Preprocedural neutrophil count predicts outcome in patients with advanced peripheral vascular disease undergoing percutaneous transluminal angioplasty

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Background: **The neutrophil count has been associated with adverse cardiovascular events after percutaneous coronary intervention. There are limited data on risk stratification of patients with advanced peripheral vascular disease (PVD) using white blood cell (WBC) subtypes. This study assessed the association of total and differential WBC counts with adverse outcome in patients with advanced PVD undergoing percutaneous transluminal angioplasty (PTA).**

Methods: **In a retrospective cohort study, consecutive de novo procedures were analyzed for patients with Rutherford category 4 or 5 PVD who underwent successful nonemergency PTA. Cardiovascular risk factors, baseline total and differential WBC counts, and angiographic data were recorded. Primary outcome was a composite of events of target vessel revascularization (repeat PTA or vascular bypass operation) or lower limb amputation.**

Results: **A total of 101 patients were studied. Their mean age was 76 ± 10 years, 54% had diabetes mellitus, 68% were hypertensive, and 12% had had previous myocardial infarction. We observed 29 events during a median period of 14 months (interquartile range, 4-26). Cox regression analysis found diabetes mellitus (odds ratio [OR], 4.67; 95% confidence interval [CI], 1.35-16.14; $P = .02$), Rutherford category 5 (OR, 4.18; 95% CI, 1.06-16.51; $P = .04$), poor tibial runoff (OR, 4.42; 95% CI, 1.16-16.82; $P = .03$), and preprocedural neutrophil count in the third tertile (OR, 10.77; 95% CI, 2.19-52.91; $P = .003$) were independent predictors of outcome.**

Conclusions: **The results suggest that the preprocedural neutrophil count could be used in global risk factor assessment of patients with advanced PVD who are being considered for PTA. The neutrophil count may reflect the burden of atherosclerosis and tissue damage, and so could identify patients who need more aggressive intervention for advanced PVD. (J Vasc Surg 2008;48:1504-8.)**

Several inflammatory markers have been shown to predict the development of symptomatic atherosclerosis.[1-3] Prospective studies have identified the risk of developing symptomatic peripheral vascular disease (PVD) is associated with plasma levels of C-reactive protein (CRP).[4,5] Risk stratification of patients with advanced PVD remains difficult when only traditional cardiovascular risk factors are used.[6] However, inflammatory markers such as CRP, D-dimer, and fibrinogen have been found to predict the risk of disease progression in patients with symptomatic PVD.[7,8]

White blood cell (WBC) count is a simple marker of inflammation associated with adverse outcomes in patients with symptomatic coronary artery disease.[9] There are limited data in the literature addressing the prognostic role of WBC count in the context of advanced PVD. The WBC count includes several subtypes of cells that are implicated in the development and progression of atherosclerotic plaques. The role of monocytes, macrophages, and T-cell lymphocytes in the formation of atherosclerotic lesions has been well described, but that of neutrophils is less well known.[10,11] Haumer et al[12] found that PVD patients with neutrophil counts in the upper tertile had a higher risk of adverse outcome relative to those in the lower tertile. We set out to identify whether the preprocedural neutrophil count is associated with an increased risk of an adverse peripheral vascular outcome in patients with advanced PVD undergoing percutaneous transluminal angioplasty (PTA).

METHODS

This retrospective cohort study included consecutive patients with PVD of Rutherford category 4 or 5 who underwent successful nonemergency PTA within a large district general hospital from 2002 to 2004. The diagnosis of PVD was assigned by means of clinical evaluation or duplex ultrasonography and confirmed by lower limb angiography. Patients with a history of surgical lower limb amputation as a consequence of PVD, or previous surgical or endovascular lower limb revascularization were excluded from the study. Patients with a history of autoimmune disease or on antibiotic therapy were also excluded.

Demographic, cardiovascular risk factors, comorbidities, and interventional data were recorded by systematic review of patient hospital records. WBC counts were measured using flow cytometry by an automated system (Advia 120, Bayer Diagnostics, Tarrytown, NJ). These indices

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Table I. Baseline clinical characteristics according to clinical outcome

Variable	*Overall (N = 101) No. (%)*[a]	*No event (n = 59), No. (%)*[a]	*Adverse event (n = 42), No. (%)*[a]	P
Age, y	76 ± 10	77 ± 10	75 ± 11	.41
Male gender	54 (53)	25 (42)	29 (69)	.01
Diabetes	55 (54)	25 (42)	30 (71)	.01
Hypertension	69 (68)	40 (68)	29 (69)	>.99
Smoker	27 (27)	14 (24)	13 (31)	.50
Angina	27 (27)	13 (22)	14 (33)	.26
Myocardial infarction	12 (12)	2 (3)	10 (24)	.003
Stroke	15 (15)	10 (17)	5 (12)	.58
Heart failure	17 (17)	5 (8)	12 (29)	.01
Renal impairment	15 (15)	4 (7)	11 (26)	.01
Rutherford category 5	69 (68)	35 (59)	34 (81)	.03
Angiographic data				
Tibial run-off score >2	60 (59)	26 (44)	34 (81)	<.001

[a]Data for age are expressed as mean ± standard deviation.

were obtained from histograms of two-dimensional light scatter signals that were converted into the WBC count and subtypes. The intra- and inter-assay coefficients of variation were <3% and <7%. Reference ranges (all expressed as count per 10^9 cells/L) were as follows: WBCs, 4.0 to 11; neutrophils, 1.7 to 7.5; lymphocytes, 1.0 to 4.5; monocytes, 0.2 to 0.8; eosinophils, 0.0 to 0.5, and basophils, 0.0 to 0.1.

A standardized protocol was used for peripheral angiography and PTA. Before PTA, patients received 3000 IU of intra-arterial heparin. All interventions were performed by experienced operators, and the PTA technique used was at the discretion of the treating interventional radiologists. Primary technical success was defined as a residual stenosis of <50% at the dilated segment.[13]

Lesions were categorized as involving the superficial femoral artery, femoral artery, or popliteal artery, according to the TransAtlantic InterSociety Consensus (TASC) categorization of arterial lesions.[14] Angiographic documentation of preprocedural tibial runoff vessels was also available for all patients. Each of the three tibial vessels was assigned a score of 0 to 2 according to the extent of luminal disease: 0, <50% stenosis; 1, 50% to 99% stenosis; 2, occluded. The sum of the scores formed the total runoff score of 0 to 6.[15] Patients were stratified into two groups of tibial runoff score 0 to 2 vs 3 to 6.

Patients were routinely followed up in outpatient clinic at 6 months for clinical re-evaluation. The primary study end point was the occurrence of a composite of adverse peripheral vascular events, including target vessel revascularization (repeat PTA or vascular bypass operation) or surgical limb amputation.

Statistical analysis. Categoric variables are expressed as frequencies and percentages, and continuous variables are expressed as mean ± standard deviation, unless otherwise stated. Odds ratios (ORs) are reported with 95% confidence intervals (CIs). For categoric variables, differences between groups were assessed using the Pearson chi-squared test or the two-tailed Fisher exact test. Continuous variables with a normal distribution were analyzed using the student *t* test, and variables with a non-normal distribution were analyzed using the Wilcoxon rank test.

Cox regression analysis was used to identify independent predictors of adverse peripheral vascular outcome. Variables were entered into the Cox regression model based on univariate association ($P < 0.1$) with the dependent variable. Kaplan-Meier survival analysis was used to compare event rate differences between tertiles of neutrophil count with the log-rank test. Statistical analysis was completed using SPSS 14.0 software (SPSS Inc, Chicago, Ill). For analyses, a value of $P \leq .05$ was considered statistically significant.

RESULTS

We studied 101 consecutive patients who were admitted with advanced PVD (Rutherford category 4 in 32, category 5 in 69), undergoing nonemergency PTA (Table I). Patients included in the study had balloon angioplasty to the superficial femoral (n = 12), femoral (n = 16), popliteal (n =26), or tibial (n = 47) artery. The mean age of the patients was 76 ± 10 years, 53% were men, 54% had diabetes mellitus, 68% had hypertension, and 27% were smokers. Their preprocedural femoral popliteal lesion morphology according to TASC criteria included type A in 33, type B in 35, type C in 22, and type D in 11. The preprocedural tibial vessels runoff scores were 0 in 10, 1 in 15, 2 in 16, 3 in 11, 4 in 29, 5 in 9, and 6 in 11.

Total and differential WBC counts were non-normally distributed. The baseline WBC counts are presented as median with 25th and 75th percentiles in Table II. During the median follow-up of 14 months (interquartile range, 4-26 months), 29 adverse events occurred, with 11 repeat PTA, 9 vascular bypass operations, and 9 below or above knee amputations. During the study period, 13 patients died.

Table III summarizes the adverse outcomes for the cohort per tertile of total and differential WBC subtypes. There was a significant association between adverse peripheral vascular outcome and both preprocedural total WBC and neutrophil counts. The unadjusted hazard ratio for

Table II. Baseline preprocedural white blood cell counts[a]

Cell type	*Median (25th–75th percentiles)*
Total WBC count	8.3 (6.7-9.9)
Neutrophils	5.7 (4.0-7.1)
Lymphocytes	1.7 (1.2-2.2)
Monocytes	0.4 (0.3-0.6)
Eosinophils	0.2 (0.1-0.3)
Basophils	0.04 (0.02-0.06)

WBC, White blood cell.
[a]All cell counts are expressed as count per 10^9 cells/L.

Table III. Adverse clinical events according to increasing tertiles of total and differential white blood cells

	Adverse events for each tertile, No. (%)			
Cell type	*1st tertile*	*2nd tertile*	*3rd tertile*	P
Total WBCs	4 (12)	9 (27)	16 (50)	.002
Neutrophils	4 (13)	8 (23)	17 (53)	.001
Lymphocytes	9 (27)	11 (33)	9 (28)	.79
Monocytes	5 (15)	9 (32)	15 (41)	.07
Eosinophils	6 (19)	10 (29)	13 (41)	.12
Basophils	9 (30)	10 (29)	10 (31)	.91

WBC, White blood cell.

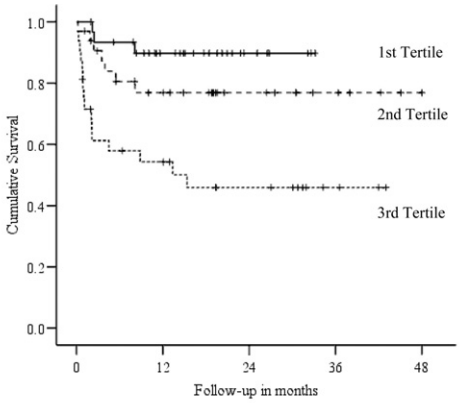
adverse peripheral vascular outcome, relative to the first tertile preprocedural total WBC count, was OR = 3.50 (95% CI, 0.85-14.42; $P = .08$) for the second tertile and OR = 9.33 (95% CI, 2.34-37.01; $P = .001$) for the third tertile. The unadjusted hazard ratio for adverse peripheral vascular outcome, relative to the first tertile preprocedural neutrophil count, was OR = 1.82 (95% CI, 0.48-6.95; $P = .38$) for the second tertile and OR = 7.65 (95% CI, 2.17-26.94; $P = .002$) for the third tertile. Figure 1 demonstrates the marked increase in adverse peripheral vascular outcome for patients with preprocedural neutrophil counts in the third tertile (log-rank test; $P < .001$).

Cox regression analysis found diabetes mellitus (OR, 4.67; 95% CI, 1.35-16.14; $P = .02$), Rutherford category 5 (OR, 4.18; 95% CI, 1.06-16.51; $P = .04$), poor tibial run-off (OR, 4.42; 95% CI, 1.16-16.82; $P = .03$), and a preprocedural neutrophil count in the third tertile (OR, 10.77; 95% CI, 2.19-52.91; $P = .003$) were independently associated with an adverse peripheral vascular outcome (Table IV).

DISCUSSION

Preprocedural neutrophil count is an independent predictor of adverse outcome in the patients with Rutherford category 4 or 5 PVD undergoing PTA. Univariate analysis showed the preprocedural WBC count was associated with adverse outcome. Indeed, studies of patients undergoing coronary angioplasty have shown that the preprocedural WBC count is independently associated with both short-term and long-term adverse outcome.[16-18] In our study, the neutrophil count in the third tertile appeared to have a greater predictive power of adverse outcome than a history of diabetes mellitus. Given that traditional risk factors do not provide a comprehensive prediction model for outcome in patients with PVD,[6] the preprocedural neutrophil count may provide valuable additional information for assessing outcome in patients with advanced PVD who are being considered for PTA.

Belch et al[19] reported that the baseline WBC count was associated with a significantly increased risk of lower limb amputation in patients with critical limb ischemia. Nearly one-fifth of our patients needed definitive surgical treatment in the form of a vascular bypass operation or surgical amputation during the follow-up period. It appears that in addition to established traditional cardiovascular risk, the preprocedural WBC and neutrophil count could be helpful in assessing whether advanced PVD can be successfully treated with PTA. Inflammation has a central role in the pathophysiology of atherosclerosis.[2,20,21] Multiple epidemiologic studies have shown associations between inflam-



Follow-up (months)	6	12	24	36	48
Number of patients at risk	101	71	57	26	8
Total Adverse Events	22 (22%)	3 (4%)	4 (7%)	0 (0%)	0 (0%)

Fig. Kaplan-Meier curves for the cumulative probability of adverse outcome by preprocedural neutrophil count tertiles.

Table IV. Cox regression analysis for predictors of adverse clinical outcome[a]

Candidate predictors	*OR (95% CI)*	P
Diabetes mellitus	4.67 (1.35-16.14)	.02
Rutherford category 5	4.18 (1.06-16.51)	.04
Tibial run-off score >2	4.42 (1.16-16.82)	.03
Pre-procedural neutrophil count (3rd vs 1st tertile)	10.77 (2.19-52.91)	.003

CI, Confidence interval; OR, odds ratio.
[a]Variables included in the model were sex, diabetes mellitus, prior myocardial infarction, heart failure, renal impairment, Rutherford category, tibial lesion, tibial runoff score >2, preprocedural neutrophil count tertiles, preprocedural WBC cell count tertiles, age, hypertension, smoking status, angina, and stroke.

matory markers and ischemic events.[22,23] It is uncertain whether markers of inflammation are causal or merely indicate atherosclerotic burden. Raised inflammatory markers may occur in response to damaged tissue or vascular endothelium caused by the severity and extent of atherosclerosis.

In patients with symptomatic PVD, there are conflicting data on the relationship between the WBC count and the ankle-brachial pressure index (ABPI). The Cardiovascular Health Study examined patients with symptomatic PVD and found an association between WBC and ABPI only among women and not men.[24] Haumer et al[12] found no significant association between the neutrophil count and the ABPI or Fontaine stage in patients with intermittent claudication or critical ischemia. However, they did show that a neutrophil count in the upper tertile was an independent predictor of major adverse cardiovascular events. Thus, neutrophil count does not simply reflect clinical information that can already be gained from measuring ABPI, but is an independent marker for the risk stratification of patients with advanced PVD.

Circulating neutrophils have been shown to release reactive species and proteolytic enzymes, which are involved in atherosclerotic plaque disruption.[25-27] Myeloperoxidase released by activated neutrophils weakens the fibrous cap of atherosclerotic plaques through activating metalloproteinases and leads to plaque rupture with consequent vessel occlusion.[28] Activated neutrophils can also aggregate with platelets and adhere to endothelial cells, which causes plugging of microvessels.[29,30] It is through plaque disruption and microvessel plugging that neutrophils could play an active role in the initiation and progression of tissue ischemia.[31]

This was an observational study, and as a result we were unable to adjust for all potentially confounding variables. Although a single baseline measurement of total and differential WBC count was used, it is plausible that serial measurement with assays of cellular activation or other inflammatory marker such as CRP and interleukin-6 may yield a different picture. Nevertheless, the simplicity and ready availability of the WBC and neutrophil count remains an attractive test for clinicians in a risk assessment model. Smoking affects WBC count with a positive dose–response relationship; however, our study was not powered to quantify the effect of smoking.

With regards to medical therapy, statins have a proven benefit in patients with PVD, in addition to their anti-inflammatory effect. The use of statins in our patient population was not recorded. We were also not able to report the ABPI for patients at follow-up because this was not routinely documented in patient notes. The decision for further intervention after the index procedure was determined by clinical symptoms.

CONCLUSIONS

The preprocedural neutrophil count was an independent predictor of adverse outcome in patients with advanced PVD undergoing nonemergency PTA. This simple and widely available test could be routinely used in clinical practice to risk stratify patients with advanced PVD being considered for PTA. Neutrophil count may reflect the burden of atherosclerosis and tissue damage, and so could identify patients who need more aggressive intervention for advanced PVD.

AUTHOR CONTRIBUTIONS

Conception and design: IT, MM, SB
Analysis and interpretation: IT, RJ, SB
Data collection: IT, RJ, MM, SB
Writing the article: IT, RJ, MM, SB
Critical revision of the article: IT, RJ, MM, SB
Final approval of the article: IT, RJ, MM, SB
Statistical analysis: IT, RJ
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