

# Association of neutrophils and future cardiovascular events in patients with peripheral artery disease

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**Objective:** We hypothesized that higher neutrophil counts are associated with an increased incidence of major adverse cardiovascular events (MACE) in patients with clinically advanced atherosclerosis.

**Methods:** We prospectively studied 398 patients (233 men; median age, 69 years) with symptomatic peripheral artery disease who were admitted to the inpatient ward of the angiology department of a tertiary care university hospital in a cohort study. Total and differential white blood cell (WBC) counts were obtained, and patients were followed for MACE, defined as myocardial infarction, percutaneous coronary interventions, coronary artery bypass grafting, stroke, carotid revascularization, and death.

**Results:** During a median follow-up of 20 months, 140 MACE occurred in 105 patients (26%). Multivariate Cox proportional hazards analysis was used to assess the association of differential WBC count parameters (in tertiles) with MACE and their interrelation with traditional cardiovascular risk factors and other parameters of inflammation. Patients with neutrophil counts >5.8 G/L (upper tertile) exhibited an increased adjusted risk for all MACE (hazards ratio [HR], 1.83;  $P = .017$ ), death (HR, 3.39;  $P = .010$ ), and the composite of myocardial infarction, stroke, and death (HR, 2.20;  $P = .012$ ) compared with patients in the lower tertile (<4.4 G/L), independently of traditional cardiovascular risk factors and levels of high-sensitivity C-reactive protein. Only neutrophils, but not eosinophils, basophils, monocytes, lymphocytes, or the total WBC count showed a significant association with cardiovascular outcome.

**Conclusion:** In patients with peripheral artery disease, neutrophil counts in the upper tertile (>5.8 G/L) indicate a substantially increased risk for major adverse cardiovascular events, adding to the prognostic information of traditional atherothrombotic risk factors and other parameters of inflammation. (*J Vasc Surg* 2005;41:610-7.)

Large observational studies have established a relation between the development of symptomatic cardiovascular disease or cardiovascular death and the white blood cell (WBC) count.<sup>1-7</sup> Furthermore, in patients with acute coronary syndromes, an elevated WBC count indicates an increased risk for short- and long-term ischemic complications and death.<sup>8-14</sup>

Leukocytes of the monocyte-macrophage lineage have a crucial pathophysiologic role in the development of atherosclerotic plaque and deposition of lipids therein. The role of granulocytes, which account for 50% to 70% of the total WBC count, in the atherothrombotic process seems less clear. In most prospective cohort studies that have provided information on differential WBC count, the number of neutrophils correlated consistently with the atherosclerotic load and adverse outcomes in ischemic conditions.<sup>8-14,20</sup>

Patients with peripheral artery disease (PAD) frequently suffer from concomitant coronary artery disease

and cerebrovascular atherosclerosis and therefore are at a particularly high risk for cardiovascular complications.<sup>15</sup> In patients with critical limb ischemia, a higher neutrophil count may predict limb loss within 6 months.<sup>16</sup> Although neutrophils are supposed to contribute to the morbidity and mortality of patients with claudication, data on the prognostic impact of differential WBC count, particularly in relation to other atherothrombotic risk factors, are largely lacking for patients with PAD.<sup>14,17</sup>

We hypothesized that higher numbers of neutrophils are associated with an increased incidence of major adverse cardiovascular events (MACE) in PAD patients and add to the prognostic information of traditional atherothrombotic risk factors. Therefore, we conducted a study to investigate the association of parameters of differential WBC count with a combined endpoint of MACE, defined as myocardial infarction, percutaneous coronary interventions, coronary artery bypass grafting (CABG), any stroke, carotid revascularization, and death in patients admitted with symptomatic PAD. Secondary objectives were to assess the association of differential WBC count and mortality as well as a composite of MI, stroke, and death.

## METHODS

**Study design.** We prospectively enrolled in a cohort study all consecutive patients with symptomatic PAD who were admitted to the inpatient ward of the angiology

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Competition of interest: none.

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department of a tertiary care university hospital from March 1, 2000 to March 1, 2001.<sup>18</sup> Study entry criterion was a >50% stenosis in the symptomatic lower limb as determined by intra-arterial angiography, which was performed according to current state-of-the-art protocols. Patients with acute infections unrelated to PAD were excluded. The study was approved by the local review board and ethics committee, and all patients gave their written informed consent.

**Patient data.** On admission, two independent observers used a standard questionnaire to record the patients' medical history and physical examination data. Clinical history and physical examination were evaluated with special attention to the cardiovascular risk factors and comorbidities of age, sex, smoking habits, hyperlipidemia, body mass index, arterial hypertension, diabetes mellitus, coronary artery disease, history of myocardial infarction, history of cerebrovascular events, and current medication. Data were checked for interobserver agreement on the day the patient was discharged. In case of discrepancies, the patient was re-evaluated by both investigators in consensus.

The ankle-brachial index (ABI) was determined by experienced vascular technologists according to a standardized and accredited protocol. On admission, routine measurement of overnight fasting blood glucose and glycated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels was done to detect undiagnosed diabetes mellitus. During the hospital stay, repeat blood pressure measurements were done to detect undiagnosed hypertensive patients. Current infections were excluded by clinical investigation, chest radiograph, and urinalysis in all patients.

**Laboratory parameters.** A complete series of routine laboratory investigations were performed, including complete blood cell count, global coagulation tests, HbA<sub>1c</sub>, and levels of fasting low-density lipoprotein (LDL) high-density lipoprotein (HDL) cholesterol, and serum creatinine. Levels of high-sensitivity C-reactive protein (hs-CRP) were determined as described previously in these patients.<sup>18</sup> Automated blood cell and differential WBC counts were performed on a Sysmex NE-8000 hematology analyzer (TOA Medical Electronics, Kobe, Japan). Samples for blood cell analysis were obtained in all patients on the morning of admission, in the absence of prior exercise, and analyzed within 1 hour. Results of automated differential WBC counts are given in G/L ( $\times 10^9/L$ ). Reference values in our laboratory are neutrophils, 2.0 to 7.5 G/L; lymphocytes, 1.0 to 4.0 G/L; monocytes, 0.0 to 1.2 G/L; eosinophils, 0.0 to 0.4 G/L; and basophils, 0.0 to 0.1 G/L.

**Study endpoints.** The primary composite study endpoint was the occurrence of the first MACE, consisting of myocardial infarction, percutaneous coronary interventions, coronary artery bypass grafting (CABG), stroke, carotid revascularization (carotid stenting or carotid endarterectomy), and death. Secondary objectives were the occurrence of death, and a composite of acute cardiovascular events (myocardial infarction, stroke, and death). To avoid a referral bias, peripheral vascular events such as the occurrence of critical limb ischemia, revascularization pro-

cedures, or amputations were not considered as study endpoints, because the patients of our study cohort were referred for the treatment of symptomatic PAD. In general, patients with claudication were admitted when a revascularization was estimated to be feasible, whereas every attempt of limb salvage was made in critical limb ischemia. Subsequent recurrence of peripheral events would have been confounded by disease stage, the location, the nature of the lesions, and most important, the treatment strategy, which was not randomized.

**Follow-up.** Patients were clinically re-evaluated routinely at 3, 6, and 12 months after hospital discharge and then annually until December 2002 at the outpatient ward of our department to record any MACE. A follow-up questionnaire was also sent to each patient during December 2002 to re-evaluate the occurrence of MACE during the entire follow-up period. Information from the follow-up questionnaire was validated by a review of the original hospital discharge reports for corresponding readmissions due to MACE. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or to the treating physicians was established.

Further information was obtained by reviewing the hospital discharge reports of any other re-admission during the follow-up period. The performance of percutaneous coronary interventions, CABG, carotid stenting, and carotid endarterectomy was validated by review of the original procedure protocols. Outcome was assessed by two independent observers who were blinded to the patients' baseline clinical and laboratory data.

**Definitions.** Diabetes mellitus was defined according to the criteria of the American Diabetes Association and was considered to be present in all patients taking antidiabetic medication.

Hyperlipidemia was defined as fasting total serum cholesterol >200 mg/dL, LDL cholesterol >130mg/dL, or serum triglycerides >180mg/dL and was considered to be present in all patients receiving lipid-lowering therapy (HMG-CoA reductase inhibitors [statins] were used routinely at our institution during the study period).

Arterial hypertension was diagnosed in patients with repeated blood pressure measurements >140/90 mm Hg and was assumed to be present in patients with a history of hypertension who were taking antihypertensive drugs.

We recorded patients' current smoking status in four categories: as non-smokers, patients smoking  $\leq 10$  cigarettes/day, 11 to 20 cigarettes/day, and >20 cigarettes/day.

For categorization of PAD, the Fontaine classification was applied. Symptomatic PAD was defined as intermittent claudication and critical limb ischemia. The latter included ischemic rest pain, ulceration, and gangrene.<sup>15</sup>

Coronary artery disease was categorized according to the Canadian Cardiovascular Society (CCS) classification,<sup>19</sup> and routine evaluation in symptomatic patients included treadmill exercise testing, dobutamine echocardi-

ography, myocardial scintigraphy, and coronary angiography in selected cases.

Myocardial infarction was defined according to the consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction<sup>20</sup> as a transient increase of laboratory markers specific of myocardial necrosis (CK-MB, or troponin T) along with ischemic symptoms, typical electrocardiographic signs such as the development of pathologic Q waves or ST segment elevation or depression, or both.

A stroke was defined as a neurologic deficit that persisted >24 hours. It was defined as minor if graded 1 to 2 or major if graded 3 to 5 on the modified Rankin stroke scale<sup>21</sup> by a neurologist. Mandatory cranial computed tomography or magnetic resonance imaging, if available, were used for confirmation of the diagnosis.

**Statistical analysis.** Continuous data are presented as the median and the interquartile range (IQR, range from the 25th to the 75th percentile). Discrete data are given as counts and percentages. We used  $\chi^2$  tests to compare proportions and Mann-Whitney *U* tests, or Kruskal-Wallis tests, as appropriate, for univariate comparison of continuous data. The Spearman correlation coefficient was calculated for the comparison of two continuous data sets. Event-free survival rates until the first MACE according to patients' baseline differential WBC count (in tertiles) are presented as Kaplan-Meier curves and compared by means of the log-rank test. Multivariate Cox proportional hazards analysis was applied to assess the effect of differential WBC counts on event free survival.

Baseline variables were entered as possible predictor variables into the model to adjust for confounding effects if they were imbalanced between patients with differential WBC count parameters in the upper tertile compared with patients in the middle and lower tertile, indicated by  $P < 0.2$ , or were established risk factors for cardiovascular events. We tested for interactions between baseline variables by stratification as well as multiplicative interaction terms and log likelihood  $\chi^2$  tests.

Results of the Cox proportional hazards model were presented as the hazard ratio (HR) and the 95% confidence interval (95% CI). A two sided  $P < 0.05$  was considered statistically significant. Calculations were performed with the Statistical Package for the Social Sciences (SPSS) version 10.0 software for Windows (SPSS Inc, Chicago, Ill) and Stata (release 8) (StataCorp, College Station, Tex).

## RESULTS

**Patients.** We studied 398 (85%) of 467 patients who were admitted with symptomatic PAD during the study period. Sixty-nine patients (15%) had to be excluded owing to missing data. Data on differential WBC count or follow-up were lacking in 56 (12%) and 13 (3%) patients, respectively. These patients with missing data closely resembled the patients with complete data with respect to clinical characteristics, without significant differences (data not shown). The median age of the 398 patients who were

eligible for the final analysis was 69 years (IQR, 59 to 76), 233 patients were men (59%), and the median ABI was 0.58 (IQR, 0.44 to 0.73). Four (1%) of 32 (8%) patients with ischemic rest pain were admitted for an acute thrombotic event and 12 (3%) of 57 (14%) patients with ischemic ulcers presented with local signs of infection.

**WBC counts.** The median total leukocyte count was 7.5 G/L (IQR, 6.2 to 9.0), consisting of median counts of 5.1 G/L for neutrophils (IQR, 3.9 to 6.3), 1.7 G/L for lymphocytes (IQR, 1.3 to 2.2), 0.4 G/L for monocytes (IQR, 0.3 to 0.5), 0.2 G/L for eosinophils (IQR, 0.1 to 0.3), and 0.1 G/L for basophils (IQR, 0 to 0.1).

Demographic data and clinical characteristics of the 398 patients according to the neutrophil count (comparing patients in the upper tertile vs patients in the middle and lower tertile) are presented in Table I. Patients with higher numbers of neutrophils were younger, had hyperlipidemia and statin therapy less frequently, and tended to have a higher frequency of diabetes mellitus (Table I).

Current smokers exhibited significantly higher neutrophil counts compared with nonsmokers, depending on the quantity of daily cigarette consumption. Median neutrophil counts in nonsmokers, smokers of  $\leq 10$  cigarettes/day, 11 to 20 cigarettes/day, and  $> 20$  cigarettes/day were 4.7 G/L (IQR, 3.8 to 5.8), 4.7 G/L (IQR, 3.7 to 6.2), 5.5 G/L (IQR, 4.8 to 6.5), and 5.7 G/L (IQR, 4.4 to 7.1), respectively ( $P < .001$ ).

Neutrophil counts showed no significant correlation with the Fontaine stage of PAD ( $P = .36$ ) or the ABI ( $r = -0.074$ ,  $P = .23$ ).

Median hs-CRP levels were 0.40 mg/dL (IQR, 0.17 to 0.86) in these patients. Neutrophil counts were significantly, but weakly, associated with hs-CRP ( $r = 0.36$ ,  $P < .001$ ), indicating that one parameter accounted for approximately 13% of the variation of the other.

**Follow-up for MACE.** During the median follow-up period of 20 months (IQR, 12 to 25), 140 MACE occurred in 105 patients (26%), with 14 myocardial infarctions, 39 percutaneous coronary interventions, 5 CABG procedures, 15 carotid artery stenting procedures, 5 carotid endarterectomies, 21 strokes, and 41 deaths. In 35 of 105 patients, a second endpoint occurred. For all analyses of event-free survival, the time interval until the first MACE in each patient was included.

**Differential WBC counts and MACE.** Analyzing the specific parameters of the differential WBC count with respect to differences of the cumulative frequency of events, we found that neutrophil counts were significantly associated with the study endpoints. Patients in the upper tertile of neutrophil counts ( $> 5.8$  G/L) had an increased risk for MACE and death compared with patients in the lower and middle tertile (Fig 1). Similarly, increasing neutrophil counts were associated with an increased risk for mortality ( $P = .0010$  by log rank) and the composite of myocardial infarction, stroke and death ( $P = .0017$  by log rank). Total WBC count ( $P = .22$ ), eosinophils ( $P = .21$ ), basophils ( $P = .82$ ), and monocytes ( $P = .58$ ) showed no significant association with cardiovascular outcome in these patients

**Table I.** Demographic data, cardiovascular risk factors and comorbidities in 398 patients with peripheral artery disease according to the baseline level of neutrophil counts (upper tertile vs. middle and lower tertile)

	Neutrophils $\geq$ 5.8 G/ L* (n = 136, 34%)	Neutrophils $\leq$ 5.8 G/ L* (n = 262, 66%)	P
Age (years)	65 (57-75)	71 (60-76)	0.017
Male sex	86 (63%)	147 (56%)	0.17
Body mass index (kg/m <sup>2</sup> )	25.8 (23.2-28.6)	25.7 (23.3-28.0)	0.95
Arterial hypertension	101 (74%)	196 (75%)	0.91
Diabetes mellitus	62 (46%)	95 (36%)	0.071
Smoking	77 (57%)	94 (36%)	<0.001
Hyperlipidemia	100 (74%)	215 (82%)	0.047
Ankle-brachial index	0.56 (0.41-0.71)	0.59 (0.45-0.75)	0.41
Critical limb ischemia	36 (27%)	53 (20%)	0.16
Coronary artery disease(CCS)			0.19
I	27 (20%)	73 (28%)	
II	26 (19%)	48 (18%)	
III	1 (1%)	6 (2%)	
IV	—	—	
History of myocardial infarction	35 (26%)	63 (24%)	0.71
History of stroke	17 (13%)	27 (10%)	0.51
Clopidogrel therapy	29 (21%)	68 (26%)	0.31
Statin therapy	61 (45%)	147 (56%)	0.033

CCS, Canadian Cardiovascular Society.

Data are given as counts and percentages or median and interquartile range (range from the 25<sup>th</sup> to the 75<sup>th</sup> percentile).

\*G/L, ( $\times 10^9$ /L).

(Fig 2). Lymphocyte counts in the lower tertile showed a trend ( $P = .065$ ) toward a better cardiovascular outcome (Fig 2).

Being aware of several possible confounding factors, we used a multivariate Cox proportional hazards model to assess the association of neutrophil counts (in tertiles) with MACE, adjusting for age (in tertiles), hyperlipidemia, smoking, arterial hypertension, diabetes mellitus, critical limb ischemia, history of myocardial infarction, history of stroke, and statin therapy (Table II). Further adjustment for the quantity of daily cigarette consumption (in four categories) did not cause a significant effect modification, and applying an interaction term *neutrophil count\*daily cigarette consumption* did not reveal a significant interaction between these variables, indicating that neutrophil counts were associated with the study endpoints independently of the current smoking status.

We further analyzed potential interactions among use of statins, hyperlipidemia, neutrophil counts, and MACE without detecting any relevant effect modifications (log likelihood ratio tests  $P > .2$ ). Patients with neutrophil counts  $>5.8$  G/L (upper tertile) exhibited a 1.83-fold increased adjusted risk (95% CI, 1.12 to 3.00;  $P = .017$ ) for MACE compared with patients in the lower tertile of neutrophil counts ( $<4.4$  G/L). Consistently, neutrophil counts  $>5.8$  G/L were associated with an increased adjusted risk for death (adjusted HR, 3.39; 95% CI, 1.34 to 8.53;  $P = .010$ ) and the composite of myocardial infarction, stroke, and death (adjusted HR, 2.20; 95% CI, 1.19 to 4.09;  $P = .012$ ).

Alternatively, calculating the adjusted incremental risk for MACE with increasing numbers of neutrophils

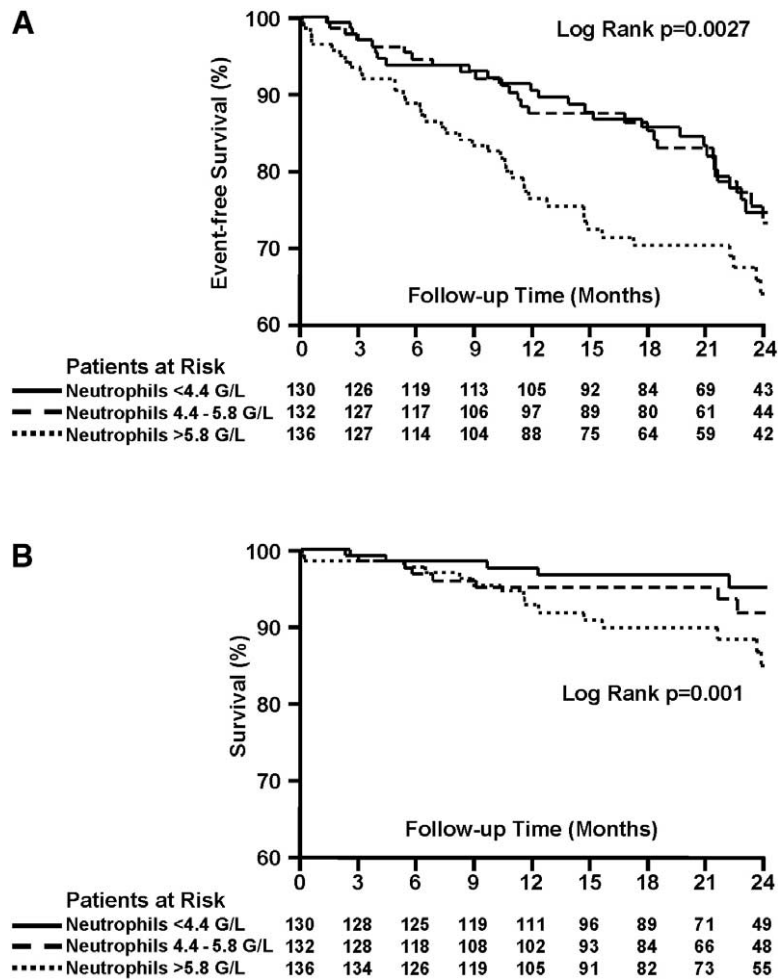
per 1 G/L, we found a HR of 1.15 (95% CI, 1.04 to 1.27;  $P = .007$ ), confirming an increasing risk with increasing numbers of peripheral neutrophils.

Increasing numbers of atherothrombotic risk factors (smoking, hyperlipidemia, arterial hypertension, and diabetes mellitus) were associated with increasing neutrophil counts ( $P = .048$  by Kruskal-Wallis test). However, we found no interaction between the number of atherothrombotic risk factors (0 to 1, 2, 3, and 4) and neutrophil counts (in tertiles) by means of a log likelihood ratio test ( $P > .20$ ). This suggests that the prognostic value of neutrophil counts is widely independent of established atherothrombotic risk factors and adds to their prognostic information. Fig 3 displays neutrophil counts in patients with and without MACE according to increasing numbers of atherothrombotic risk factors.

To assess the interrelation of neutrophil counts with other markers of inflammation, we additionally included hs-CRP levels (in tertiles) in the final multivariate model and tested for interaction between these parameters. The overall effect size of neutrophils on MACE was slightly attenuated by adjustment for hs-CRP (adjusted HRs, 0.94 and 1.69 for the middle and upper tertile of neutrophils compared with the lower tertile,  $P = .82$  and  $P = .043$ , respectively), but there was no significant statistical interaction between neutrophils and hs-CRP (log likelihood ratio  $\chi^2$   $P > .2$ ), indicating that neutrophils predicted MACE independently of hs-CRP levels.

## DISCUSSION

This longitudinal study supports a repeatedly described strong positive association of an elevated neutrophil count



**Fig 1.** A, Cumulative event-free survival defined as freedom from myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, any stroke, carotid revascularization and death, and (B) cumulative survival in 398 patients according to the baseline neutrophil count (in tertiles).

within the normal range with the risk for atherosclerotic ischemic events<sup>4-9,14</sup> and further confirms that observation in patients with PAD. In these patients, neutrophil counts in the upper tertile (>5.8 G/L) indicated a substantially increased adjusted risk for cardiovascular events during a relatively short median follow-up period of 20 months. This association was widely independent of traditional cardiovascular risk factors and hs-CRP levels and thus adds to their prognostic information.

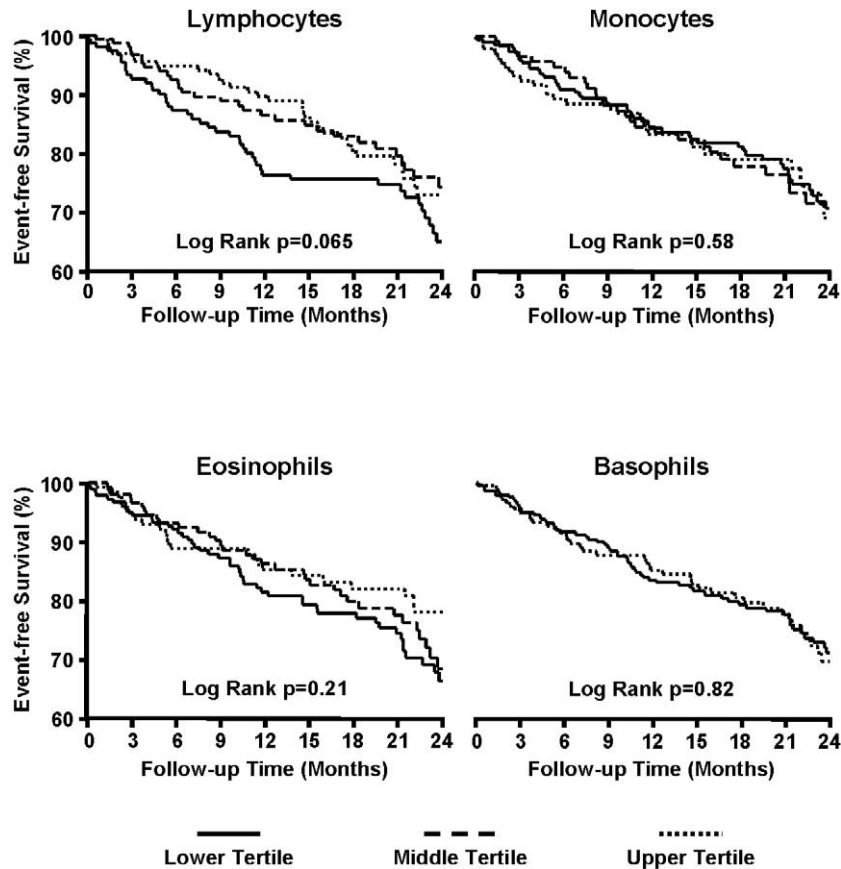
Contrary to previous reports,<sup>1-5,7,8,10-14</sup> we found no significant association of the total leukocyte count with cardiovascular outcome in our patients. This fact may represent a statistical type II error attributable to a nonsignificant trend of lower lymphocyte counts towards a better cardiovascular outcome.

Patients suffering from PAD bear a considerable risk of myocardial infarction, stroke, or cardiovascular death that is directly related to the severity of disease.<sup>15</sup> The excessive cardiovascular event rate of 26% within 20 months in our

patient sample confirmed that this population has to be considered at highest risk for poor outcome and underlines the need for additional parameters for risk stratification. An approximately twofold increase in risk in individuals with a single baseline neutrophil count in the top third compares well with both the relative risks attributable to traditional risk factors and the contribution of WBC count, as determined in large cohort studies.<sup>3,4</sup>

In our cohort, univariate comparison showed no significant association of critical leg ischemia or severity of PAD, as indicated by ABI, with neutrophil count. In the Cardiovascular Health Study, statistical significance was only found for the relation between subclinical PAD, as defined by an asymptomatic reduction of the ABI <0.9, and WBC count in both men and women, or clinically overt PAD in women but not in men.<sup>22</sup>

The nature of the association of neutrophil counts with MACE remains unclear. Despite the detailed insight into the inflammatory processes involved in the initiation, pro-



**Fig 2.** Cumulative event-free survival, defined as freedom from myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, any stroke, carotid revascularization and death, in 398 patients according to baseline eosinophils, basophils, lymphocytes and monocytes (in tertiles).

**Table II.** Multivariate Cox proportional hazards model to assess the association of neutrophil counts with major cardiovascular adverse events (myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, any stroke, carotid revascularization and death) in 398 patients with atherosclerosis

	Hazard Ratio	95% Confidence interval	P
Univariate model			
<4.4 G/L (lower tertile)	1.0	—	—
4.4-5.8 G/L (middle tertile)	1.06	0.62-1.81	0.83
>5.8 G/L (upper tertile)	1.97	1.23-3.17	0.005
Model adjustments for age (in tertiles) and sex			
<4.4 G/L (lower tertile)	1.0	—	—
4.4-5.8 G/L (middle tertile)	1.04	0.61-1.79	0.88
>5.8 G/L (upper tertile)	1.99	1.23-3.20	0.005
Model adjustments for comorbidities†			
<4.4 G/L (lower tertile)	1.0	—	—
4.4-5.8 G/L (middle tertile)	0.96	0.56-1.66	0.99
>5.8 G/L (upper tertile)	1.83	1.12-3.00	0.017

\*G/L, ( $\times 10^9/L$ ).

†Age (in tertiles), diabetes, smoking, hyperlipidemia, hypertension, critical limb ischemia, history of stroke, history of myocardial infarction, and statin therapy.

gression, and complications of atherosclerotic vascular disease, it is not yet possible to determine if elevated markers of inflammation within the normal range are a causative factor rather than merely an indicator of cardiovascular disease.<sup>23,24</sup> Several epidemiologic observations have established WBC count as a predictor of coronary heart disease, stroke, and mortality that is independently associated with multiple coronary risk factors.<sup>1,3,4,10,25,26</sup>

Whereas leukocytes of the monocyte-macrophage lineage have a crucial role in the development of atherosclerotic plaque and deposition of lipids therein, the role of granulocytes in the atherothrombotic process seems less clear. In a French trial, a high monocyte count seemed to predict the premature occurrence of a coronary event in men aged 29 to 52 years.<sup>27</sup> However, in most of the prospective cohort studies that have provided information on differential WBC count, the number of neutrophils correlated primarily and consistently in a positive manner with the atherosclerotic load and ischemic conditions.<sup>4-9,14</sup> Acute ischemic syndromes are accompanied by markers indicating an activation of neutrophils.<sup>28-31</sup>

In addition to the release of reactive substances such as superoxide radicals, proteolytic enzymes, and arachidonic

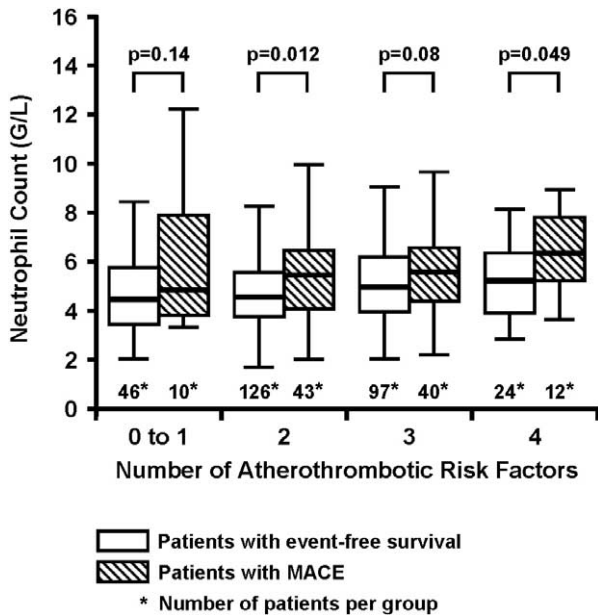


Fig 3. Neutrophil counts in patients with and without major adverse cardiovascular events (MACE) according to the presence of increasing numbers of the atherothrombotic risk factors of smoking, hyperlipidemia, arterial hypertension, and diabetes mellitus.

acid metabolites, activated neutrophils may have a major role in plaque disruption, as suggested by the direct viewing of neutrophils invading plaque in an animal model<sup>32</sup> or the recent demonstration of neutrophil infiltration of culprit lesions in acute coronary syndromes.<sup>33</sup> Furthermore, neutrophils share with platelets the ability to aggregate with either cell type or endothelial cells upon stimulation<sup>34-36</sup>; vice versa, activation of the endothelium leads to the adherence of both leukocytes and platelets. By plugging microvessels, these aggregates may contribute significantly to the outcome in ischemic conditions.<sup>2,9,12,17,29,34</sup>

Although we can only speculate on the pathophysiologic significance of an elevated neutrophil count in atherosclerotic disease, it seems reasonable to suppose that a higher number of circulating neutrophils will at least increase the chance of their activation by altered endothelial cells and will thereby accelerate the build-up of either microvascular or macrovascular occlusive plugs. We therefore believe that our finding of an independent association of MACE with neutrophil count only, instead of the whole WBC count, is relevant for the prediction of MACE in patients with PAD. In contrast to previous studies, however, we found no relation between total WBC count and cardiovascular outcome in our patients. Presumably, this rather unexpected finding can be explained by the statistically nonsignificant finding of a reduced event-free survival with a lower lymphocyte count.

**Study limitations.** Because we included all consecutive patients who were admitted to a tertiary care university hospital due to symptomatic PAD, we do not believe that

referral bias was a relevant issue. The positive dose-response relationship between cigarette smoking and the WBC is well established and complex in nature.<sup>37,38</sup> As we did not perform any objective measurements, such as the determination of blood cotinine, serum thiocyanate, or expired carbon monoxide, to either confirm a nonsmoker status as reported by our patients or quantify the smoking intensity, we cannot rule out passive smoking, low-intensity smoking (eg, <3 cigarettes/day), or nonreported smoking as confounders of our data. We also made no differentiation between former smokers and never smokers. However, the expected increase in leukocyte count associated with various measures of greater smoking intensity is of small magnitude and explains only a small percentage of the variance in the WBC count.<sup>37</sup> The relative increase of neutrophils was shown to reach a statistically significant level only in smokers who smoked at least one pack daily<sup>38</sup> and might therefore not further affect our results, which were adjusted for the reported smoking status and derived from tertiles of neutrophil counts.

We cannot rule out an effect of statins on WBC counts as described previously<sup>39</sup>; however, we corrected for use of statins in our multivariate model and did not observe any statistical interaction between statin use, neutrophils, and MACE, suggesting that statins did not impact the predictive value of neutrophil counts.

**Conclusion.** Neutrophil counts in the upper tertile (>5.8 G/L) indicate an increased risk for major cardiovascular adverse events in patients with PAD independently from traditional risk factors and the level of inflammation as measured by hs-CRP. This simple test with a wide availability and a high interassay precision may therefore add to the risk stratification in these patients.

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