical analyses were performed using GraphPad Prism v8.4 (GraphPad Software, San Diego, CA) and the “survival” package in R (52). Statistical differences between the failure and maturation groups were assessed using Wilcoxon matched pairs signed rank tests and paired t tests. The Benjamini–Hochberg method (also known as FDR) was used to correct for multiple testing (53). An FDR<0.1 was considered statistically significant. Odds ratios (OR) were calculated using conditional logistic regressions accounting for paired strata.

Results
Demographics, Clinical, and AVF Characteristics of the Study Population

The preoperative plasma levels of 74 cytokines were quantified by ELISA (catalog no. RAB0096–1KT; Sigma–Aldrich, St. Louis, MO) in 1:20,000 diluted plasma according to the manufacturer’s recommendations.

Quantification limit value. High-sensitivity CRP was measured in 64 out of 74 tests in one of the samples. The baseline levels were assessed using Wilcoxon matched pairs signed rank tests and paired t tests. In addition, at least 89%–93% of those already on hemodialysis and followed by our team used either the Optiflux

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A

B

Interleukins

Chemokines

Growth Factors

and Others

Figure 2. | Preoperative profile of plasma cytokines in AVF patients. (A) Distribution of cytokine levels in 62 ESKD patients. Bars indicate the median plasma concentration. (B) Spearman’s correlation of 74 cytokines and high-sensitivity C-reactive protein (blue arrow). The legend indicates the correlation coefficient.

F160NR or F180NR high-flux dialyzers. The types of AVF

created and the diameter of the native vein selected for sur-

gery were also similar in both arms of the study (Table 1).

Lastly, none of the study participants were on hemodia-

filtration at the time of AVF creation or during the period of

maturation.

Preoperative Cytokines Levels and Association with

AVF Failure

Plasma cytokine levels in the patient population ranged

from ≤1 pg/ml (IL-2, IL-4, IL-10, vascular endothelial
growth factor A) to ≥10,000 pg/ml (MIG/CXCL9, PDGF-

AB/BB, SDF-1α/β, TGFβ1; Figure 2A, Supplemental Table

1). For the most part, there were no significant correlations

between cytokines in circulation or with respect to hs-CRP

(Figure 2B), suggesting different cell sources, clinical char-

acteristics, or disease processes responsible for their eleva-

tion. Exceptions included a cluster of growth factors

(PDGF-AA, PDGF-AB/BB, TGFβ1, and TGFβ2) and small

groups of interleukins and other cytokines (sCD40L and

ENA-78; IL-3 and IL-4; IL-1β, IL-17A, and IFN-α2; IL-13,

TNFβ, and monocyte chemoattractant protein-3; IL-2 and

IFN-γ). An extreme outlier was detected in 27 out of 74

tests (Grubb’s test alpha = 0.0001; Figure 1A), and this pair

was removed from the corresponding linear regression

models and comparative analyses.

Seven cytokines were significantly higher in individuals

with AVF maturation failure than in matching controls

(Figure 3A, Supplemental Table 1), including highly abun-

dant factors with median levels ≥500 pg/ml (MDC,

RANTES, and SDF-1α/β). Five cytokines (G-CSF, MDC,

RANTES, SDF-1α/β, and TGFα) also demonstrated an

incremental association by logistic regression (Table 2),

which remained significant after adjusting for hs-CRP and
dialysis vintage, with the exception of TGFα. This suggests

that the higher these factors are found in blood, the higher

the likelihood of AVF maturation failure.

Interestingly, higher plasma levels of G-CSF were

significantly associated with women (β coefficient 0.430

[95% confidence interval 0.140 to 0.720]), smoking history

(0.359 [0.066 to 0.651]), and predialysis status (0.433 [0.155
to 0.710]; Figure 3B). The latter was also associated with

increased IL-6 (0.335 [0.053 to 0.618]) and MDC (0.343

[0.052 to 0.635]). Increased thrombopoietin (TPO) levels

were associated with smoking history (0.323 [0.044 to

0.602]) but negatively associated with age (−0.363 [−0.638 to

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were associated with smoking history (0.323 [0.044 to

0.602]) but negatively associated with age (−0.363 [−0.638 to
likely reflecting reduced liver function in older individuals. Lastly, higher levels of both RANTES (0.355 \([0.062 to 0.647]\)) and SDF-1\(\alpha/b\) (0.346 \([0.050 to 0.642]\)) were significantly associated with dialysis vintage. The lack of significant correlations between these cytokines (Figure 2B) and the different effects of baseline covariates on their plasma concentrations (Figure 3B) highlight the complexity of clinical factors in the hemodialysis population and the multifactorial mechanisms that may contribute to AVF failure.

Discussion

Systemic inflammation is considered a catalyst for postoperative vascular complications. Various inflammatory cytokines have shown significant associations with vascular proliferative processes such as restenosis after percutaneous coronary interventions and vein graft failure (3,4,54). Therefore, it is plausible to hypothesize a positive relationship between systemic cytokine concentrations and increased risk of AVF failure. Herein, we identified a group of circulating cytokines with preoperative levels significantly elevated in patients with AVF maturation failure (G-CSF, IL-6, MDC, RANTES, SDF-1\(\alpha/b\), TGF\(\alpha\), and TPO). Out of these, G-CSF, MDC, RANTES, SDF-1\(\alpha/b\), and TGF\(\alpha\) also showed an incremental association in logistic regressions (i.e., the higher the cytokine level, the higher the risk of failure).

Failure of the AVF happens secondary to a combination of wall fibrosis and excessive intimal expansion (55). However, the mechanisms that initiate or contribute to maladaptive remodeling early after AVF creation remain unknown (56). We have found cytokines associated with AVF failure that have been previously implicated in adverse vascular remodeling. IL-6, for instance, activates endothelial cells (ECs) and fibroblasts, induces SMC proliferation and migration, and enhances immune cell infiltration (57). PBMCs from patients with AVF failure produce more IL-6 than dialysis age-matched controls (58). Interestingly, activation of the IL-6 receptor in ECs of the adventitial neovasculature is commonly found in stenotic AVFs (58). TGF\(\alpha\), in turn, is proangiogenic and serves as a transducer of mechanosensitive NF\(\kappa\)B proinflammatory...
signaling in response to high blood pressure (59–61). G-CSF increases proliferation and migration of ECs and SMCs, inhibits nitric oxide signaling, and is chemotactic for immune cells (8,62,63). There is also increasing evidence of SMCs, inhibits nitric oxide signaling, and is chemotactic for G-CSF increases proliferation and migration of ECs and immune cells (8,62,63). There is also increasing evidence of SMCs, inhibits nitric oxide signaling, and is chemotactic for G-CSF increases proliferation and migration of ECs and immune cells (8,62,63). There is also increasing evidence of SMCs, inhibits nitric oxide signaling, and is chemotactic for G-CSF increases proliferation and migration of ECs and immune cells (8,62,63). There is also increasing evidence of SMCs, inhibits nitric oxide signaling, and is chemotactic for G-CSF increases proliferation and migration of ECs and immune cells (8,62,63).

Despite the lack of correlation between the plasma levels of all of these factors, they share potential vascular effects in common such as immune cell infiltration, neovascularization, and platelet aggregation. The role of immune cell infiltration in early AVF remodeling is not clear at the moment (56). However, an exaggerated response to injury may affect the proper healing of the vascular wall. Adventitial vascularization increases significantly after access creation (79), but how much it contributes to wall remodeling is also an open question. Animal studies support a role for hypoxia-driven angiogenic signaling in intimal expansion (80,81), but prominent vascularization of the intima is not evident in human two-stage AVFs (79). Nonetheless, excessive vascularization early after surgery (and that resolves later on) may be linked to myofibroblast activation and fibrotic remodeling as a response to oxidative stress. Early thrombosis is a relatively infrequent complication (2%–5%) after AVF creation surgery, but it can occur in association with small vein diameter, stenosis, and poor intraoperative thrill (82,83).

The sources and triggers of these circulating factors are not clear at the moment because many of them are ubiquitously produced by hematopoietic and nonhematopoietic cells (58,59,70,76,84–86). Immune cell phenotyping studies are needed to assess activation of leukocyte populations

Table 2. Associations of plasma cytokines with AVF maturation failure by logistic regression

| Cytokine     | Odds Ratio (Confidence Interval) | Odds Ratio (Confidence Interval) | Main Cellular Sources | Potential Vascular Effects |
|--------------|----------------------------------|----------------------------------|-----------------------|---------------------------|
| G-CSF        | 1.71 (1.05 to 2.79)              | 1.64 (1.02 to 2.64)              | Monocytes/macrophages, bone marrow stromal cells, endothelial cells, fibroblasts | Hematopoietic stem cell mobilization, endothelial cell activation, proangiogenic, prothrombotic |
| IL-6         | 1.15 (0.98 to 1.36)              | 1.14 (0.96 to 1.36)              | Monocytes/macrophages, endothelial cells, smooth muscle cells, fibroblasts | Proinflammatory, immune cell recruitment, endothelial cell activation and dysfunction, proinflammatory |
| MDC          | 1.60 (1.08 to 2.38)              | 1.76 (1.12 to 2.78)              | Dendritic cells, macrophages, NK cells, B cells, T cells | Proinflammatory, platelet aggregation, immune cell recruitment |
| RANTES       | 1.55 (1.10 to 2.17)              | 1.45 (1.03 to 2.06)              | T cells, macrophages, platelets, adipocytes | Proinflammatory, proangiogenic, immune cell recruitment, SMC proliferation and synthetic switch |
| SDF-1α/β     | 1.18 (1.04 to 1.33)              | 1.31 (1.07 to 1.60)              | Bone marrow stromal cells, monocytes, liver | Progenitor cell mobilization, proangiogenic, platelet aggregation, proinflammatory, SMC proliferation and chemotaxis |
| TGFα         | 1.39 (1.003 to 1.92)             | 1.34 (0.98 to 1.83)              | Monocytes/macrophages, epithelial cells | Proangiogenic, endothelial cell migration, mechanosensitive signaling |
| TPO          | 1.10 (0.97 to 1.25)              | 1.08 (0.96 to 1.22)              | Liver, kidney | Platelet priming, proangiogenic |

CI, confidence interval; G-CSF, granulocyte colony-stimulating factor; IL-6, interleukin 6; MDC, macrophage-derived chemokine; OR, odds ratio; RANTES, regulated on activation, normal T cell expressed and secreted; SDF-1α/β, stromal cell-derived factor 1 alpha and beta; TGFα, transforming growth factor alpha; TPO, thrombopoietin.

aORs and CIs indicate increased risk per 1 pg/ml increment of IL-6 and TGFα; 10 pg/ml increment of G-CSF; 100 pg/ml increment of MDC, RANTES, and TPO; and 1000 pg/ml increment of SDF-1α/β.

bAdjusted by high-sensitivity C-reactive protein and hemodialysis vintage.

cReferences for cellular sources and vascular effects are cited in the Discussion.
Disclosures
L.H. Salman reports research funding from Albany Medical Center, Roach funds, and Transonic, Inc.; a patent application for the use of 4-methylumbelliferone in diabetic kidney disease; and other interests/relationships with the American Society of Nephrology, Data Safety Monitoring Board—Phraxis, and the Renal Physican Association. R.I. Vazquez-Padron reports being a scientific advisor for Kidney360 and Scientific Reports, and has other interests/relationships with the National Institutes of Health and American Heart Association study sections. All remaining authors have nothing to disclose.

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