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# Prognostic value of neutrophils in patients with asymptomatic carotid artery disease

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ABSTRACT

*Background:* Inflammation is associated with atherosclerotic disease. In this context, it has been shown that an increased neutrophil count is a risk factor for cardiovascular events in patients with coronary and peripheral artery disease. However, the impact of neutrophils on long-term mortality in patients with carotid atherosclerosis is not yet fully understood.
*Methods:* We prospectively studied 853 of 1268 consecutive patients with neurologically asymptomatic carotid stenosis for all-cause and cardiovascular death, respectively.
*Results:* During a median follow-up time of 6.3 years (IQR 5.8–6.7 years) a total of 203 deaths (23.8%), including 134 cardiovascular deaths (15.7%), were recorded. An increase of 1 G/L of neutrophil count indicated an increased risk for all-cause mortality of 1.20 (CI [95%] 1.10–1.31, $P < 0.001$) and of cardiovascular death of 1.30 (CI 1.17–1.45, $P < 0.001$), respectively. For the second to the fourth quartile of the neutrophil count, adjusted hazard ratios for all-cause mortality were 1.12 (CI, 0.71–1.75), 1.46 (CI, 0.96–2.21), and 1.76 (CI, 1.15–2.69; $P = 0.03$ for trend); and 1.41 (CI, 0.80–2.49), 1.53 (CI, 0.88–2.68), and 2.54 (CI, 1.49–4.33; $P < 0.01$ for trend) for cardiovascular mortality, compared to the lowest quartile, respectively. Patients with baseline carotid stenosis of more than 50% and/or increased neutrophil count (≥median), had a 1.9–2.4 fold increase in risk of (CV-) death, compared to patients with carotid narrowing of less than 50% and/or neutrophil count less than the median ($P < 0.001$). After adjusting for cardiovascular risk factors, only neutrophils, but not eosinophils, basophils, monocytes, lymphocytes, or the total leukocyte count showed a significant association with long-term mortality. No significant association was found between white blood cell subtypes with either baseline degree or progression during a 6 month follow-up of carotid stenosis.
*Conclusion:* The baseline neutrophil count was an independent predictor for all-cause and cardiovascular mortality in neurologically asymptomatic patients with carotid stenosis. Thus, the measurement of neutrophils could provide prognostic information on outcome in patients at risk.


## 1. Introduction

Atherosclerosis and cardiovascular disease are the leading cause of death worldwide [1]. Smooth muscle cell proliferation and lipid deposition in response to endothelial injury were the main theories to explain atherogenesis [2]. However, the discovery of immune cells in atherosclerotic plaques created a paradigm shift in the understanding of atherosclerosis. It has become well established that the development and progression of atherosclerotic plaques is mainly controlled by inflammatory and immune mechanisms [3]. Blood monocytes and T-cells adhere to dysfunctional endothelial cells and undergo directed diapedesis into the intima [4,5]. Within the intima, monocytes turn into activated macrophages that produce inflammatory cytokines which stimulate the generation of endothelial adhesion molecules, proteases, and other mediators [6]. Lymphocytes switch to activated inflammatory cells (T-helper cells 1, T-helper cells 2 and Regulatory T-cells) that secrete cytokines and chemokines [4,7]. Activated dendritic cells contribute to T-cell

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recruitment and activation within the plaque [8]. Recent studies indicate a close relationship between the number of circulating activated neutrophils, the coronary and carotid artery disease, respectively, and their presence in atherosclerotic plaques, suggesting that neutrophils may have a pivotal role in the early development of atherosclerosis [9,10]. Neutrophils release superoxide and pro-inflammatory mediators that may affect endothelial cells, and promote or amplify the recruitment of other inflammatory cells [11]. Furthermore, neutrophils may support monocyte adhesion and mobilization to the site of inflammation [12]. However, more research is necessary to uncover the mechanisms by which neutrophils may contribute to atherogenesis. Our understanding of the complex role of immune and inflammatory cells in the progression and initiation of atherosclerosis has led to advances in both diagnostic and prognostic approaches. Studies have confirmed an association of biomarkers for inflammation and outcome in various patient populations with chronic atherosclerotic diseases [13–15]. In this context, we previously showed, that C-reactive Protein (CRP) is associated with the clinical outcome in asymptomatic patients with carotid artery disease [15]. Inflammatory biomarkers can help the physician in risk stratification and selection of the most efficient therapy. Increases in white blood cells (WBC) have been associated with adverse outcome in the general population [16], in patients with coronary heart disease [17], in acute coronary syndromes [18], in patients with acute myocardial infarctions [19], in patients after PCI [20], and in those with chronic heart failure [21]. In this context, we already showed that neutrophils are an independent predictor of short-term cardiovascular outcome in patients with symptomatic peripheral arterial disease [22]. Up to now, the impact of neutrophil count on long-term mortality in patients with carotid atherosclerosis has not been fully elucidated. Therefore, it was the aim of this study to investigate whether the neutrophil count is associated with long-term mortality in neurologically asymptomatic patients with carotid artery disease.

## 2. Methods

Between March 2002 and March 2003 we prospectively enrolled 1268 consecutive Caucasian patients, who underwent duplex ultrasound investigations of the extracranial carotid arteries in the Inflammation in Carotid Arteries Risk for Atherosclerosis Study (ICARAS). Study design and patients characteristics have been published previously [15]. In brief, patients underwent baseline carotid ultrasound (US) investigations and a second US examination after 6–9 months. Blood tests and physical examinations were performed at baseline and after a 6–9 months follow-up of carotid stenosis. Only patients with atherosclerotic carotid narrowing, who were neurologically asymptomatic – defined as absence of transient ischemic attacks, amaurosis fugax, or stroke within 12 months before inclusion – as assessed by a neurologist, were included. Patients with active malignancies, current infectious or inflammatory diseases, symptomatic carotid artery disease that necessitated revascularization therapy, patients after bilateral carotid occlusions, bilateral stent implantation or bilateral carotid endarterectomy as well as patients with a recent cardiovascular event (myocardial infarction, stroke, coronary revascularization and/or peripheral vascular surgery) within the preceding 6 months were not included in ICARAS. The rationale to exclude the latter patients was to avoid an impact of acute cardiovascular events on inflammatory parameters which might reflect the acute event rather than chronic condition of carotid atherosclerosis. The study complied with the Declaration of Helsinki and was approved by the institutional review board of the Medical University of Vienna. All patients gave their written informed consent.

### 2.1. Clinical and laboratory data

After enrollment, the medical history and data from physical examination were recorded. All recorded parameters were ascertained for completeness and exactness by two independent observers. Clinical history and physical examination were assessed 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. Blood was drawn and analyzed directly without freezing according to local standard laboratory procedures. Blood cell and differential white blood cell count were analyzed in a Sysmex XE-2100 automated cell counter (Sysmex Corporation, Norderstedt, Germany). Treating physicians and sonographers were blinded for all laboratory and demographic parameters.

### 2.2. Ultrasound

Duplex ultrasound examinations were performed on an Acuson 128 XP10 with a 7.5-MHz linear array probe (Acuson, Malvern, Pennsylvania). The degree of carotid narrowing was quantified as previously described [15]. Briefly, duplex grading of the carotid stenosis was determined by measuring the peak systolic and end-diastolic velocities in the internal carotid arteries (ICAs) and common carotid arteries.

## 3. Definitions

Definitions of risk factors and comorbidities were published previously [15]. Briefly, hypertension was diagnosed in patients with blood pressure values above 140/90 mm Hg and was considered present in patients taking antihypertensive drugs. Patients with a fasting blood glucose level of $>$126 mg/dL (7.0 mmol/L), a glycohemoglobin A1c $>$6.5% and patients under anti-diabetic therapy were considered diabetic. The family history of atherosclerotic disease was considered positive if its presence had been verified in a first-degree relative.

## 4. Study end point and surveillance protocol

All-cause death was the primary study end point, cardiovascular death was the secondary objective. Cardiovascular and all-cause mortality were assessed by screening the national register of death for the specific cause of death (according to the “International Statistical Classification of Diseases and Related Health Problems, 10th Revision”). To prevent study subjects being lost to follow-up due to migration or other causes, telephone contact to the patients or their relatives was established to check on the patients’ vital status. This was done if a patient had not been re-examined at our outpatient department within 12 months. None of the patients were lost to follow-up for the final analysis. In 43% of deaths the underlying cause was assessed by autopsy.

## 5. Statistical methods

Continuous data are presented as median and interquartile range. Discrete data are given as counts and percentages. We used *t*-tests and Mann–Whitney-tests for comparison of neutrophil numbers in patients who died from all-cause and cardiovascular mortality, respectively, compared to survivors as well as Spearman correlation coefficients ($r_s$) for univariate analyses, as appropriate. Time-dependent variables were analyzed using the Kaplan–Meier method and compared by the log-rank test. For this purpose, levels