Published in final edited form as:

J Surg Res. 2011 May 1; 167(1): 140–150. doi:10.1016/j.jss.2009.06.014.

Elevated Monocytes in Patients with Critical Limb Ischemia Diminish after Bypass Surgery

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Abstract

Introduction—Mononuclear cells (MNC) increase neovascularization and ulcer healing after injection into an ischemic extremity. Circulating MNC are composed of lymphocytes (85%), monocytes (15%) and endothelial progenitor cells (EPC; 0.03%). We hypothesized that ischemic limbs secrete paracrine signals to recruit bone marrow-derived monocytes and EPC into the circulation, such that patients with critical limb ischemia (CLI) have increased circulating monocytes compared to control patients. We also hypothesized that circulating monocytes and EPC recruitment decrease after resolution of ischemia with successful revascularization.

Methods—We reviewed the records of all patients at the VA Connecticut Healthcare System undergoing lower extremity peripheral bypass surgery between 2002 and 2007, only including patients with both preoperative and postoperative complete blood counts with differentials.

Results—Patients with CLI (n=24) had elevated preoperative monocyte counts compared to control patients (n=8) (0.753±0.04 vs. 0.516±0.05; p=0.0046), whereas the preoperative lymphocyte counts were not significantly different. After revascularization, ischemic patients had decreased monocyte counts compared to control patients (-20% vs. +55%; p=.0003), although lymphocyte counts were unchanged in both groups. Diabetic patients also had reduced postoperative monocyte counts (-32% vs. +13%; p=0.035). Multivariable logistic regression demonstrated that the only factor that independently predicted reduced postoperative monocyte count was preoperative CLI (p=0.038).

Conclusions—Patients with CLI have increased numbers of circulating monocytes, and the monocyte number decreases with resolution of ischemia after successful revascularization. Circulating monocytes may be a clinically useful perioperative marker in patients with CLI undergoing vascular surgery.

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Keywords

Blood Cells; Ischemia; Claudication; Monocyte; Peripheral Vascular Disease

Introduction

The therapeutic effect of mononuclear cells (MNC) in patients with peripheral arterial disease (PAD) is being reported with increasing frequency. Tateishi-Yuyama and colleagues reported increased ankle-brachial index (ABI), transcutaneous oxygen pressure, and pain-free walking time 4 weeks after injection of autologous bone marrow-derived MNC into the ischemic extremity of patients with PAD.¹ Bartsch and colleagues reported early and mid-term results of the TAM-PAD study showing significant symptomatic improvement after combined intraarterial and intramuscular injection of bone marrow-derived MNC in patients with claudication; pain-free walking distance increased 3.7-fold, and the ABI was significantly improved, even after 13 months.^{2,3} In contrast to studies evaluating bone marrow-derived cellular therapy, Ishida and colleagues used peripheral blood mononuclear cells in patients with PAD. These MNC were mobilized with G-CSF and then harvested and injected intramuscularly. After four weeks they observed a significant improvement in ABI, healing of ischemic ulcers, and increased mean maximum walking distance.⁴

MNC are a heterogeneous population composed of both CD34+ (hematopoietic) and CD34- (lymphocytic and monocytic) cells that can be differentially isolated in the laboratory using gradient centrifugation. In most patients, the circulating MNC population consists of approximately 85% lymphocytes, 15% monocytes, and 0.03% endothelial progenitor cells (EPC), which are thought to be a component of the monocyte fraction.⁵ The therapeutic properties of the MNC population have classically been attributed to the CD34+ EPC component, since EPC are thought to be protective in both acute and chronic vascular injury. In fact, Saigawa and colleagues noted that the clinical effectiveness of autologous bone marrow implantation, as measured by an improvement in ABI, was strongly correlated with the number of CD34+ cells delivered to the ischemic limb.⁶

However the MNC monocyte fraction may also play an important role, either separately or in conjunction with EPC. Studies have shown that patients with PAD have increased inflammatory markers, including monocyte chemoattractant protein-1 (MCP-1), a paracrine factor implicated in recruitment of monocytes.^{7,8} Interestingly, MCP-1 is also associated with angiogenesis in ischemic hindlimb animal models. Seidler and colleagues reported that intra-arterial infusion of MCP-1 in a pig model after unilateral femoral artery occlusion stimulated collateral artery growth.^{9,10} In a human study of patients with PAD, elevated monocytes were the only white blood cell type that independently correlated with the presence of atherosclerotic PAD.¹¹ These studies suggest a therapeutic role for monocytes in patients with ischemia.

We believe that monocytes may play an important role in stimulating angiogenesis and relieving limb ischemia. Since ischemia, or other factors related to patient presentation such as infection or nonhealing ulcers, may increase circulating monocytes in patients with PAD, it is possible that in patients with severe ischemia, the ischemic areas secrete paracrine factors such as MCP-1 to recruit bone marrow-derived MNC, including monocytes, into the circulation. We hypothesize that these monocytes are recruited into the circulation to levels that are detectable with conventional laboratory testing. We also examined whether relief of ischemia, and/or infection, and/or wound healing, by successful surgical revascularization diminishes the stimulus for monocyte recruitment and, consequently, the numbers of detectable monocytes in the peripheral circulation.

Methods

The records of all consecutive lower extremity peripheral bypass surgery cases performed at the VA Connecticut Healthcare Systems, West Haven, CT, between July 2002 and June 2007 were reviewed. This study was approved by the Institutional Review Board at the VA Connecticut Healthcare Systems.

Patients were selected for this study if they had lower extremity peripheral bypass surgery that was functionally successful. Patients were included in the study if they had a preoperative complete blood count (CBC) with differential recorded within one year prior to bypass surgery as well as a postoperative CBC with differential recorded between four months and 1.5 years after performance of the bypass surgery. Lab results between the time of surgery and up to 4 months after the procedure were not included. The primary study time frame is defined for each patient as the time bounded by the dates of the preoperative and postoperative lab tests.

Patients were excluded from the study if the surgery was a revision procedure, in cases where staged surgeries for bilateral lower extremities resulted in overlapping lab tests, and if the bypass surgery was an inflow procedure, i.e. with a target vessel proximal to the above-knee (AK) popliteal artery. In addition, patients having procedures complicated by limb-threatening graft failure within the study time frame were excluded; for example, if graft thrombosis required graft revision or amputation, or if persistent graft infection required graft removal or lifetime suppressive antibiotics. However, patients with graft thrombosis that did not result in limb-threatening ischemia were included in the study. Death prior to a postoperative CBC would exclude the patient due to insufficient data, and amputation would exclude the patient due to graft failure and unsuccessful resolution of limb ischemia at the time of the postoperative CBC.

Patient risk factors were determined by chart review. Demographic variables were grouped into patient characteristics, comorbidities, medications, surgical variables, and preoperative lab values. Patient characteristics included the following: age, BMI, race, sex, and smoking status (never, prior, or current smoker). Patient comorbidities included: anesthesia ASA grade, diabetes status, presence of heart disease (either coronary artery disease or arrhythmia), ejection fraction (EF, presumed normal or > 50% in patients with no heart disease), hypertension, chronic obstructive pulmonary disease (COPD), and renal disease. Patient medications were also analyzed, and these included: statin therapy, ACE-inhibitors, beta-blockers, calcium channel blockers, antiplatelet agents (including aspirin) and anticoagulation therapy. Surgical variables included the following: type of graft (native or prosthetic), outflow vessel (above-knee (AK) popliteal, below-knee (BK) popliteal, or tibial), affected extremity (left or right), and preoperative minor infection, defined as cellulitis, toe gangrene, or osteomyelitis localized to the foot. Preoperative lab values included the components of the CBC with differential, as well as creatinine as a measure of renal function and albumin as a measure of global synthetic function.

Outcome variables were grouped by time frame. The peri-operative time frame was defined as events within one month of surgery, and included peri-operative myocardial infarction (MI) and stroke. Events within the study time frame were defined as events between the time of surgery and the date of the postoperative CBC; these included postoperative minor infection, defined as local wound infection or cellulitis, toe gangrene, or osteomyelitis confined to the foot, but without graft infection; and graft status (patent or thrombosed). Outcomes through final follow-up occurred after the postoperative CBC date until the date of last contact with the patient. These events included graft status (patent or thrombosed), limb status (intact or proximal amputation, consisting of AKA or BKA), and mortality. All

patient information with respect to the outcome variables was gathered until the date of last patient contact, which extended beyond the primary study time frame.

The primary outcome variable of interest in this study was the monocyte count ratio. Each cell count was derived from the CBC by multiplying the white blood cell (WBC) count by the percentage of cells in the differential. Each cell ratio was calculated by dividing the postoperative cell count by the preoperative cell count; a ratio greater than one corresponds to an increase in circulating cells postoperatively, whereas a ratio less than one corresponds to a decrease in circulating cells postoperatively.

Results are reported as mean $\pm$ SEM. Categorical variables were analyzed using Pearson's Chi-Square or the Fisher exact test. Continuous variables were analyzed using ANOVA. Survival data was analyzed using Kaplan-Meier statistics, and the curves were compared using the log rank test. The effect of multiple patient risk factors on the dependent variable of interest was analyzed by multivariable logistic regression. All tests were 2-tailed and p values ≤ 0.05 were considered statistically significant. (Statview 5.0, SAS Institute, Cary, NC).

Results

Demographics

There were a total of 110 lower extremity peripheral bypass surgery cases performed at the VA Connecticut Healthcare System between July 2002 and June 2007. A total of 32 cases from the initial 110 charts reviewed met the inclusion, but not the exclusion, criteria and were the subject of this study.

Three-fourths of the study population (n=24) had baseline critical limb ischemia (CLI; Fontaine Stage III-IV), and presented with rest pain, chronic foot ulceration, and/or gangrene requiring operative intervention, and a mean preoperative ankle-brachial index (ABI) of 0.58 ± 0.05 . One-fourth of our study population (n=8) did not present with signs of CLI, i.e. had no baseline ischemia and a mean preoperative ABI of 0.88 ± 0.13 (p=0.01), and were designated the control group. Half of these patients (n=4) had bypass surgery for exclusion of an asymptomatic popliteal aneurysm and the other half (n=4) had surgery for relief of intermittent claudication (Fontaine Stage II).

The demographics of these patients are listed in Table 1. The mean age of CLI patients was 67.6 ± 1.7 years and the mean age of control patients was 72.6 ± 5.1 years; there was no significant difference in age between the two groups (p=0.242). All of the patients were men, consistent with studies conducted within the VA system. All of the patients were either Caucasian (n=26) or African American (n=6) but there was no difference between the two groups based on race. Diabetes (n=10) was more prevalent in the group with CLI as compared to control patients (41.7% vs. 0%; p=0.035). Smoking was also distributed unequally; of the patients who had never smoked (n=3) none were in the CLI group (0% of CLI patients vs. 37.5% of controls; p=0.003). Of the patients who were prior smokers but had quit before the surgery (n=14) there was little difference between groups (41.7% of CLI patients vs. 50% of controls); and of the patients who were current smokers at the time of the surgery (n=15) the majority were in the CLI group (58.3% of CLI patients vs. 12.5% of controls; p=0.003). There was no significant difference between the two groups for age, sex, statin therapy, type of graft, outflow vessel, or operative extremity (Table 1).

Patients with CLI had increased numbers of circulating monocytes per high-powered field (0.753 ± 0.04 vs. 0.516 ± 0.05 ; p=0.0046) but lymphocyte numbers were not significantly different (1.979 ± 0.14 vs. 1.912 ± 0.22 ; p=0.814). These data are consistent with our

hypothesis that patients with baseline critical ischemia have increased number of circulating monocytes recruited into the circulation. Patients with CLI also had elevated preoperative WBC counts (9.517 ± 0.56 vs. 7.225 ± 1.10 ; $p=0.055$) and neutrophil counts (6.458 ± 0.53 vs. 4.612 ± 0.93 ; $p=0.092$) compared to control patients, but these differences were not statistically significant (Table 1).

Outcomes

Postoperative outcomes are presented in Table 2. Follow-up was complete in all patients; however, the mean follow-up time was slightly shorter in patients with ischemia compared to control patients (2.14 years vs. 3.26 years; $p=0.042$). Consistent with the study design, all deaths and amputations occurred outside of the study time frame, which was defined as the time bounded by the dates of the preoperative and postoperative CBC. Although both deaths ($n=9$) and amputations ($n=4$) occurred only in patients with CLI (deaths 37.5% vs. 0%; $p=0.070$; amputations 16.7% vs. 0%; $p=0.550$), the increased mortality and morbidity in the CLI group were not statistically significant (Table 2). The mean postoperative ABI was not increased compared to preoperative values in the control group (1.11 ± 0.08 vs 0.88 ± 0.13 ; $p=0.09$), but was increased in patients with CLI (0.83 ± 0.06 vs. 0.58 ± 0.05 ; $p=0.008$).

The fate of the grafts was examined both within the study time frame as well as through the date of complete follow-up. Of the patients who had thrombosed grafts within the study time frame ($n=4$) all were in the CLI group (16.7% of CLI patients vs. 0% of controls; $p=0.550$). After the study time frame, the majority of patients who had thrombosed grafts (total $n=9$) were in the CLI group ($n=8$ or 33.3% of CLI patients vs. $n=1$ or 12.5% of controls; $p=0.386$; Table 2).

The survival curves for mortality rate, amputation rate, and thrombosis rate are shown in Figure 1. Patients with CLI had increased mortality compared to control patients ($p=0.032$; Figure 1A); all deaths ($n=9$) occurred in CLI patients within three years of the surgery; cumulative survival was 47% at three years.

There was no difference in cumulative limb salvage between groups ($p=0.168$; Figure 1B). All amputations ($n=4$) occurred in CLI patients within two years of the surgery; cumulative limb salvage was 76% at two years. There was no difference in cumulative primary graft patency between groups ($p=0.186$; Figure 1C). There were a total of 9 thrombosed grafts; $n=8$ in the CLI group and $n=1$ in the control group, with all graft thromboses occurring within two years of the surgery. Primary graft patency was 58% at two years in patients with CLI compared to 87.5% at two years in control patients ($p=0.186$; Figure 1C).

Monocyte Counts

The primary dependent variable of interest was the monocyte ratio, and on univariable analysis only four risk factors had a significant effect on this ratio. The univariable analyses are summarized in Table 3. Consistent with our hypothesis, patients with CLI had decreased monocyte counts after revascularization compared to patients without CLI (-20% vs. +55%; $p=.0003$). In addition, diabetic patients also had significantly reduced postoperative monocyte counts compared to patients without diabetes (-32% vs. +13%; $p=0.035$). Both hypertension and use of a beta-blocker were also associated with reduced postoperative monocyte ratios (Table 3). Age greater than or less than 70, statin therapy, smoking status, type of graft, outflow vessel, operative extremity, graft status, and mortality had no significant effect on the postoperative monocyte count (Table 3).

Figure 2 shows the effect of CLI on peripheral blood cell populations. Since circulating mononuclear cells consist of both monocytes and lymphocytes, we determined the effect of CLI on both the monocyte and the lymphocyte counts (Figure 2). Unlike the diminished

monocyte ratio observed in patients with CLI (-20% vs. +55%; $p=0.0003$; Figure 2A), lymphocyte ratios were unchanged in both ischemic and control patients (-10% vs. +1%; $p=0.404$; Figure 2B). In patients with CLI, both the WBC and neutrophil counts were also significantly decreased after revascularization (Figure 2). The diminished WBC ratio in patients with CLI (-14% vs. +26%; $p=0.008$; Figure 2C) paralleled the diminished neutrophil ratio (-9% vs. +39%; $p=0.037$; Figure 2D).

To determine the significance of the risk factors identified by univariable analysis, we performed multivariable logistic regression to identify which of these risk factors independently predicted diminished postoperative monocyte count (Table 4). Our results demonstrate that the only factor that independently predicted reduced postoperative monocyte count was preoperative CLI ($p=0.038$). Age, thrombosed graft, native graft, smoking status, diabetes, statin therapy, tibial outflow, and mortality did not predict diminished postoperative monocyte count (Table 4).

Discussion

We report two findings consistent with our hypothesis. First, the preoperative monocyte count was elevated in patients with CLI compared to control patients with no baseline critical ischemia. Second, the monocyte count decreased significantly after successful bypass surgery in patients with CLI compared to control patients. In addition to limb ischemia, diabetes was also associated with decreased monocyte counts after bypass surgery. However, using multivariable analysis, only CLI independently predicted a decreased monocyte count after bypass surgery.

We believe that one of our most significant findings was that the preoperative monocyte count was elevated in patients with CLI and reduced preoperative ABI, compared to control patients with normal ABI. This observation is consistent with our hypothesis that patients with baseline CLI secrete paracrine factors such as MCP-1 that stimulate monocyte release from the bone marrow, promoting angiogenesis and relief of ischemia. Notably, the monocytes were the only WBC component that had significantly different preoperative values when comparing patients with CLI to controls ($p=0.0046$; Table 1). These results also agree with the report from Nasir and colleagues that demonstrated that elevated circulating monocytes were the only WBC fraction that correlated significantly and independently with the presence of PAD.¹¹ Further studies that quantify the degree of ischemia may lead to a quantitative correlation between the magnitude of ischemia and the degree of elevation of the preoperative monocyte count, although it is also possible that the monocyte elevation is due to associated factors such as infection and nonhealing wounds, rather than the ischemia per se.

We believe that increased recruitment of monocytes in patients with CLI is intimately related to recruitment of EPC, and that circulating numbers of EPC correlate inversely with the severity of PAD. We propose that monocytes are recruited in parallel with EPC, perhaps via the same paracrine mechanism. This possibility is supported by the recent study by Sieveking and colleagues, who isolated and defined two different populations of putative EPC, early endothelial progenitor cells (early EPC) and late outgrowth endothelial cells (late OEC). Early EPC, which appear in culture after 4 to 7 days, are similar to those originally described by Asahara and colleagues,¹² augment angiogenesis in a paracrine fashion, and have been used in therapeutic studies.¹³ In contrast, late OEC appear in culture after 14 to 21 days, form colonies with high proliferation rates, and are capable of forming vascular networks in vitro. These two different populations have been previously classified as EPC since they express endothelial markers.¹⁴ This lineage is represented in Figure 3. It has been proposed that early EPC are not true progenitors of endothelial cells but are actually

monocytic cells capable of indirectly facilitating angiogenesis in a paracrine fashion.¹⁴ These results confirm an important study by Yoder and colleagues that reported that early EPC are cells of low proliferative potential, are hematopoietic in origin, and differentiate into macrophages rather than endothelial cells in culture.¹⁵

Thus it is entirely possible that monocytic cells (called “early EPC” by Sieveking, but which may not be progenitor cells, despite the nomenclature) stimulate true EPC (called “late OEC” by Sieveking) to respond to ischemia. This possibility correlates with our observation that monocytes are increased in patients with ischemia and decreased after resolution of ischemia, since recruitment of EPC depends on both monocytes and on the severity of vascular injury.

Furthermore, in patients with CLI that is severe enough to require peripheral arterial bypass surgery, endogenous mechanisms are necessarily insufficient to relieve the ischemia, despite elevated levels of MNC, monocytes, and other paracrine factors. This suggests that the monocyte may not be the ultimate effector cell responsible for healing, and reinforces the theory that the EPC is the effector cell. It is possible that elderly and chronically ill patients with deficient reserves of EPC may experience greater increases in monocytes in the setting of ischemia due to ineffective EPC recruitment or other monocyte-EPC interactions.

Two demographic variables that were significantly different in ischemic patients compared to controls were diabetes and smoking. Diabetes was significantly more prevalent in the group with CLI as compared to control patients, and smoking status was also distributed unequally, with smokers being at greater risk for more severe disease. For these variables, our results parallel the known risk factors for PAD and CLI with remarkable accuracy.¹⁶

In addition, we found that monocytes, but not lymphocytes, decreased in patients with CLI after successful revascularization with a surgical bypass procedure (Figure 2). This decrease in monocytes after resolution of ischemia is consistent with our hypothesis that monocytes recruit EPC to promote healing in the setting of ischemia. These results are also consistent with the report from Seidler and colleagues describing the angiogenesis response to MCP-1 in ischemic tissue.¹⁰ When resolution of ischemia is achieved, the demand for active cell populations that target vessel healing is diminished, decreasing both circulating MCP-1 and monocytes. However, since the lymphocyte ratio did not change postoperatively in either the CLI patients or the control patients, we believe that our results strongly suggest that it is the monocyte fraction, not the lymphocyte fraction, of the mononuclear cell population which is responsive to ischemia.

In addition to a decreased postoperative monocyte count in patients with CLI, we found that the postoperative WBC and neutrophil counts were also diminished in these patients (Figure 2). Since neutrophils comprise approximately 85% of circulating WBC, we believe that the diminished postoperative neutrophil count is primarily responsible for the diminished WBC count. However, since neutrophils are not mononuclear cells, they cannot account for the decrease in monocyte count. We speculate that the diminished WBC and neutrophil counts in patients with successful limb revascularization reflects the resolution of preoperative inflammation present in these critically ill patients. This is consistent with the slightly elevated pre-operative WBC counts in patients with CLI, as well as elevated rates of smoking and diabetes, compared to control patients (Table 1). In addition, we believe that the increased inflammation in patients with CLI may account for their diminished long-term survival (Figure 1A). However, routine laboratory studies of inflammation, such as with the erythrocyte sedimentation rate or hsCRP, was not performed in this group of patients.

One distinct advantage of our control group is that it is composed of patients without baseline ischemia who underwent peripheral bypass surgery. This is an unusual patient

population, since the vast majority of patients who undergo open limb revascularization procedures in our patient population have CLI. The incidental discovery of asymptomatic popliteal aneurysms is also not common. In addition, surgery is not the first line treatment for patients with claudication, and is recommended only in patients whose severe claudication interferes significantly with their activities or with their work. The advantage of our control group is that it allows comparison of patients with no baseline ischemia to patients with critical ischemia before and after the same intervention. We believe that the small increases in monocyte, WBC, and neutrophil ratios in control patients (Figure 2) may reflect natural variation in this small number of patients; additional studies with larger numbers of patients will clarify whether these increases are truly significant.

A major limitation of this study is the small number of patients. This is primarily due to the exclusion of large numbers of patients because they did not have a CBC with differential performed as part of their perioperative laboratory studies. However, enough patients were present for us to detect consistent decreases in postoperative monocyte counts. Since laboratory tests may be ordered more frequently for patients admitted to the hospital, selection of an asymptomatic control group may be difficult in retrospective studies. In addition, our study design selected control patients without limb-threatening graft failure, so our survival data (Figure 1) was biased towards patients with better outcomes and is not generalizable to all patients with limb revascularization in our institution; on the other hand, selection of patients with patent grafts, or only mild graft failure, is necessary to determine whether relief of ischemia influences the monocyte count. Additional studies that are not retrospective must be conducted to confirm the results of this study. In addition, further studies may allow correlation of circulating cytokines and other factors to the level of ischemia and to the circulating monocyte count. Finally, we measured the postoperative CBC at a minimum of four months after surgery to minimize effects of transient postoperative changes associated with injury, inflammation, and stress of the surgical procedure. Although we were limited to data obtained in retrospective format, and therefore had no control over the dates of test selection, we believe that the random sampling of both preoperative and postoperative tests increases the power of our findings, as there is no test selection bias.

Our study suggests that CLI is associated with increased circulating monocytes and that relief of CLI after successful revascularization results in diminished numbers of monocytes. We believe that this is due to the resolution of ischemia and a subsequent decrease in the demand for regenerative stem cells recruited from the bone marrow. However, the true effector cell remains unclear: of the components of the mononuclear cell fraction, our results strongly suggest a role for monocytes rather than lymphocytes. However, as EPCs are thought to be a component of the monocyte population, it remains to be determined whether the protective and healing effect is due to monocyte activity, EPC activity, or the coordinated activity of both cell types. It is reasonable to conclude, however, that circulating monocytes may be a clinically useful perioperative marker in patients with PAD.

Acknowledgments

Supported by a Doris Duke Clinical Research Fellowship, the NIH Career Development award HL079927, the American Vascular Association William J. von Liebig Award, as well as with resources and the use of facilities at the VA Connecticut Healthcare System, West Haven, CT.

References

1. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a

- pilot study and a randomised controlled trial. *Lancet*. 2002; 360(9331):427–435. [PubMed: 12241713]
2. Bartsch T, Brehm M, Zeus T, Strauer BE. Autologous mononuclear stem cell transplantation in patients with peripheral occlusive arterial disease. *J Cardiovasc Nurs*. 2006; 21(6):430–432. [PubMed: 17293730]
 3. Bartsch T, Brehm M, Zeus T, Kogler G, Wernet P, Strauer BE. Transplantation of autologous mononuclear bone marrow stem cells in patients with peripheral arterial disease (the TAM-PAD study). *Clin Res Cardiol*. 2007; 96(12):891–899. [PubMed: 17694378]
 4. Ishida A, Ohya Y, Sakuda H, Ohshiro K, Higashiuesato Y, Nakaema M, Matsubara S, Yakabi S, Kakihana A, Ueda M, Miyagi C, Yamane N, Koja K, Komori K, Takishita S. Autologous peripheral blood mononuclear cell implantation for patients with peripheral arterial disease improves limb ischemia. *Circ J*. 2005; 69(10):1260–1265. [PubMed: 16195628]
 5. Dong C, Goldschmidt-Clermont PJ. Endothelial progenitor cells: a promising therapeutic alternative for cardiovascular disease. *J Interv Cardiol*. 2007; 20(2):93–99. [PubMed: 17391216]
 6. Saigawa T, Kato K, Ozawa T, Toba K, Makiyama Y, Minagawa S, Hashimoto S, Furukawa T, Nakamura Y, Hanawa H, Kodama M, Yoshimura N, Fujiwara H, Namura O, Sogawa M, Hayashi J, Aizawa Y. Clinical application of bone marrow implantation in patients with arteriosclerosis obliterans, and the association between efficacy and the number of implanted bone marrow cells. *Circ J*. 2004; 68(12):1189–1193. [PubMed: 15564705]
 7. Petrkova J, Szotkowska J, Hermanova Z, Lukl J, Petrek M. Monocyte chemoattractant protein-1 in patients with peripheral arterial disease. *Mediators Inflamm*. 2004; 13(1):39–43. [PubMed: 15203564]
 8. Hoogeveen RC, Morrison A, Boerwinkle E, Miles JS, Rhodes CE, Sharrett AR, Ballantyne CM. Plasma MCP-1 level and risk for peripheral arterial disease and incident coronary heart disease: Atherosclerosis Risk in Communities study. *Atherosclerosis*. 2005; 183(2):301–307. [PubMed: 16285993]
 9. Muhs A, Lenter MC, Seidler RW, Zweigerdt R, Kirchengast M, Weser R, Ruediger M, Guth B. Nonviral monocyte chemoattractant protein-1 gene transfer improves arteriogenesis after femoral artery occlusion. *Gene Ther*. 2004; 11(23):1685–1693. [PubMed: 15470479]
 10. Seidler RW, Lenter MC, Guth BD, Doods H. Short-term intra-arterial infusion of monocyte chemoattractant protein-1 results in sustained collateral artery growth. *J Cardiovasc Pharmacol Ther*. 2007; 12(1):61–68. [PubMed: 17495259]
 11. Nasir K, Guallar E, Navas-Acien A, Criqui MH, Lima JA. Relationship of monocyte count and peripheral arterial disease: results from the National Health and Nutrition Examination Survey 1999–2002. *Arterioscler Thromb Vasc Biol*. 2005; 25(9):1966–1971. [PubMed: 15976323]
 12. 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(5302):964–967. [PubMed: 9020076]
 13. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A*. 2000; 97(7):3422–3427. [PubMed: 10725398]
 14. Sieveking DP, Buckle A, Celermajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. *J Am Coll Cardiol*. 2008; 51(6):660–668. [PubMed: 18261686]
 15. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood*. 2007; 109(5):1801–1809. [PubMed: 17053059]
 16. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *J Vasc Surg*. 2007; 45(Suppl S):S5–S67. [PubMed: 17223489]
 17. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res*. 2004; 95(4):343–353. [PubMed: 15321944]

18. Shantsila E, Watson T, Tse HF, Lip GY. New insights on endothelial progenitor cell subpopulations and their angiogenic properties. *J Am Coll Cardiol.* 2008; 51(6):669–671. [PubMed: 18261687]

Figure 1. Survival curves. A) Kaplan-Meier plot of cumulative patient survival. B) Kaplan-Meier plot of cumulative limb salvage. C) Kaplan-Meier plot of cumulative primary graft patency.

Figure 2.

Effect of critical limb ischemia (CLI) on peripheral blood cell populations. A) Bar graph shows the monocyte ratio in patients without and with CLI. B) Bar graph shows the lymphocyte ratio in patients without and with CLI. C) Bar graph shows the WBC ratio in patients without and with CLI. D) Bar graph shows the neutrophil ratio in patients without and with CLI.

Figure 3.

EPC and monocyte lineage diagram. Bold arrows show the pathways most commonly cited. Dashed arrow shows the pathway for possible paracrine stimulation. Adapted from Urbich¹⁷ and Shantsila.¹⁸ VE cadherin = vascular endothelial cadherin; VEGF-R2 = vascular endothelial growth factor receptor 2; vWF = von Willebrand factor; early “EPC” = early endothelial progenitor cell, found to be a monocyte, not a progenitor cell, by Sieveking and colleagues;¹⁴ question mark on early “EPC” monocyte cell membrane suggests specific markers are unknown; late outgrowth endothelial cell, found to be a true endothelial progenitor cell by Sieveking and colleagues.¹⁴

Table 1

Demographic Variables

Variable	Total Number	Total (%)	Control	Control (%)	Ischemic	Ischemic (%)	P value
Total patients	32		8		24		
Patient Characteristics							
Age (years)	68.9 ± 1.8		72.6 ± 5.1		67.6 ± 1.7		0.2423
BMI	27.4		28.3 ± 1.1		27.1 ± 1.1		0.5553
Race							
Caucasian	26	81.3%	8	100.0%	18	75.0%	0.2964
African American	6	18.8%	0	0.0%	6	25.0%	
Sex							
Male	32	100.0%	8	100.0%	24	100.0%	>0.9999
Female	0	0.0%	0	0.0%	0	0.0%	
Smoking Status							
Never a Smoker	3	9.4%	3	37.5%	0	0.0%	0.0028
Prior Smoker	14	43.8%	4	50.0%	10	41.7%	
Current Smoker	15	46.9%	1	12.5%	14	58.3%	
Patient Comorbidities							
Anesthesia ASA	3.16		2.63 ± 0.18		3.33 ± 0.10		0.0013
Diabetes							
No	22	68.8%	8	100.0%	14	58.3%	0.0353
Yes	10	31.3%	0	0.0%	10	41.7%	
Heart Disease							
No	13	40.6%	3	37.5%	10	41.7%	>0.9999
Yes	19	59.4%	5	62.5%	14	58.3%	
Ejection Fraction < 50%							
No	23	71.9%	5	62.5%	18	75.0%	0.6545
Yes	9	28.1%	3	37.5%	6	25.0%	
Hypertension							
No	3	9.4%	2	25.0%	1	4.2%	0.1468
Yes	29	90.6%	6	75.0%	23	95.8%	
COPD							

Variable	Total Number	Total (%)	Control	Control (%)	Ischemic	Ischemic (%)	P value
No	24	75.0%	7	87.5%	17	70.8%	0.6417
Yes	8	25.0%	1	12.5%	7	29.2%	
Renal Disease							
No	23	71.9%	7	87.5%	16	66.7%	0.3858
Yes	9	28.1%	1	12.5%	8	33.3%	
Patient Medications							
Statin Therapy							
No	11	34.4%	3	37.5%	8	33.3%	> 0.9999
Yes	21	65.6%	5	62.5%	16	66.7%	
ACE-Inhibitor							
No	18	56.3%	4	50.0%	14	58.3%	0.7035
Yes	14	43.8%	4	50.0%	10	41.7%	
Beta-Blocker							
No	8	25.0%	4	50.0%	4	16.7%	0.1522
Yes	24	75.0%	4	50.0%	20	83.3%	
Calcium Channel Blocker							
No	19	59.4%	5	62.5%	14	58.3%	> 0.9999
Yes	13	40.6%	3	37.5%	10	41.7%	
Antiplatelet Agent							
No	14	43.8%	4	50.0%	10	41.7%	0.7035
Yes	18	56.3%	4	50.0%	14	58.3%	
Anticoagulation							
No	26	81.3%	7	87.5%	19	79.2%	> 0.9999
Yes	6	18.8%	1	12.5%	6	25.0%	
Surgical Variables							
Type of Graft							
Native Vein	19	59.4%	4	50.0%	15	62.5%	0.6838
Prosthetic	13	40.6%	4	50.0%	9	37.5%	
Outflow Vessel							
AK Popliteal	10	31.3%	4	50.0%	6	25.0%	0.1091
BK Popliteal	13	40.6%	4	50.0%	9	37.5%	

Variable	Total Number	Total (%)	Control	Control (%)	Ischemic	Ischemic (%)	P value
Tibial	9	28.1%	0	0.0%	9	37.5%	
Operative Extremity							
Left	10	31.3%	1	12.5%	9	37.5%	0.3803
Right	22	68.8%	7	87.5%	15	62.5%	
Preoperative Minor Infection							
No	28	87.5%	8	100.0%	20	83.3%	0.5497
Yes	4	12.5%	0	0.0%	4	16.7%	
Preoperative Lab Values							
WBC Count (thousand cells per cubic mm)			7.225 ± 1.10		9.517 ± 0.56		0.0554
Lymphocyte Count			1.912 ± 0.22		1.979 ± 0.14		0.8143
Neutrophil Count			4.612 ± 0.93		6.458 ± 0.53		0.0923
Monocyte Count			0.516 ± 0.05		0.753 ± 0.04		0.0046
Eosinophil Count			0.147 ± 0.03		0.254 ± 0.05		0.2627
Basophil Count			0.032 ± 0.01		0.063 ± 0.01		0.1902
Hemoglobin (g/dL)			13.50 ± 0.63		13.58 ± 0.42		0.9190
Hematocrit (%)			40.18 ± 1.91		40.52 ± 1.14		0.8798
Platelets (thousand cells per cubic mm)			200.1 ± 16.34		263.3 ± 17.63		0.0592
Creatinine (mg/dL)			1.275 ± 0.20		1.646 ± 0.35		0.5595
Albumin (g/dL)			4.108 ± 0.23		3.904 ± 0.15		0.5393

Table 2

Outcome Variables

Variable	Total Number	Total (%)	Control	Control (%)	Ischemic	Ischemic (%)	P value
Total patients	32			8		24	
Mean Follow-Up Time (years)	2.42		3.26		2.14		0.0421
Peri-operative Outcomes							
Peri-operative MI							
No	32	100.0%	8	100.0%	24	100%	>0.9999
Yes	0	0.0%	0	0.0%	0	0.0%	
Peri-operative Stroke							
No	32	100.0%	8	100.0%	24	100%	>0.9999
Yes	0	0.0%	0	0.0%	0	0.0%	
Outcomes Within Study Time Frame							
Postoperative Minor Infection							
No	16	50.0%	5	62.5%	11	45.8%	0.6851
Yes	16	50.0%	3	37.5%	13	54.2%	
Graft Status							
Patent	28	87.5%	8	100.0%	20	83.3%	0.5497
Thrombosed	4	12.5%	0	0.0%	4	16.7%	
Outcomes Through Final Follow -Up							
Graft Status:							
Patent	23	71.9%	7	87.5%	16	66.7%	0.3858
Thrombosed	9	28.1%	1	12.5%	8	33.3%	
Limb Status:							
Intact	28	87.5%	8	100.0%	20	83.3%	0.5497
AKA or BKA	4	12.5%	0	0.0%	4	16.7%	
Mortality:							
Alive	23	71.9%	8	100.0%	15	62.5%	0.0699
Dead	9	28.1%	0	0.0%	9	37.5%	

Table 3

Univariable Analysis for Factors Affecting Monocyte Ratio

Variable	Risk Factor	Mean Monocyte Ratio	P-value
Patient Characteristics			
Preoperative CLI	No	1.554	0.0003
	Yes	0.799	
Age (years)	< 70	1.077	0.3726
	> 70	0.898	
Race	Caucasian African	1.076	0.0608
	American	0.604	
BMI	< 25	0.909	0.6244
	> 25	1.019	
Smoking Status	Never a Smoker	1.182	0.5655
	Prior Smoker	1.063	
	Current Smoker	0.878	
Patient Comorbidities			
Anesthesia ASA	< 3.5	1.038	0.3865
	> 3.5	0.837	
Diabetes	No	1.127	0.0348
	Yes	0.682	
Heart Disease	No	1.066	0.5228
	Yes	0.934	
Ejection Fraction < 50%	No	1.022	0.5895
	Yes	0.901	
Hypertension	No	1.707	0.0165
	Yes	0.913	
COPD	No	1.066	0.1748
	Yes	0.753	
Renal Disease	No	1.064	0.2229
	Yes	0.793	
Patient Medications			
Statin Therapy	No	0.975	0.9288
	Yes	0.994	
ACE-Inhibitor	No	0.919	0.4408
	Yes	1.076	
Beta-Blocker	No	1.377	0.0202
	Yes	0.858	
Calcium Channel Blocker	No	1.065	0.3560
	Yes	0.876	
Antiplatelet Agent	No	1.157	0.1320
	Yes	0.856	
Anticoagulation	No	1.018	0.5388

Variable	Risk Factor	Mean Monocyte Ratio	P-value
	Yes	0.859	
Surgical Variables			
Type of Graft	Native Vein	0.940	0.5693
	Prosthetic	1.057	
Outflow Vessel	AK Popliteal	1.192	0.0740
	BK Popliteal	1.071	
	Tibial	0.641	
Operative Extremity	Left	0.996	0.9552
	Right	0.984	
Preoperative Minor Infection	No	1.033	0.2368
	Yes	0.675	
Postoperative Minor Infection	No	1.047	0.5595
	Yes	0.929	
Graft Status	Patent	1.002	0.7126
(within study time frame)	Thrombosed	0.889	
Mortality	Alive	1.088	0.1055
(after study time frame)	Dead	0.732	

Table 4

Multivariable Logistic Regression Analysis

Variable	P-Value	Exp (Coef)	95% Lower	95% Upper
Age	0.5506	1.046	0.903	1.211
Thrombosed Graft	0.1212	0.021	1.62E-04	2.775
Native Graft	0.2664	0.186	0.01	3.619
Preoperative CLI	0.0377	91.883	1.291	6538
Smoking Status	0.9155	1.245	0.022	71.239
Diabetes	0.6373	0.423	0.012	15.169
Statin Therapy	0.5023	0.348	0.016	7.596
Tibial Outflow	0.9962	4.77E+08	0	∞
Mortality	0.534	0.317	0.008	11.852