Sustained Elevation in Monocyte Levels in Diabetic Patients

Abstract

Context: Diabetes and atherosclerosis are both pro-inflammatory states that may lead to elevations in monocyte levels. It was previously demonstrated that there is a reduction in monocyte levels after infra-inguinal bypass in patients with critical limb ischemia (CLI). We hypothesized that patients with diabetes would not realize the same reduction in monocyte levels post-bypass as patients without diabetes.

Objective: To determine whether patients with diabetes would have a sustained sub-clinical inflammation after resolution of CLI with infra-inguinal bypass, as marked by a persistent elevation of monocyte levels.

Design: Patients undergoing lower extremity vascular bypass surgery between 2003 and 2013 at the Syracuse VA Medical Center were retrospectively reviewed. Pre- and post-operative leukocyte count with differential were recorded for each patient and stratified according to the presence of diabetes.

Results: Patients with CLI and no bypass failure (n=43) were included for analysis of the primary outcome, change in monocyte level. Diabetic (DM+) patients (n=27) and non-diabetic (DM-) patients (n=16) had similar pre-operative leukocyte counts and differential (p>0.05). In DM-, there was a 5.6% decrease in monocyte count post-operatively, whereas there was an elevation in monocytes in DM+ (+20.8%; p<0.05). The overall rate of complications was significantly greater and the time to develop complications significantly less in DM+ (p<0.05).

Conclusion: Diabetic patients have a persistent elevation in monocyte levels even after infra-inguinal vascular bypass as compared with non-diabetic patients. This suggests diabetic patients have persistent sub-clinical inflammation even upon resolution of critical limb ischemia.

Keywords: Infra-inguinal bypass; Monocyte; Diabetes; Smoking

Abbreviations: CLI: Critical Limb Ischemia; DM+: Patients with Diabetes; DM-: Patients without Diabetes; PAD: Peripheral Arterial Disease

Introduction

The role of monocytes on the development of peripheral arterial disease (PAD) and critical limb ischemia (CLI) has been a source of great interest given the potential for therapeutic opportunities [1, 2]. It has previously been demonstrated that monocyte levels decrease after revascularization [2], consistent with the fact that they play an integral role in the early stages of atherosclerosis initiation, plaque rupture, and remodeling [3, 4]. The presence of diabetes potentiates this process through a combination of increasing oxidative stress [5] and non-enzymatic glycation of molecules which activate inflammatory mediators and macrophages and encourage atheroma formation [3]. The endothelial cells of diabetics secrete cytokines that inhibit the synthesis of new collagen by vascular smooth muscle cells and promote the production of matrix metalloproteinases, allowing plaques to rupture more easily and form thrombi [5]. Over time, this process leads to occlusion of the vessel, decreased arterial perfusion, and ultimately CLI and limb loss. Diabetes induces
a pro-inflammatory state with a tendency toward monocyte recruitment and activation, suggesting that patients with diabetes may have ongoing sub-clinical inflammation as measured by monocyte elevation.

In this study, we investigated monocyte levels before and after surgical revascularization in patients with CLI. Specifically, we hypothesized that patients with diabetes would have a sustained elevation of monocyte levels after vascular bypass procedures as compared with patients without diabetes. This preliminary retrospective study demonstrates that monocyte levels do not necessarily decrease after revascularization in patients with diabetes. This serves as a foundation for future prospective studies investigating monocyte levels in patients with and without diabetes after bypass, including measuring the ratio of pro-inflammatory to anti-inflammatory monocytes, as a potential monitoring tool.

**Methods**

All patients who underwent a lower extremity bypass surgery between 2003 and 2013 at the Syracuse VA Medical Center were reviewed retrospectively. Patients who underwent inflow procedures including femoral-femoral bypass or aortobifemoral/axillofemoral bypasses were excluded from this study. Leukocyte levels with differentials including neutrophils, lymphocytes, monocytes, eosinophils and basophils were recorded at two time points: pre-operative and post-operative. The pre-operative time point closest to the day of the procedure was recorded and post-operative leukocyte counts nearest to one year post-operatively were preferentially selected, however there was a wide time range, consistent with the nature of a retrospective study (Figure 1). Patients were excluded from the study if they did not have a leukocyte count with differential for both data points. Patients requiring more than one bypass during the study period were included only once for analysis, but only the data from their most recent bypass was analyzed. If the pre-operative leukocyte count was within one year of a previous bypass, the patient was excluded from the study. For each cell count within the differential, the percent change between pre- and post-operative values was calculated.

Patients were classified according to Fontaine Stage in order to stratify whether or not they had CLI. Patients with Fontaine stage III (rest pain) or IV (ischemic ulcers or gangrene) were classified as having CLI [6]. Since the primary objective of the study was to analyze the response of monocyte levels in diabetic patients with CLI after bypass, patients with Fontaine stage I (asymptomatic disease) or II (intermittent claudication) were excluded from the study [6]. Patients with a failed graft, as defined by one of: post-operative graft occlusion, classification as Fontaine stage III or IV post-operatively, or limb loss were excluded from the study so as not to include secondarily elevated monocyte levels. Patients were classified as diabetic (DM+) if they (a) had a current diagnosis of diabetes at the time of the surgery, (b) regularly took an anti-hyperglycemic agent or (c) had a hemoglobin A1C (HgbA1c) level greater than 6.5%. Patients who did not meet any of these criteria were classified as non-diabetic (DM-).

Patient factors including demographics, co-morbidities, surgical variables, medications and pre-operative lab values were also elicited by chart review. Patient demographics included: age at time of surgery, race, and gender. Co-morbidities included: smoking status, body mass index (BMI), presence of heart disease, hypertension, renal disease and chronic obstructive pulmonary disease (COPD). Surgical variables included: side of bypass, type of graft, and inflow and outflow vessel. Medications analyzed included: statins, ACE-inhibitors, beta-blockers, calcium channel blockers and antiplatelet/anticoagulation therapy. Pre-operative lab values included: leukocyte counts with differential, albumin, serum creatinine and HgbA1c. Outcome measures including pre- and post-operative ankle-brachial indices (ABI), myocardial infarction, new arrhythmia, stroke, minor infections, cellulitis, osteomyelitis, ulceration or gangrene of the revascularized limb, graft status, limb status and mortality were also recorded. Mean follow-up time and time to death were also recorded.

As mentioned previously, patients who developed bypass failure,
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