Published in final edited form as:
Intensive Care Med. 2012 March ; 38(3): 429–436. doi:10.1007/s00134-012-2480-9.

Circulating endothelial progenitor cells inversely associate with organ dysfunction in sepsis

Sushma K. Cribbs,
Division of Pulmonary, Allergy, and Critical Care Medicine, Grady Memorial Hospital, Emory University School of Medicine, 49 Jesse Hill Jr Drive, SE (FOB), Pulmonary, Atlanta, GA 30303, USA, Tel.: +1-404-6160821

Diane J. Sutcliffe,
Division of Cardiology, Emory University, Atlanta, GA, USA

William R. Taylor,
Division of Cardiology, Emory University, Atlanta, GA, USA

Mauricio Rojas,
Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Kirk A. Easley,
Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Li Tang,
Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Kenneth L. Brigham, and
Division of Pulmonary, Allergy, and Critical Care Medicine, Emory University, Atlanta, GA, USA

Greg S. Martin
Division of Pulmonary, Allergy, and Critical Care Medicine, Grady Memorial Hospital, Emory University School of Medicine, 49 Jesse Hill Jr Drive, SE (FOB), Pulmonary, Atlanta, GA 30303, USA, Tel.: +1-404-6160821

Sushma K. Cribbs: skomaku@emory.edu

Abstract

Purpose—Endothelial dysfunction is a primary contributor to sepsis-related organ dysfunction and death. In sepsis animal models, endothelial progenitor cells (EPC) have contributed to vascular repair. The role of endothelial progenitor cells as a biomarker for organ dysfunction is still unknown. We hypothesized that circulating numbers of endothelial progenitor cells would be associated with improved outcomes in sepsis.

Methods—Prospective, observational single-center cohort study in adult intensive care units at Grady Memorial Hospital, an affiliate of Emory University, from July 2007 through April 2009. Peripheral blood was obtained from 95 patients with sepsis, 37 intensive care unit controls, and 51

Correspondence to: Sushma K. Cribbs, skomaku@emory.edu.

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-012-2480-9) contains supplementary material, which is available to authorized users.

healthy controls, of whom only 86 patients with sepsis were used in the analysis because we were not able to obtain enough blood in 9 sepsis patients. Clinical data were obtained, and organ dysfunction was measured by Sepsis-Related Organ Failure Assessment (SOFA) score. Endothelial progenitor cells were assessed by a colony-forming unit (CFU) assay in which peripheral blood mononuclear cells were isolated using Ficoll density-gradient centrifugation and cultured in growth media.

Results—The patients with sepsis had significantly lower mean endothelial progenitor cell colony counts compared with intensive care unit controls ($p = 0.035$) and healthy controls ($p = 0.0005$). There was no difference in colony counts between ICU controls and healthy controls ($p = 0.81$). In the sepsis patients, EPC CFU numbers inversely associated with SOFA score, adjusting for mortality ($r^2 = 0.05$, $p = 0.04$).

Conclusion—Increased circulating endothelial progenitor cells inversely correlate with organ dysfunction in sepsis patients.

Keywords

Sepsis; Septic shock; Endothelium; Progenitor cells; SOFA; Outcomes

Introduction

Sepsis is an acute inflammatory response to infection, leading to systemic illness and organ dysfunction [1]. In the USA, sepsis is a leading cause of death in the intensive care unit (ICU), and the 10th leading cause of death overall [2]. The severity of the inflammatory response may cause organ dysfunction, a primary determinant of survival. However, the development of organ dysfunction is highly variable in sepsis patients and not well predicted by clinical, physiological or biochemical data [3].

Of the mechanisms that lead to sepsis-related organ dysfunction and death, alterations in vascular function are a primary contributor. Early descriptions of endotoxin-induced vascular reactions showed changes in hemodynamics that were influential in accounting for the poor responses of animals to endotoxin [4]. More recently, alterations in microvasculature have been reported in human subjects with sepsis [5, 6] and have been correlated with disease severity and organ failure. Damage to the endothelium can result in multi-organ failure, leading to increased sepsis severity, and ultimately increased mortality.

Endothelial progenitor cells (EPC) are a specific subtype of hematopoietic stem cells that have been isolated from circulating mononuclear cells [7], bone marrow [8], and cord blood [9]. EPC have been shown to migrate from the bone marrow to the peripheral circulation, where they contribute to vascular repair [7]. When injected into animal models of ischemia, they are incorporated into sites of neovascularization [7, 10, 11]. Intracoronary EPC infusion has even improved outcomes in patients with acute myocardial infarction [12] and chronic post-infarction heart failure [13]. Although persistent evidence has shown that EPC from the bone marrow may contribute to endothelial repair, it is unclear if EPC are involved in vascular dysfunction inherent to sepsis or whether EPC can contribute to repair of damaged organs in patients with sepsis, and if this repair will result in improved clinical outcomes.

To date, little is known regarding the role of EPC in critical illnesses, specifically the relationship of EPC to organ dysfunction and survival in sepsis. Based upon previous data in acute lung injury (ALI) patients [14], we hypothesize that EPC, measured by a colony-forming unit assay, will be differentially circulated in sepsis patients and will associate with survival. Although Rafat et al. [15] noted that septic patients had significantly higher number of circulating EPC than nonseptic ICU patients and healthy controls, the role of EPC

in prevention of or recovery from *organ dysfunction*, a major determinant of mortality, was not evaluated and is still unknown. In addition, we propose a culture-based methodology for measuring EPC as opposed to the flow cytometry method used by Rafat and colleagues. Understanding the function of EPC in sepsis and sepsis-related organ dysfunction may provide innovative diagnostic and therapeutic approaches in this devastating disease.

Materials and methods

Patient characteristics

Patients meeting the American College of Chest Physicians (ACCP)/Society of Critical Care Medicine (SCCM) consensus definition of sepsis, severe sepsis, or septic shock were enrolled from the adult intensive care units (ICU) at Grady Memorial Hospital, an affiliate of Emory University, from July 2007 through April 2009 [1]. ICU patients not meeting the diagnostic criteria for sepsis, severe sepsis, or septic shock were enrolled as control subjects (hereafter referred to as "ICU control subjects"). Patients who were <18 years of age, pregnant, neutropenic (defined as absolute neutrophil count <1,000 cells/mm^3), or with history of hematological malignancy or bone marrow transplantation, acute coronary syndrome or acute myocardial infarction within the past 2 months, previous surgical procedure requiring general anesthesia within the past 2 weeks, or currently hospitalized for traumatic injuries were excluded. In addition, healthy individuals were recruited from the general population (hereafter referred to as "healthy control subjects"). These individuals had no significant past medical history and were on no medications. Informed consent was obtained from the subjects or from designated surrogates before enrollment into the study. The study was approved by the Institutional Review Board at Emory University and the research oversight committee of Grady Memorial Hospital.

At enrollment, all sepsis and ICU control subjects had demographic and physiologic information collected including Acute Physiology and Chronic Health Evaluation (APACHE) II and Sepsis-Related Organ Failure Assessment (SOFA) scores. Source of infection was determined and ALI was diagnosed when patients met the American–European Consensus Conference (AECC) definition [16].

EPC colony isolation

Please see Supplementary Appendix for description of EPC colony isolation.

Analysis

Endothelial progenitor cell CFU counts were compared between the sepsis, ICU control, and healthy control subjects using the Kruskal–Wallis test, and the three pairwise comparisons were made using the Wilcoxon rank-sum test (Fig. 2). Since the healthy control subjects were younger than the sepsis and ICU control subjects, log EPC CFU counts were regressed on age using linear regression, and pairwise comparisons between the three age-adjusted EPC CFU count means were made using *t* tests with Bonferroni correction. In Table 1, two-sample Wilcoxon rank-sum tests were performed to compare continuous demographic characteristics between sepsis and ICU control subjects. Pearson χ^2 tests were used to compare categorical characteristics. Logistic regression was performed to quantify the relationship between binary outcomes and EPC counts. SOFA scores for the survivors and nonsurvivors among sepsis patients were compared by regressing on EPC counts using analysis of covariance (ANCOVA) with a common linear slope. An adjusted mean was calculated for each subgroup. This regression model was refitted adjusting for age, gender, and presence or absence of shock. When multiple comparisons were made, Bonferroni corrections were performed to address multiple testing.

All statistical analyses were conducted using SAS 9.2 (SAS Inc.). All reported p values were two-sided, and p values of 0.05 or less or smaller than a threshold calculated with Bonferroni correction were considered statistically significant.

Results

Demographics of patients with sepsis and ICU control patients

Ninety-five patients with sepsis and 37 ICU control patients were enrolled (Table 1), of whom 86 patients with sepsis and all ICU controls were used in the analysis because we were not able to obtain enough blood for EPC in 9 sepsis patients. ICU control subjects were hospitalized for a variety of disorders. Further detail on ICU control subjects is given in Supplementary Appendix. Sepsis patients and ICU controls were similar except sepsis patients had higher illness severity (APACHE II) and organ dysfunction (SOFA) scores, as well as higher mortality (26% versus 5%, $p = 0.008$). Sepsis patients also had longer ICU and hospital lengths of stay and higher white blood cell counts. Fifty-one healthy volunteers without any comorbidity were also enrolled with a median age of 30 years [interquartile range (IQR) 16–28 years]. A second EPC sampling was done on a subgroup of sepsis patients (see Supplementary Appendix).

Morphology of endothelial progenitor cell colonies

Morphologically, the EPC CFUs from the sepsis subjects were similar to those from previous investigators [14, 17], as shown in the photomicrograph taken on day 7 of a CFU on a fibronectin-coated plate in Fig. 1a. We assessed the ability of the cells to incorporate acetylated low-density lipoprotein (LDL) and to bind to endothelial specific lectin [*Ulex europaeus* agglutinin I (UEA) fluorescein isothiocyanate (FITC)] via immunostaining. We showed that the cells stained for both of these markers, consistent with an endothelial lineage (Fig. 1b) [7, 17].

EPC quantitation and relationship to organ dysfunction

The absolute numbers of CFUs (per well) present in the entire cohort of patients is shown in Fig. 2. The patients with sepsis had significantly lower EPC CFU counts (median 10; 25–75th percentiles: 2–21) compared with ICU controls (median 21.5; 25–75th percentiles: 8.5–36.5; $p = 0.035$) and healthy controls (median 22; 25–75th percentiles: 16–28; $p = 0.0005$), and there was no difference in EPC CFU counts between ICU controls and healthy controls ($p = 0.81$, Bonferroni threshold 0.01). In the sepsis patients, EPC CFU numbers inversely associated with SOFA score, adjusting for mortality ($r^2 = 0.05$, $p = 0.04$, Fig. 3). The relationship between the outcome total SOFA score and the predictor EPC CFU count is linear, and as the EPC CFU count decreased, there was a small increase in the total SOFA score. Specifically, for a 10-cell decline in EPC counts measured by CFU, total SOFA score increased by 0.5 points [95% confidence interval (CI) (0.03–0.93), $p = 0.04$]. The EPC-adjusted mean total SOFA score was 7.8 for survivors and 11.5 for nonsurvivors, and these estimates were similar when adjusting for age and gender. The relationship between EPC and SOFA was similar in presence or absence of shock ($p = 0.003$). The median EPC CFU count for survivors ($n = 65$) was similar to nonsurvivors ($n = 21$) [10 (IQR 2–20) versus 12 (IQR 1–21), respectively, $p = 0.93$]. In sepsis patients, EPC CFU counts did not correlate with ALI [odds ratio (OR) 0.99, 95% CI (0.96–1.01), $p = 0.27$], mortality [OR 0.99, 95% CI (0.97–1.02), $p = 0.78$], shock [OR 1.00, 95% CI (0.98–1.03), $p = 0.74$], chronic alcohol abuse [OR 0.98, 95% CI (0.95–1.01), $p = 0.28$], or APACHE II score ($r = 0.04$, $p = 0.74$). The median EPC CFU count for patients with ALI ($n = 33$) was similar to patients without ALI ($n = 53$) [12 (IQR 0–20) versus 10 (IQR 2–25), respectively, $p = 0.55$]. ICU control data are given in the Supplementary Appendix.

Discussion

The results from this study showed that EPC, as measured by a colony-forming assay, are significantly lower in sepsis patients compared with ICU and healthy control subjects and that the number of circulating EPC is inversely associated with the severity of organ dysfunction in sepsis patients. Specifically, greater EPC CFU counts correlated with lower SOFA scores, irrespective of presence of shock or subsequent survival. In sepsis patients with prolonged ICU stay, the number of circulating EPC CFU count did not change at day 28. These data support the hypothesis that mobilization of EPC from the bone marrow abrogates organ dysfunction, potentially through interactions at the vascular endothelium; however, the association between EPC and SOFA score is very weak, and its exact clinical significance remains unclear at this time.

The last several years have seen an enormous increase in the amount of preclinical and clinical data regarding the prognostic and therapeutic benefits of stem cells in sepsis [14, 15, 18–21]. The pathophysiologic changes associated with critical illness may lead to apoptosis and necrosis of endothelial cells (EC) from the vasculature and recruitment of EPC from the bone marrow. Rafat et al. [15] measured levels of circulating EPC in a cohort of 32 patients within 48 h after sepsis onset. Identifying EPC by fluorescence-activated cell sorting (FACS) using antibodies against CD34, CD133, and vascular endothelial growth factor receptor-2 (VEGFR-2), they observed that the number of circulating EPC was significantly higher in patients with sepsis versus nonseptic ICU patients and healthy controls [15]. They also noted that sepsis survivors had significantly greater number of EPC than nonsurvivors, suggesting not only that vascular damage induces release of EPC in circulation, but that circulating EPC may be protective and predict clinical outcome in critically ill patients [15]. Becchi et al. also demonstrated that $CD34^+$ EPC in patients with sepsis had a fourfold increase compared with healthy controls (45 ± 4.5 versus $12 \pm 3.6\%$, $p < 0.001$). Most interestingly, this increase was already evident at 6 h from diagnosis [22].

In contrast to Rafat, Becchi, and colleagues, the current study showed that EPC were significantly *lower* in sepsis patients compared with the control population. These results are likely explained by differences in the techniques used for EPC identification. The flow cytometry technique utilized by Rafat et al. contrasts to our cell-culture technique, and these techniques may identify different progenitor cell types. Cell-culturing techniques may offer a measurement of EPC functionality that FACS analysis does not. With cell-culture methods, two distinct phenotypes of EPC have been described, namely early and late outgrowth EPC [23, 24]. The early outgrowth EPC are seeded onto fibronectin-coated plates in the presence of growth factors that promote EPC growth and colony formation (CFU) after 5–7 days, while late outgrowth EPC are plated onto collagen I-coated plates and give rise to colonies after 14–21 days. Early outgrowth EPC have low proliferative capacity, and although these cells may incorporate into the endothelial monolayer, they fail to form perfused vessels in vivo, whereas late outgrowth EPC have a high proliferative rate and can be maintained in culture extensively. Recent studies have further identified these cells as $CD34^+$ $CD45^-$ precursors [25]. Thus, while sepsis patients may have increased mobilization of EPC into the peripheral circulation secondary to vascular dysfunction as noted previously [15], the early outgrowth EPC that were measured in this study may be poorly functioning and unable to form endothelial colonies, resulting in lower EPC CFU numbers in sepsis patients. In a large systematic comparison of EPC enumerated by both commonly used culture-based techniques and EPC surface markers measured by FACS [26], the authors found that culture-based assays are less precise and display more daily variability than do assays based on cell surface markers. The authors also found that EPC defined by $CD133^+$ and $CD34^+$ did not correlate with the culture-based assay [26], again providing evidence for the lack of consistent results between this study and others that utilized cell surface markers

[15]. These results indicate that a uniform definition of EPCs is required to clarify their origins.

The current study also demonstrated that increasing EPC CFU numbers were inversely associated with organ dysfunction. Others utilizing the same cell-culturing techniques have demonstrated that greater EPC CFU counts were associated with survival in patients with ALI, one of the most common organ dysfunctions in sepsis [14], concluding that adequate mobilization of EPC from bone marrow in ALI could contribute to repair and recovery of damaged pulmonary endothelium [14]. The same authors also looked at CFU in a sepsis population, and analysis revealed that, in contrast to this study, septic patients had greater CFU counts compared with healthy controls. However, there were only 17 patients with severe sepsis enrolled (in contrast to the 86 septic patients enrolled in this study), which may account for the differences seen. However, those with CFU count ≥48 per volume had overall better survival compared with those with CFU count <48 [27], and similarly, we have shown that higher EPC CFU numbers are associated with improved organ function.

This study has important limiting factors. First, EPC have been analyzed and studied in a variety of ways [7, 28–30], and considerable debate exists about which method correctly identifies an EPC. The limited knowledge of unique stains and markers specific for EPC makes contamination with non-EPC possible in both cell-culture and flow cytometry techniques. In addition, cell-culture techniques depend on the formation of EPC colonies, which may not occur in particular disease states. It is likely that methodological differences in EPC phenotyping contributed to the varied results seen in previous sepsis studies [15]. However, the method used in this study has been previously used and validated by others [14, 17], and EPC CFU numbers in healthy controls were similar to those reported previously [14], suggesting compatible techniques. Second, although data collection was completed using the same method in all patients and blood was drawn within 72 h of meeting criteria for sepsis, it is possible that circulating EPC numbers changed within the 72-h timeframe; however, culturing of EPC requires at least 4–7 days, so it is less likely when measuring EPC through culture techniques that there is significant variability within 72 h. In addition, others have reported that EPC have not changed even within 7 days [14], and we were able to demonstrate in this study that CFU numbers remained stable over 28 days. Third, sepsis patients are a heterogeneous population with varied etiologies of disease, organ dysfunction, and illness severity. These distinctions may have limited the prognostic potential of these cells. However, despite these disparities, a significant association was seen between organ dysfunction and EPC CFU numbers in patients with sepsis. In addition, the trend towards significance in ICU controls suggests that EPC biological relevance may not be specific to sepsis but rather reflect organ dysfunction in all critically ill patients. Fourth, there was a significant age discrepancy between the healthy controls and the two patient groups. Given that EPC are well documented to change with age [31], this may have influenced the results. However, significant changes were observed between the sepsis patients and the ICU control subjects, and there were no age discrepancies between these two groups. Fifth, although certain medications known to influence EPC numbers were excluded in the study, it is difficult to know exactly what other medications (i.e., corticosteroids) influenced EPC CFU counts until further studies are done to examine these medications and their particular influence on EPC [32, 33]. There is similar controversy with regard to blood transfusions, and a recent study found that there was no significant difference in peripheral EPC numbers in trauma patients who received less than 1,800 mL blood (approximately 6–7 units) compared with controls [34]. It is less likely that the sepsis patients in our study received more than 1,800 mL blood prior to EPC measurement. Sixth, ICU control subjects in this study had a range of neurological disorders along with other respiratory disorders including asthma and chronic obstructive pulmonary disease (COPD). Because of the variation in techniques described above and the undeveloped nature of this

field, it is difficult to determine exactly what an appropriate ICU control group is in this emerging field. Studies have shown conflicting results with regard to neurologic disorders [35, 36] and asthma/COPD [36, 37]. Some have found that EPC are not decreased in COPD [38], while other investigators have shown that EPC are even influenced by whether patients are currently in a COPD exacerbation versus stable disease [39]. Thus, it is unclear at this time how these various diseases affected circulating EPC numbers in these patients. As the field of EPC matures and further large studies of these cells are completed (utilizing both flow cytometry and culture techniques), then it may be possible to know the optimal control group for studies of sepsis or other critically ill patients. Finally, although further functional assays were not performed in this study, we believe, along with others [14], that the cell-culturing technique utilized in this study demonstrates the ability of these cells to form vascular colonies through the migration and proliferation of these immature cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Tina Holden, Judith Schmidt, Joel Andrews, Mona Brown, Edwin Omohwo, Kavita Demla, Elizabeth Ju, Constance Anani, and Jaber El-Bashir for their assistance with patient enrollment, and Susan Gregory and Neeta Shenvi for their assistance with database management.

Funding sources NIH T32 HL076118, University Research Committee funding from Emory University.

References

1. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/ Society of Critical Care Medicine. Chest. 1992; 101:1644–1655. [PubMed: 1303622]
2. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 2003; 348:1546–1554. [PubMed: 12700374]
3. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR, Payen D. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med. 2006; 34:344–353. [PubMed: 16424713]
4. Gilbert RP. Mechanisms of the hemodynamic effects of endotoxin. Physiol Rev. 1960; 40:245–279. [PubMed: 13850016]
5. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med. 2004; 32:1825–1831. [PubMed: 15343008]
6. Trzeciak S, McCoy JV, Phillip DR, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med. 2008; 34:2210–2217. [PubMed: 18594793]
7. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275:964–967. [PubMed: 9020076]
8. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest. 2002; 109:337–346. [PubMed: 11827993]
9. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, Imaizumi T. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest. 2000; 105:1527–1536. [PubMed: 10841511]

10. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999; 85:221–228. [PubMed: 10436164]
11. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia-and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med. 1999; 5:434–438. [PubMed: 10202935]
12. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcoronary transplantation of progenitor cells after myocardial infarction. N Engl J Med. 2006; 355:1222–1232. [PubMed: 16990385]
13. Assmus B, Fischer-Rasokat U, Honold J, Seeger FH, Fichtlscherer S, Tonn T, Seifried E, Schachinger V, Dimmeler S, Zeiher AM. Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure: results of the TOPCARE-CHD Registry. Circ Res. 2007; 100:1234–1241. [PubMed: 17379833]
14. Burnham EL, Taylor WR, Quyyumi AA, Rojas M, Brigham KL, Moss M. Increased circulating endothelial progenitor cells are associated with survival in acute lung injury. Am J Respir Crit Care Med. 2005; 172:854–860. [PubMed: 15976374]
15. Rafat N, Hanusch C, Brinkkoetter PT, Schulte J, Brade J, Zijlstra JG, van der Woude FJ, van Ackern K, Yard BA, Beck GC. Increased circulating endothelial progenitor cells in septic patients: correlation with survival. Crit Care Med. 2007; 35:1677–1684. [PubMed: 17522579]
16. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994; 149:818–824. [PubMed: 7509706]
17. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med. 2003; 348:593–600. [PubMed: 12584367]
18. Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol. 2007; 179:1855–1863. [PubMed: 17641052]
19. Mutunga M, Fulton B, Bullock R, Batchelor A, Gascoigne A, Gillespie JI, Baudouin SV. Circulating endothelial cells in patients with septic shock. Am J Respir Crit Care Med. 2001; 163:195–200. [PubMed: 11208646]
20. Xu J, Woods CR, Mora AL, Joodi R, Brigham KL, Iyer S, Rojas M. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. Am J Physiol Lung Cell Mol Physiol. 2007; 293:L131–L141. [PubMed: 17416739]
21. Lee JW, Fang X, Gupta N, Serikov V, Matthay MA. Allogeneic human mesenchymal stem cells for treatment of *E. coli* endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci USA. 2009; 106:16357–16362. [PubMed: 19721001]
22. Becchi C, Pillozzi S, Fabbri LP, Al Malyan M, Cacciapuoti C, Della BC, Nucera M, Masselli M, Boncinelli S, Arcangeli A, Amedei A. The increase of endothelial progenitor cells in the peripheral blood: a new parameter for detecting onset and severity of sepsis. Int J Immunopathol Pharmacol. 2008; 21:697–705. [PubMed: 18831938]
23. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest. 2000; 105:71–77. [PubMed: 10619863]
24. Zampetaki A, Kirton JP, Xu Q. Vascular repair by endothelial progenitor cells. Cardiovasc Res. 2008; 78:413–421. [PubMed: 18349136]
25. Timmermans F, Van Hauwermeiren F, De Smedt M, Raedt R, Plasschaert F, De Buyzere ML, Gillebert TC, Plum J, Vandekerckhove B. Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors. Arterioscler Thromb Vasc Biol. 2007; 27:1572–1579. [PubMed: 17495235]

26. Povsic TJ, Zavodni KL, Vainorius E, Kherani JF, Goldschmidt-Clermont PJ, Peterson ED. Common endothelial progenitor cell assays identify discrete endothelial progenitor cell populations. Am Heart J. 2009; 157:335–344. [PubMed: 19185643]
27. Burnham EL, Mealer M, Gaydos J, Majka S, Moss M. Acute lung injury but not sepsis is associated with increased colony formation by peripheral blood mononuclear cells. Am J Respir Cell Mol Biol. 2010; 43:326–333. [PubMed: 19843706]
28. Dimmeler S, Zeiher AM. Endothelial cell apoptosis in angiogenesis and vessel regression. Circ Res. 2000; 87:434–439. [PubMed: 10988233]
29. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood. 2004; 104:2752–2760. [PubMed: 15226175]
30. Prater DN, Case J, Ingram DA, Yoder MC. Working hypothesis to redefine endothelial progenitor cells. Leukemia. 2007; 21:1141–1149. [PubMed: 17392816]
31. Thijssen DH, Vos JB, Verseyden C, van Zonneveld AJ, Smits P, Sweep FC, Hopman MT, de Boer HC. Haematopoietic stem cells and endothelial progenitor cells in healthy men: effect of aging and training. Aging Cell. 2006; 5:495–503. [PubMed: 17081158]
32. Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, Saemann M, Schwarzinger I, Buranyi B, Steiner G, Smolen JS. Endothelial progenitor cells in active rheumatoid arthritis: effects of tumour necrosis factor and glucocorticoid therapy. Ann Rheum Dis. 2007; 66:1284–1288. [PubMed: 17293363]
33. Soler MJ, Martinez-Estrada OM, Puig-Mari JM, Marco-Feliu D, Oliveras A, Vila J, Mir M, Orfila A, Vilaro S, Lloveras J. Circulating endothelial progenitor cells after kidney transplantation. Am J Transplant. 2005; 5:2154–2159. [PubMed: 16095494]
34. Liu L, Wei H, Chen F, Wang J, Dong JF, Zhang J. Endothelial progenitor cells correlate with clinical outcome of traumatic brain injury. Crit Care Med. 2011; 39:1760–1765. [PubMed: 21460712]
35. Lee ST, Chu K, Jung KH, Park HK, Kim DH, Bahn JJ, Kim JH, Oh MJ, Lee SK, Kim M, Roh JK. Reduced circulating angiogenic cells in Alzheimer disease. Neurology. 2009; 72:1858–1863. [PubMed: 19470969]
36. Sobrino T, Hurtado O, Moro MA, Rodriguez-Yanez M, Castellanos M, Brea D, Moldes O, Blanco M, Arenillas JF, Leira R, Davalos A, Lizasoain I, Castillo J. The increase of circulating endothelial progenitor cells after acute ischemic stroke is associated with good outcome. Stroke. 2007; 38:2759–2764. [PubMed: 17761925]
37. Huertas A, Testa U, Riccioni R, Petrucci E, Riti V, Savi D, Serra P, Bonsignore MR, Palange P. Bone marrow-derived progenitors are greatly reduced in patients with severe COPD and low-BMI. Respir Physiol Neurobiol. 2010; 170:23–31. [PubMed: 19895908]
38. Caramori G, Rigolin GM, Mazzoni F, Leprotti S, Campioni P, Papi A. Circulating endothelial stem cells are not decreased in pulmonary emphysema or COPD. Thorax. 2010; 65:554–555. [PubMed: 20522859]
39. Sala E, Villena C, Balaguer C, Rios A, Fernandez-Palomeque C, Cosio BG, Garcia J, Noguera A, Agusti A. Abnormal levels of circulating endothelial progenitor cells during exacerbations of COPD. Lung. 2010; 188:331–338. [PubMed: 20082199]

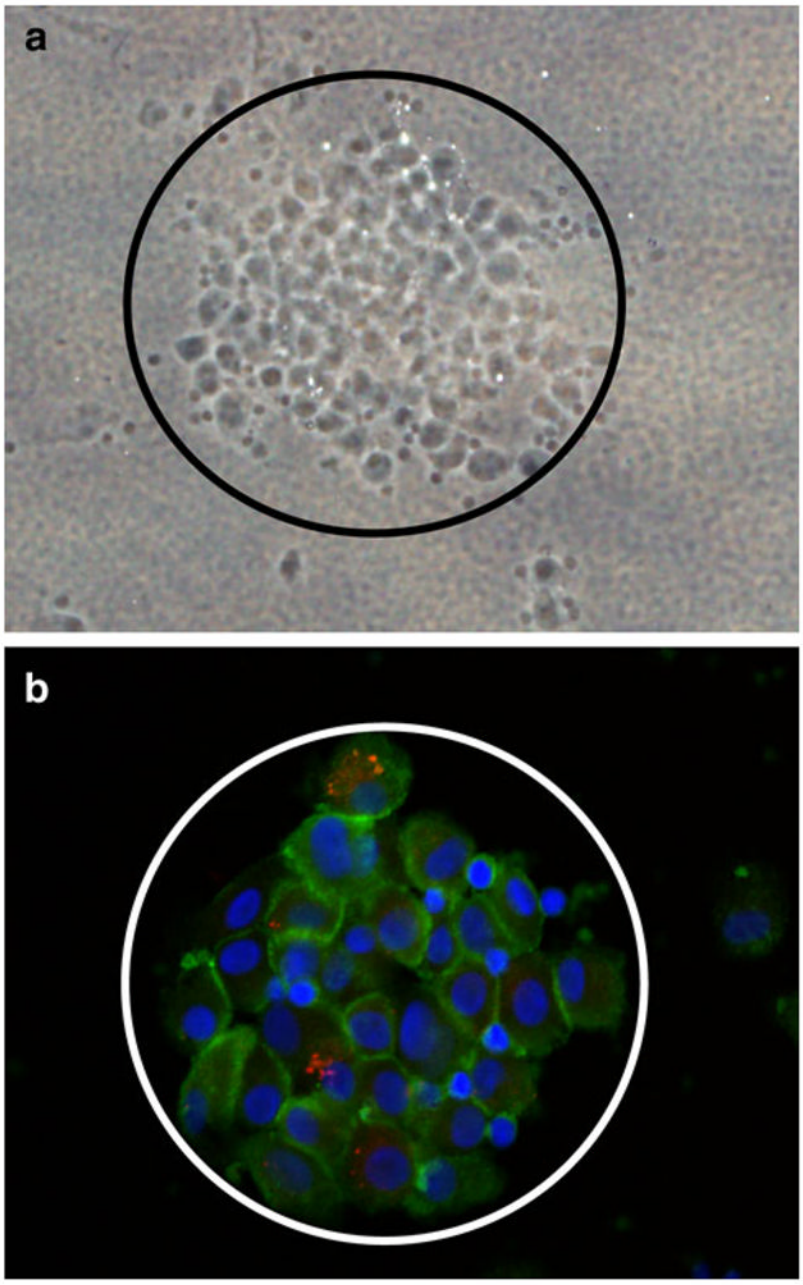

Fig. 1.

a Phase-contrast microscopy of endothelial progenitor cell (EPC) colonies on day 7 from a patient with sepsis. After the initial preplating step, nonadherent cells were collected from the supernatant of the culture and plated again on new fibronectin-coated wells (seen here). Colonies consist of a central cluster of rounded cells surrounded by thin, flat cells (×10). **b** Endothelial progenitor cell (EPC) colony from patient with sepsis. Similar to Fig. 1, nonadherent cells were collected from the supernatant of the culture after an initial plating step, and replated again on new fibronectin-coated wells. This picture depicts an overlapping fluorescent microscopic image of dil-acetylated low-density lipoprotein (LDL) uptake in *red*, *Ulex europaeus* lectin binding in *green*, and 4′,6-diamidino-2-phenylindole (DAPI), a *blue* nuclear stain (×10)

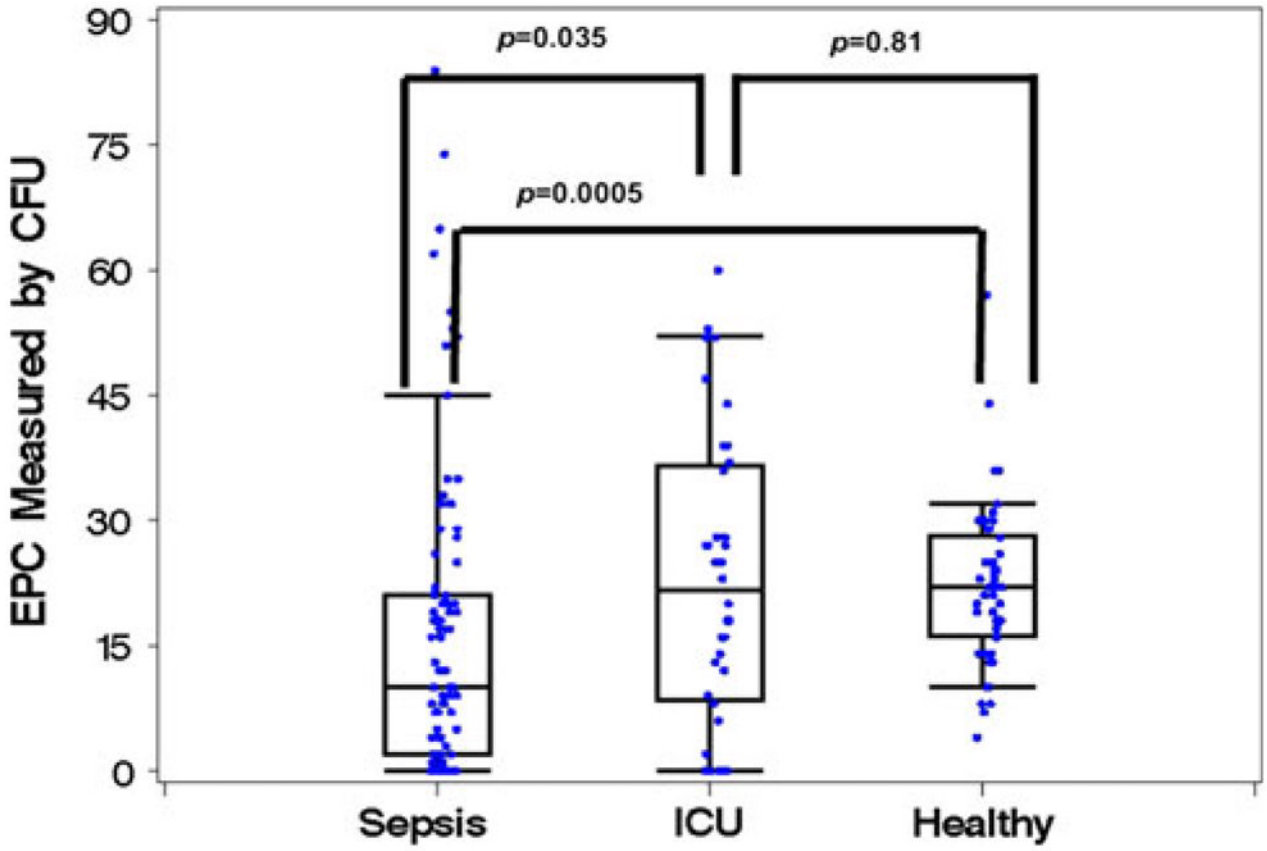

Fig. 2.

Numbers of EPC colony-forming units (CFU) from patients with sepsis ($n = 86$), ICU control subjects ($n = 37$), and healthy control subjects ($n = 49$). As compared with ICU controls, EPC CFU counts were significantly lower in sepsis patients ($p = 0.035$). There was no significant difference in EPC CFU counts between ICU controls and healthy controls. *Boxes* represent the median, 25th and 75th percentiles. The *whiskers* are the 10th and 90th percentiles. *Blue dots* represent individual data values

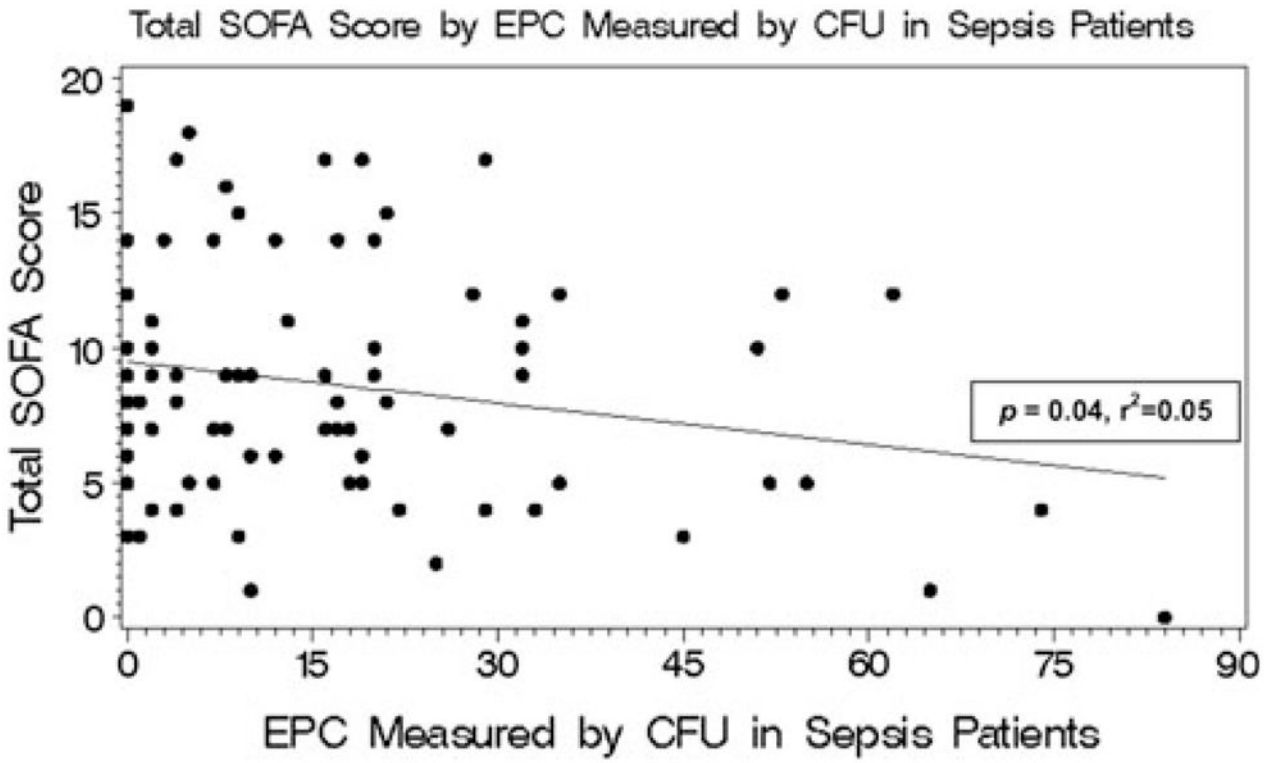

Fig. 3.

In patients with sepsis ($n = 86$), EPC CFU numbers inversely associated with Sepsis-Related Organ Failure Assessment (SOFA) score, adjusting for mortality, assuming an equal slope. Specifically, for a 10-cell decline in EPC counts measured by CFU, total SOFA score increased by 0.5 points ($p = 0.04$). *Black dots* represent individual data values, and the *line* is the fitted regression line. Results were similar after adjustments for presence or absence of shock ($p = 0.003$). p-Value was calculated from analysis of covariance (ANCOVA)

Table 1

Characteristics of patients with sepsis and intensive care unit control patients enrolled in study

Variable	Sepsis subjects	ICU control subjects	*p*-Value
Number of subjects (*N*)	95	37	
Age (years)[a]	51 (44–62)	55 (39–61)	0.9375
Gender (male, %)	57%	62%	0.5776
Race (%)			0.4865
White	18	11	
Black	82	89	
Source of infection, *n* (%)			
Blood	14 (15%)		
Pneumonia	53 (56%)		
Urine	14 (15%)		
GI	7 (7%)		
CSF	1 (1%)		
Skin and soft tissue	6 (6%)		
WBC (10^9/L)[a]	11,900 (8,045–14,900)	9,200 (7,440–12,800)	0.0378
CFU (per well)[a]	10 (2–21)	21.5 (8.5–36.5)	0.0347
SOFA score[a]	8 (5–12)	2 (1–4)	< 0.0001
APACHE II score[a]	19 (15–26)	9 (6–14)	< 0.0001
ICU LOS (days)[a]	8 (4–13)	2 (1–4)	< 0.0001
Hospital LOS (days)[a]	17 (10–28)	9 (4–16)	0.0002
Death (%)	26%	5%	0.0075

APACHE Acute Physiology and Chronic Health Evaluation, *CFU* colony-forming unit, *ICU* intensive care unit, *WBC* white blood count, *LOS* length of stay, *SOFA* Sepsis-Related Organ Failure Assessment, *GI* gastrointestinal, *CSF* cerebrospinal fluid

[a]Results presented as medians with interquartile range