ORIGINAL RESEARCH

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Vascular progenitor recruitment in critically ill patients with acute kidney injury

Abstract

Purpose: Endothelial-like vascular progenitor cells (VPCs) are blood-derived angiogenic precursors that can facilitate vascular repair. The mobilization of peripheral blood VPCs and their role in recovery were investigated in patients with acute kidney injury (AKI) in the intensive care unit (ICU) setting.

Methods: Blood samples were drawn on days 0, 3, 7 and 14 in 38 patients admitted to ICU: 30 with AKI and in eight controls with normal renal function. Circulating VPC levels were quantified by the early outgrowth cell cluster-forming assay and/or by flow cytometry.

Results: AKI patients (16 males, mean age 62.4) were classified as Risk (R, n=5), Injury (I, n=11) and Failure (F, n=14) according to the RIFLE criteria. VPC clusters increased over time following the diagnosis of AKI ($p < 0.01$ for day 0 *vs.* day 14) while VPC clusters were higher at enrollment in control patients and decreased over time ($p = 0.02$). Greater mobilization of VPCs occurred in patients with more severe AKI at enrollment (I and F categories compared with R, $p = 0.05$). A trend towards greater mobilization of VPC clusters was observed in patients with improved renal function ($p = 0.07$).

Conclusion: Time-dependent increases in circulating VPCs occur in critically ill patients with established AKI. Greater mobilization of VPCs may be associated with recovery of renal function, suggesting a potential role for VPCs in repair after kidney injury.

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Acute kidney injury (AKI) represents a significant clinical problem, affecting up to 5% of hospitalized patients, and is associated with a mortality rate of ~50% [1]. Pathophysiological processes contributing to AKI are complex, and include vascular injury, inflammatory responses, oxidative stress and tubular damage [2-4]. In the critical care setting, most cases of established AKI are secondary to prolonged hypotension or occur in the presence of sepsis [5]. Microvascular blood flow can be altered by inflammatory mediators, contributing to acute tubular necrosis (ATN). In these cases, recovery of renal function is dependent upon restoration of blood flow and repair of injured endothelial cells [6], as well as regeneration of functional nephrons and proliferation of tubular epithelial cells of the cortex and outer medulla. To date, the treatment of ATN has been largely supportive, and includes renal replacement with dialysis until sufficient recovery of renal function occurs. Unfortunately, treatments to enhance regeneration of nephron segments and pharmacologic interventions focused on improving renal hemodynamics have not demonstrated a significant benefit. Recognizing that mortality rates for AKI remain high and are unchanged over the past several decades, there remains a critical need for novel approaches to improve the rate of functional renal recovery.

Endothelial-like vascular progenitor cells (VPCs) were first described by Asahara *et al.* as CD34+ human circulating cells expressing endothelial cell characteristics [7]. Using an *in vitro* cell culture assay under angiogenic conditions, VPC clusters can be readily enumerated from peripheral blood samples [8]. VPC clusters correlate with important clinical outcomes in vascular health, including decreased probability of myocardial infarction [9] and stroke [10]. Vascular precursors and angiogenic progenitors with varying immunophenotypes and biological characteristics appear to contribute to angiogenesis and organ repair in both animal models and human studies of ischemic injury [8,11,12]. Further, altered endothelial function has been implicated in the development of multiple organ failure [13] and increased circulating levels of VPCs have been observed in critically ill patients with acute lung injury [14] and sepsis [15] and correlated with improved survival.

With regards to renal injury, vascular progenitors appear to home and incorporate into sites of active neovascularization in the kidney [16,17]. Patschan *et al.* showed that in mice with renal ischemia, VPCs are rapidly mobilized. They initially home to the spleen, and subsequently accumulate in the medullopapillary region of the kidney [18]. Intravenous injection of these angiogenic cells into mice with acute renal ischemia is associated with partial protection from injury. In athymic nude

rats with ischemic renal injury, Brodsky *et al.* demonstrated that intravenous infusion of human umbilical vein endothelial cells caused an improvement in renal function and renal capillary blood flow [19]. Although it appears that blood-derived vascular progenitors may play a role in improving kidney function after ischemic or nephrotoxic injury in experimental models, the role of VPCs in modulating renal function in humans in the aftermath of AKI has not been examined.

In this study, the mobilization of VPCs following AKI was examined in a prospective cohort of 30 critically ill patients to test the hypothesis that VPC mobilization represents an adaptive response involved in renal endothelial cell repair and thus would be associated with improvement of renal and global functioning.

Methods

Clinical Research Protocol

We enrolled 30 patients (> 18 years of age) with AKI who were admitted to the intensive care unit (ICU) at a large tertiary care hospital in Canada (The Ottawa Hospital, Ottawa, Ontario, Canada). The control population consisted of eight patients with normal renal function enrolled within 48 hours of admission to ICU. Renal function was determined by serum creatinine concentration and calculation of estimated glomerular filtration rate (eGFR) [20]. Patients were enrolled within 48 hours of developing AKI and following correction of any hypovolemia (mean arterial pressure > 65 mm Hg, central venous pressure > 8 mm Hg, and agreement from the treating physician that adequate fluid resuscitation had been administered to correct any hypovolemia). All patients or patient delegates provided informed consent prior to enrollment, and the study protocol was approved by The Ottawa Hospital Research Ethics Board. Blood samples were drawn (two 6 mLs BD Vacutainer spray-coated K₂EDTA tubes) at the time of enrollment (time 0) and at 3, 7 and 14 days afterwards for analysis of VPC levels (described below), complete blood counts, and serum biochemistry. Blood samples were drawn at those time points as long as patients were in the hospital and blood sampling ceased upon discharge from the hospital. Subjects were excluded if they were pregnant, not expected to survive beyond 48 hours, if AKI was attributed to urinary tract obstruction, if there was an episode of AKI in the eight weeks prior to enrollment, if the white blood count was < 3.0 x 10⁹/L, if patients had received chemotherapy in the 30 days prior to enrollment, if patients had received hematopoietic cytokines in the 14 days prior to enrollment, or if there was a history of bone marrow dysfunction such as hematological malignancy or myeloprolif-

TABLE 1. RIFLE criteria for evaluating renal injury.

Class	Glomerular filtration rate criteria	Urine output criteria
Risk	Increase in serum Cr $\geq 1.5\times$ baseline or decrease in GFR $\geq 25\%$	<0.5 ml/kg/hour x 6 hours
Injury	Increase in serum Cr $\geq 2.0\times$ baseline or decrease in GFR $\geq 50\%$	<0.5 ml/kg/hour x 12 hours
Failure	Increase in serum Cr $\geq 3.0\times$ baseline or decrease in GFR $\geq 75\%$ or an absolute serum creatinine ≥ 354 $\mu\text{mol/L}$ with an acute increase of ≥ 44 $\mu\text{mol/L}$	<0.3 ml/kg/hour x 24 hours or anuria x 12 hours

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erative disorder. Baseline serum creatinine was estimated from laboratory records, obtained from results reported within 12 months of admission to the ICU. For patients for whom a previous serum creatinine level was not available, baseline serum creatinine was calculated using the Modification of Diet in Renal Disease (MDRD) equation [20], assuming a baseline eGFR of 75 ml/min/1.73 m².

Patients were assigned to categories of AKI using the risk, injury, failure, loss of kidney function and end-stage kidney disease (RIFLE) criteria at each timepoint [1,21] (see Table 1). The requirement for persistent renal dysfunction precluded us from assigning any patients to the L or E categories. For some analyses, patients were divided into low risk (RIFLE score Risk) and high risk (RIFLE score Injury or Failure) categories, and further stratified based on presence or absence of improvement in renal function. Improvement was defined as any decrease in the severity of AKI by RIFLE score at any of the follow-up time points in comparison with the category at enrollment without subsequent reclassification to a worse category at later time points. Global recovery was assessed in a similar manner using clinical SOFA (Sepsis Related Organ Failure Assessment) scores [22]. Global improvement was defined as any decrease in the number of organs with a SOFA score ≥ 2 at any of the follow-up time points in comparison to the number of organs with a SOFA score ≥ 2 at enrollment. Moreover, patients categorized with global improvement did not have any subsequent increase in the number of organs with a SOFA score ≥ 2 at subsequent time points. An analysis was also performed using modified SOFA scores that were calculated without the renal component to assess for non-renal global recovery.

The primary objective of this study is to describe the VPC response in critically ill patients with AKI. The secondary objectives are to examine the association between VPC response and global improvement and recovery of renal function, as well as study the effects of plasma EPO levels on VPC mobilization, in critically ill patients with AKI.

VPC Culture Assay

VPCs were evaluated by cell culture assay using an adaptation of the method of Hill *et al.* [23]. In brief, 5×10^6 mononuclear cells from fresh peripheral blood samples were plated into a 9.6-cm² well of a fibronectin-coated plastic tissue culture dish (BioCoat; BD Biosciences, Bedford, MA, USA) in Endocult media (Stem Cell Technologies, Vancouver, BC, Canada) and incubated at 37°C in room air with 5% CO₂ and greater than 85% humidity. After 48 hours, nonadherent cells were removed, washed, and replated at 1.0×10^6 cells per 2.0 cm² well in fibronectin-coated dishes. VPC clusters were enumerated after 7 days of culture and defined by the presence of both cell clusters (minimum of 30 cells per cluster) and associated elongated, projecting cells (minimum of 3 attached projecting cells per cluster) by use of an inverted microscope. Counting of VPC clusters and data analyses were performed in a blinded fashion.

Cell Populations Enriched for VPCs Measured by Flow Cytometry

Antibodies used for flow cytometry included anti-VEGFR2 conjugated to fluorescein isothiocyanate (clone 89106, R&D Systems, Minneapolis, MN, USA), anti-CD133 conjugated to phycoerythrin (PE)-R (clone AC133, Miltenyi Biotec Inc., Auburn, CA, USA), anti-CD45 conjugated to PE Texas Red (clone J.33, Beckman Coulter Inc., Brea, CA, USA) and anti-CD34 conjugated to PE Cy7 (clone 581, Beckman Coulter Inc.). All samples were processed by incubating undiluted whole blood with an appropriate quantity of antibody, determined in preliminary experiments. The samples were incubated in the dark for 30 minutes, lysed with 1 ml of IO Test 3 solution (Beckman Coulter Inc.) that had been diluted 1:10 as recommended by the manufacturer. The samples were run immediately on a MPL FC 500 cytometer (Beckman Coulter Inc.). All data were analyzed using Kaluza software (Beckman Coulter Inc.). Putative VPCs were considered to be positive for CD34, CD45, and VEGFR2 in accordance with previous studies [24]. Early hematopoietic precursors were positive for CD45 and CD34 and were enumerated in accordance with the

TABLE 2. Characteristics of study patients and controls at enrollment

Patient Characteristics	AKI Cohort	Control	P value
N	30	8	
Age (years)	62.4±3.6	58.8±3.5	NS
Male gender, n (%)	16 (53)	4 (50)	NS
Hemoglobin (g/L)	102.1±3.3	101.1±8.4	NS
Initial APACHE II Score	23.1±1.2	20.6±1.9	NS
Serum creatinine (μmol/L)	246±26	66.3±4.3	0.01
Diagnosis at ICU admission n (%)			<0.001*
Sepsis	19 (63)	0 (0)	
H1N1	5 (17)	0 (0)	
Pneumonia	3 (10)	3 (37.5)	
Respiratory Failure	3 (10)	3 (37.5)	
Other	0 (0)	2 (25)	

* p value calculated using ANOVA for multiple variables

TABLE 3: Patient baseline characteristics and outcomes according to the category of AKI

Patient Characteristics	Cohort	Risk	Injury	Failure	P Value
N	30	5	12	13	
Age (years)	62.4±3.6	57.6±5.5	65.8±6.1	61.0±6.1	NS
Male gender, n (%)	16 (53)	3 (60)	6 (50)	7 (54)	NS
Hemoglobin (g/L)	102.1±3.3	100.9±3.8	96.2±4.2	104.8±5.9	NS
Initial # organs with SOFA Score ≥ 2	2.9±0.2	2.4±0.4	2.3±0.4	3.8±0.3	0.009
Serum creatinine (μmol/L)	246±26	157±14	171±8	348±47	0.001
Diagnosis at ICU admission n (%)					NS
Sepsis	19 (63)	3 (60)	9 (75)	7 (54)	
H1N1	5 (17)	1 (20)	2 (16)	2 (15)	
Pneumonia	3 (10)	1 (20)	1 (8)	1 (8)	
Respiratory Failure	3 (10)	0 (0)	0 (0)	3 (23)	
Presumed cause of AKI n (%)					NS
Septic shock	25 (83)	5 (100)	11 (92)	9 (69)	
Hypovolemic shock	4 (13)	0 (0)	1 (8)	3 (23)	
Cardiogenic shock	1 (4)	0 (0)	0 (0)	1 (8)	
Outcomes					
Length of Hospital Stay (days) ^a	16.6±2.1	17.4±3.4	29.9±5.1	34.0±6.0	NS
Treatment n (%)					0.001
CRRT	8 (27)	0 (0)	0 (0)	8 (62)	
Hemodialysis	5 (17)	0 (0)	0 (0)	5 (38)	
Recovery/Death n (%)					
Renal Recovery	11 (37)	2 (40)	5 (42)	4 (31)	1.0
SOFA Recovery	11 (37)	3 (60)	3 (25)	5 (38)	0.4
Death	5 (17)	0 (0)	3 (25)	2 (15)	0.9

^a length of hospital stay was measured from the day of enrollment in the study until hospital discharge or until death.

SOFA – Sepsis-related Organ Failure Assessment; CRRT – continuous renal replacement therapy; AKI – acute kidney injury; H1N1 – influenza strain

FIGURE 1. (A) VPC mobilization of control subjects (black bars) compared with patients with AKI (white bars). (* $p=0.02$ between VPC levels on day 14 in the two groups; ** $p<0.01$ between enrollment and time 14 in the patients with AKI). (B) VPC mobilization in Risk, Injury, and Failure patients over course of study. (* $p=0.05$ between day 14 and day 0 for the I and F groups; ** $p=0.001$ between VPC levels in group R vs groups I and F).

guidelines published by the International Society for Hematology and Graft Engineering [25].

Erythropoietin Assays

Plasma samples were collected at the time of enrollment and at the last timepoint in the study for each patient (day 7 or day 14). Erythropoietin (EPO) levels were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems) and optical densities were quantified using an MRX plate reader (Dynatec Laboratories, El Paso, TX, USA). Each sample was analyzed in duplicate and procedures were performed in accordance with the manufacturer's instructions.

FIGURE 2. Time-dependent VPC mobilization in patients who improve in RIFLE score ($n=12$, white bars, * $p=0.04$ in comparing VPC levels on Day 0 and Day 14) and in patients who did not improve in RIFLE score or had worsened RIFLE score ($n=18$, black bars, $p=0.24$ in comparing VPC levels on Day 0 and Day 14). (** $p=0.07$ in comparison of VPC levels on Day 14 between the two groups).

Statistics

Mean values are reported $\pm$ standard error of the mean (SEM). Unpaired Student's t test was used to compare means with a significance threshold of 0.05 and assuming unequal variance. Proportions were compared by use of chi-square analysis with a significance threshold of 0.05. One-way ANOVA was performed for comparison of means involving more than two samples with a significance threshold of 0.05.

Results

Demographic data

Characteristics of patients with AKI and controls are summarized in Table 2. The proportion of male subjects in the AKI group and control group was similar (53% vs 50%, $p=NS$). The mean initial serum creatinine value was significantly elevated in the AKI cohort compared with controls (246 ± 26 vs. 66 ± 4.3 $\mu\text{mol/L}$, respectively, $p=0.01$). Patient age, gender distribution and baseline hemoglobin levels were similar between patients with AKI and controls. Initial APACHE II and SOFA scores were not different between the AKI group and control patients.

Septic shock was the most common primary cause of AKI (25 patients, 83%). The mean baseline serum creatinine for all patients in the AKI group prior to enrollment in the study was 109 ± 51 $\mu\text{mol/L}$ and was not different between patients with

R, I, or F categories of AKI (Table 3). Patients classified as Risk, however, had lower SOFA scores ($p=0.004$) compared with patients in the Injury or Failure categories at time of enrolment. As expected, serum creatinine levels differed significantly between the three groups of patients in the AKI group, with highest values in the Failure patients compared to the R and I groups ($p=0.001$). Only patients in the Failure category required continuous renal replacement therapy (CRRT) or hemodialysis ($n=13$, $p=0.001$ versus Risk and Injury groups). A trend towards shorter hospital stay was noted in the Risk sub-group ($p>0.05$) in comparison with the other categories of AKI, but was not different from control patients.

RIFLE criteria were evaluated at each time point in patients admitted to ICU with AKI. Two of the five patients (40%) in the Risk group improved over the time course of the study, compared with five of 12 (42%) and four of 13 patients (31%) in the Injury and Failure groups, respectively ($p=NS$). No patient died in the Risk category, whereas the mortality rate was 25% in the Injury category (three of 12 patients) and 15% in the failure group (two of 13 patients) ($p=0.9$, Injury *vs.* Failure). Improvements in SOFA scores over time were noted in 3 (25%), 4 (25%) and 8 (62%) patients in the Risk, Injury and Failure groups, respectively ($p=0.8$).

Mobilization of VPCs

In the entire cohort of patients with AKI, a significant time-dependent and linear increase of VPC cluster numbers was observed from time 0 to 14 days ($p<0.01$, Figure 1A). In contrast, in control subjects VPC cluster levels were highest at day 0 and decreased with time, such that by day 14 levels were significantly lower than those in the cohort of AKI patients (Figure 1A, $p<0.001$); however, the pattern of VPC mobilization over time in patients with AKI was different for the patients in the Risk, Injury, and Failure groups (Figure 1B). Time-dependent increases in VPCs were seen in both the Injury and Failure groups ($p=0.05$ for comparison of VPC levels on day 14 versus day 0 in Injury and Failure groups). In contrast, patients in the Risk category demonstrated a distinct VPC mobilization pattern, with highest VPC levels at time 0 and a trend towards decreasing levels over the ensuing 14 days. Interestingly, patients in the Risk category had significantly higher levels of VPC clusters at the time of enrollment compared with patients in the Injury or Failure groups ($p=0.001$).

VPC mobilization and recovery of renal and global function

In patients with a decrease in RIFLE score over the course of the study ($n=12$) there was a significant linear increase in VPC

clusters over time (Figure 2) ($p=0.04$ between days 0 and 14). In contrast, there was no significant change in VPC cluster numbers in patients who had no improvement or increased severity in the RIFLE score ($n=18$) ($p=0.24$). Furthermore, there was a trend towards greater numbers of VPC clusters on day 14 in patients who had an improvement in RIFLE category, compared to patients who did not show improvement ($p=0.07$).

SOFA scores were determined for each patient with AKI at all time points in the study to assess the overall clinical status of patients, in accordance with previous studies, (18) to provide a general sense of whether VPC levels were correlated with overall clinical improvement. Patients who demonstrated improvement in SOFA score ($n=12$) and patients without improvement in the SOFA scores ($n=18$) had similar overall increases in VPC levels during the study period but no significant difference was observed in VPC levels between the two groups. Moreover, calculation of modified SOFA scores that omitted the renal score, and repeat analysis of VPCs in patients that improved compared with those that did not improve revealed no significant difference (data not shown).

Angiogenic precursors and recovery in patients with AKI

Flow cytometry was used to enumerate angiogenic precursors in blood from patients with AKI at each time point in the study. In contrast to VPC cluster determinations, CD45+CD34+VEGFR2+ angiogenic precursors did not increase over 14 days in patients with AKI (Figure 3). Indeed, there was a non-significant trend towards a decrease in the number of circulating angiogenic precursors from day 0 to day 14 in the entire cohort ($p=0.10$). There were no observed differences in CD45+CD34+VEGFR2+ cells among patients with R, I or F categories of AKI. Moreover, the number of angiogenic precursors in peripheral blood did not correlate with improvement in renal function. No changes in CD45+CD34+hematopoietic progenitor levels were observed by flow cytometry throughout the study period ($p=0.47$). Enumeration of angiogenic and hematopoietic precursors by flow cytometry was not performed for control subjects.

Plasma erythropoietin levels

AKI may be associated with hypoxic damage to the renal tubulointerstitium and subsequent impact on erythropoietin (EPO) production through hypoxia-inducible factors [26,27]. EPO is known to stimulate VPC mobilization and endothelial progenitor function and has been associated with improvement in kidney function in experimental models [28-30]. EPO

FIGURE 3. Angiogenic precursor mobilization in patients with AKI. CD34+CD45+VEGFR2+ angiogenic precursors were enumerated by flow cytometry and did not significantly change over 14 days after AKI in these patients.

levels were measured by ELISA in plasma samples collected at the time of enrollment and on the last sample available in the study in patients with AKI (day 7 or day 14). EPO levels were significantly increased at time 0 in patients categorized as Failure in comparison to patients in Risk and Injury groups (Figure 4). EPO levels returned to similar levels by day 14 in all patient groups, regardless of the category of AKI.

Discussion

The results of our study indicate that critically ill patients with AKI have a distinct pattern of vascular progenitor mobilization, as measured using the VPC cluster assay: vascular progenitors increased gradually over 14 days. In comparison, critically ill patients without AKI had higher initial levels of vascular progenitors that decreased gradually over the same time period. Patients with more severe renal damage appeared to have greater mobilization of vascular progenitors over time. Moreover, mobilization of VPCs may be enhanced in patients with improvement of renal function over the study period. Enumerating subpopulations of cells from peripheral blood enriched for putative angiogenic precursors did not show an association with the category of AKI or extent of recovery in the follow-up period. Our results suggest that mobilization of angiogenic cells following AKI may be a target for investigating new approaches to improve the outcome of patients with AKI.

Repair of tissue injury is a complex process involving the release of inflammatory and angiogenic cytokines and homing of cells involved in repair, including mature angiogenic monocytes and vascular precursors. Vascular precursors may originate from bone marrow, vessel walls, or circulating monocytes

FIGURE 4. Plasma EPO levels and RIFLE score. EPO was measured by ELISA and levels were increased in the Failure group at day 0 (* $p=0.05$ vs. Risk and Injury). EPO levels in all groups were similar at day 14.

and likely all these precursors contribute to the aggregation of cells in the VPC cluster assay [31,32]. Cell types that make up the vascular repair response remain incompletely understood and the functional status of important cell types may be best captured by the VPC cluster assay as opposed to simply enumerating cell types by flow cytometry.

Many studies have enumerated different subpopulations of cells from peripheral blood using flow cytometry and have reported variable results in terms of association with clinical outcome, including clinical studies of myocardial infarction [33, 34] and stroke [35]. Taken together, investigators have been unable to clearly identify a dominant cell population associated with vascular repair. In our study, the VPC cluster assay seemed to correlate with improvement of renal function but specific subpopulations of angiogenic precursors were not associated with clinical improvement. The VPC cluster assay may better represent the heterogeneous cell populations that comprise the repair response in acute injury and better encompass the interactions between multiple cell types. Strunk *et al.* recently reported on the nature of cells within VPC clusters and confirmed that cells are predominantly T lymphocytes and monocytes and that these cells induce the aggregation of other non-clonal immune effector cells [36]. Soluble factors, however, produced in colony assays can support vascular network formation *in vitro* [36]. Our own work and other reports confirm the importance of monocytic cells within VPC clusters and in the process of vascular repair [31,37,38]. The correlation of VPC clusters with various indicators of vascular health underscores the relevance of the VPC assay as a diagnostic indicator of vascular competence. In particular, VPC clusters have correlated with cardiovascular risk factors [23], cardiovascular mortality

in high risk patients [9] and survival in patients with sepsis [14] and are increased following numerous types of injury including stroke [10], severe trauma [39], and radiation-induced toxicity in cancer patients [40]. Moreover, infusing stem cell products with increased VPCs was associated with reduced toxicity after hematopoietic stem cell transplantation [41].

Importantly, the degree of renal damage was a key factor in distinguishing a pattern of increased VPC cluster formation after AKI. Patients with lesser degrees of renal compromise had VPC cluster levels that decreased over time with a pattern that was similar to that seen in the control group of acutely ill patients admitted to ICU but without renal injury. Thus, the increase in VPC clusters in the Injury and Failure categories with time may be specific to patients with AKI, and not generalizable to all patients with critical illness. Importantly, the underlying cause for admission to ICU for non-renal illnesses may be relevant. Few of the patients in our control group had infections and sepsis has been associated with changes in VPC cluster formation [15], in addition to factors associated with AKI, which may have influenced our results. The dynamic changes in VPCs and the differences noted according to severity of AKI suggest that sepsis alone may not account for the increasing number of VPC clusters observed in patients with more severe AKI.

Our data do not allow us to draw conclusions regarding possible mechanisms of renal repair; however, the observation that EPO levels and VPC mobilization are increased in patients with more severe AKI suggests that renal ischemia may stimulate EPO production and a more pronounced vascular repair response. EPO contributes to increased VPC cluster formation and the mobilization of vascular precursors with greater angiogenic function [42,43] and can contribute to recovery in animal models of kidney injury [28-30]. The role of EPO in VPC mobilization in AKI and any possible role for EPO in clinical improvement requires further study.

A major limitation of our study is the small number of patients enrolled. It is not possible to account for the influences of potential competing variables such as comorbidities and concomitant medication usage, which could influence both the category of AKI and the degree of recovery in our patients. Advanced age [44], diabetes [45], smoking [46] and certain medications such as HMG-coenzyme reductase inhibitors [47] are known to influence the number of VPC clusters in the peripheral blood. Larger cohorts of patients are needed to perform multivariable analysis of factors influencing recovery following AKI and to more fully assess the preliminary observations obtained in our study.

Taken together, our data suggest that AKI is associated with the mobilization of cells that contribute to increased VPC cluster formation. Furthermore, greater VPC mobilization over time appears to correlate with renal recovery in critically ill patients with AKI. Further studies should aim to determine if VPC mobilization plays a direct role in recovery of renal function or simply increase in response to factors released during the recovery period.

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