Increased leucocyte count could predict coronary artery calcification in patients free of clinically apparent cardiovascular disease

Bilinen kardiyovasküler hastalığı olmayanlarda artmış lökosit sayısı koroner arter kalsifikasyonu için belirleyici olabilir

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ABSTRACT

Objectives: Several studies have demonstrated that inflammation plays a major role in the development of atherosclerosis and that the inflammatory process might also be involved in coronary artery calcification (CAC). The main purpose of this study was to investigate the relation between leucocyte count and CAC and to determine whether a higher leucocyte count could indicate subclinical atherosclerosis in patients without overt cardiovascular disease.

Study design: A total of 284 consecutive patients (156 men, 128 women) without established cardiovascular disease were enrolled. CAC was measured using cardiac computed tomography. Leucocyte count was measured via routine blood examination.

Results: Patients with CAC had higher leucocyte counts compared to those without calcification (7.87±1.85 *vs.* 6.01±1.84; p<0.001). Logistic regression analysis identified the following as independent predictors of CAC: leucocyte count (odds ratio [OR]: 1.7, 95% confidence interval [CI]: 1.3-2.1), smoking (OR: 2.4, 95% CI: 1.2-4.6) and age (OR: 1.2, 95% CI: 1.1-2.3). There was also a significant correlation between CAC and leucocyte count (r=0.57, p<0.001).

Conclusion: We demonstrated that leucocytes may play an important role in the evolution of CAC and may be used in the detection of subclinical atherosclerosis in asymptomatic subjects.

ÖZET

Amaç: Yapılan çeşitli çalışmalarda, enflamasyonun ateroskleroz gelişiminde önemli bir rol aldığı ve enflamatuvar sürecin aynı zamanda koroner arter kalsifikasyonu (KAK) ile de ilişkili olabileceği gösterilmiştir. Bu çalışmanın temel amacı, belirgin kardiyovasküler hastalığı olmayan hastalarda lökosit sayısı ile KAK arasındaki ilişkiyi ve artmış lökosit sayısının subklinik aterosklerozun bir göstergesi olup olamayacağını araştırmaktır.

Çalışma planı: Kardiyovasküler hastalığı olmayan ardışık 284 hasta (156 erkek, 128 kadın) çalışmaya alındı. KAK bilgisayarlı kardiyak tomografi ile değerlendirilidi. Lökosit sayısı rutin kan incelemesi yoluyla ölçüldü.

Bulgular: Lökosit sayısı, KAK olanlarda olmayanlara göre anlamlı olarak yüksek bulundu (7.87±1.85 ve 6.01±1.84; p<0.001). Lojistik regresyon analizde lökosit sayısı (odds oranı [OO]: 1.7, %95 güven aralığı [GA]: 1.3-2.1), sigara içimi (OO: 2.4, %95 GA: 1.2-4.6) ve yaş (OO: 1.2, %95 GA: 1.1-2.3) KAK'nin bağımsız öngördürücüleri olarak bulundu. Lökosit sayısı ile KAK arasında anlamlı ilişki saptandı (r=0.57, p<0.001).

Sonuç: Lökosit KAK gelişiminde önemli rol oynayabilir ve artmış lökosit sayısı subklinik aterosklerozun göstergesi olabilir.

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Although atherosclerotic calcification begins as early as the second decade of life, just after fatty streak formation, it is generally not detected due to the absence of clinical symptoms.[1] Traditional cardiovascular risk factors and conventional risk assessment using the Framingham score may also significantly underestimate the extent of atherosclerosis.[2] Therefore, several noninvasive imaging technologies have evolved which make it possible to identify subclinical atherosclerosis before symptoms appear or major vascular events occur.[3]

Coronary artery calcification has long been known to occur as part of the atherosclerotic process. CAC can be quantified using cardiac computed tomography, and its occurrence is correlated with the extent and severity of atherosclerotic disease.[4] Moreover, CAC assessed by CT is now widely accepted as a surrogate marker of subclinical atherosclerosis.[5]

Inflammation is believed to play a major role in the initiation and progression of atherosclerosis and in the development of its clinical complications.[6] Leukocyte count is a marker of inflammation that is widely available in clinical practice. However, no studies have investigated the association between leucocyte count and coronary calcification.

The main purpose of this study was to investigate the relation between leucocyte count and coronary calcification and to determine whether higher leucocyte counts could identify patients with subclinical atherosclerosis.

PATIENTS AND METHODS

Study population

The study population consisted of 284 subjects free of clinically apparent cardiovascular disease who underwent coronary calcium score measurement. The criteria used to determine which patients undergo CAC measurement vary among physicians; patients with Framingham risk scores placing them in the intermediate risk group, a family history of coronary artery disease, or multiple risk factors may undergo CAC measurement. In this study, none of the patients underwent coronary angiography or any stress tests before CAC. Hypertension was defined as an average systolic blood pressure ≥140 mmHg and a diastolic blood pressure ≥90 mmHg or use of antihypertensive medication. Diabetes was defined as a fasting glucose level ≥126 mg/dl or use of any hypoglycemic medication. Hypercholesterolemia was defined as total cholesterol ≥200 mg/dl or use of lipid lowering medication. Patients were excluded if they had active infection or systemic diseases causing an increase in leucocytes. The local ethics committee approved the study.

Abbreviations:

CAC	*Coronary artery calcification*
CAD	*Coronary artery disease*
CT	*Computed tomography*

Cardiac computed tomography

All patients were scanned using similar commercially-available 64-detector MDCT scanners (Aquilion, Toshiba Medical Systems, Tochigi, Japan). The calcium score (CS) scans were obtained using standard techniques with slice collimation 4 x 3.0 mm, 300 mA, 120 kV, and gantry rotation time 0.4 s. Offline analyses at remote workstations with dedicated cardiac analysis software (Vitrea2 version 3.0.9.1, Vital Images, Minnetonka, Minnesota, USA) were used to calculate Agatston CS.

Statistical analysis

Continuous variables were expressed as mean±SD, and categorical variables were expressed as percentages. Comparison of categorical and continuous variables between groups was performed using the chi-square test, the Independent sample T test and the Mann-Whitney U test, as appropriate. Logistic regression analysis was used to identify independent determinants of CAC. Variables with p values ≤0.1 were selected for logistic regression analysis. The Mann-Whitney U test was performed to compare CAC scores of patients with leucocyte groups. Spearman's test was performed to investigate the correlation between CAC and leucocyte count. Statistical analysis was performed using SPSS 14.0. p values ≤0.05 were considered significant.

RESULTS

Two-hundred eighty-four patients were enrolled in this study. Patients were divided into two groups

Table 1. Clinical and demographic characteristics of patients with and without calcification

	CAC (–) (n=118)			CAC (+) (n=166)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			53±8			61±10	**<0.001**
Gender							
Male	62			94			
Female	56			72			0.32
Hypertension	60	50		83	50		0.88
Dyslipidemia	45	38		68	40		0.64
Diabetes mellitus	24	20		61	36		**0.003**
Smoking	18	14		77	44		**<0.001**
ACEI and ARB	40	31		51	30		0.85
CaCB	18	14		21	13		0.53
Beta blockers	12	10		18	12		0.60
Lipid lowering drugs	38	32		62	37		0.37
Total cholesterol			211±45			218±36	0.21
HDL-cholesterol			41±6			39±8	0.15
LDL-cholesterol			121±28			129±37	0.11
Triglycerides			168±67			172±87	0.68
Hemoglobin			13.3±1.3			13.6±1.2	0.06
White blood cell			6.01±1.84			7.87±1.85	**<0.001**
Platelets			243±58			235±69	0.31
BUN (mg/dl)			19±10			20±10	0.64
Creatine (mg/dl)			0.8±0.2			0.9±0.3	0.10

ARB: Angiotensin receptor blocker; ACEI: Angiotensin converting enzyme inhibitor; CAC: Coronary artery calcification score; CaCB: Calcium channel blockers.

according to CAC status. The CAC (–) group included patients with non-detectable coronary calcification. The CAC (+) group consisted of patients with any coronary calcification. The clinical and demographic characteristics of the two groups are detailed in Table 1. Patients with calcification had higher leucocyte counts (7.87±1.85 *vs.* 6.01±1.84; p<0.001; Fig. 1), and were more likely to have a history of diabetes and smoking (Table 1). Logistic regression analysis identified leucocyte count (odds ratio [OR]): 1.7, 95% confidence interval (CI): 1.3-2.1), smoking (OR: 2.3, 95% CI: 1.2-4.6) and age (OR: 1.2, 95% CI: 1.1-2.3) as independent predictors of coronary artery calcification (Table 2).

Spearman's correlation analysis also determined a significant correlation between CAC and leucocyte count (r=0.57, p<0.001; Fig. 2).

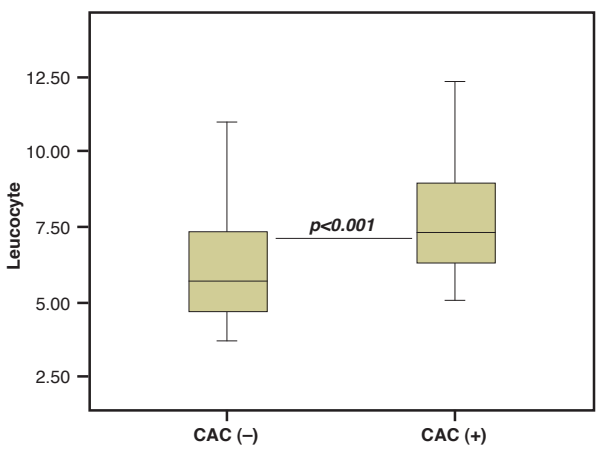

Figure 1. Leucocyte count in patients with and without coronary artery calcification.

In order to investigate the association between leucocyte levels and coronary calcification, pa-

Table 2. Logistic regression analysis of coronary artery calcification

Variable	*p*	OR	95% CI
Leukocytes	<0.001	1.7	1.3-2.1
Age (years)	0.001	1.2	1.1-2.3
Smoking, n (%)	0.003	2.3	1.2-4.6

CI: Confidence interval; OR: Odds ratio.

tients were divided into two subgroups according to leucocyte count. The number of patients with low (leucocyte count <7) and high leucocyte counts (leucocyte count ≥7) were 139 and 145, respectively. CAC measurements for patients with low and high leucocyte counts were 16±35 and 126±181, respectively (p<0.001; Fig. 3).

DISCUSSION

In this study, we determined a significant and independent association between leucocyte count and coronary artery calcification in patients free of clinically apparent cardiovascular disease. We also found that patients with higher leucocyte counts had increased atherosclerotic burdens.

Inflammation has been demonstrated to be a prominent feature of atherosclerotic disease, as well as, its clinical manifestations. Intensive studies have been performed, but the mechanisms behind coronary calcification are still unclear. There may be a mechanistic link between the pathological processes leading to calcification and those leading to atherosclerosis. Over the past two decades, data have emerged showing that immune cells are involved in the pathogenesis of atherosclerotic plaques. The accumulation and continued recruitment of leukocytes are associated with the development of plaques. Leukocytes may influence the development of CAD through their ability to cause proteolytic and oxidative damage to coronary arteries. Stimulated neutrophils are known to release proteolytic neutral proteases that promote the detachment of endothelial cells from vessel walls and the adherence of platelets to subendothelial collagen and fibronectin.[7] Activated neutrophils also release large amounts of the chemotactic agent leukotriene B4 in patients with stable angina,[8] secrete large amounts of inflammatory mediators,[9] and release superoxide anions in hyperlipidemic patients.[10]

The relationship between inflammatory markers and coronary artery calcification has been investigated previously.[11] CRP is one of the most studied inflammatory markers[12-15] and has been investigated in different populations with conflicting results. Other markers include fibrinogen,[16] lipoprotein-associated phospholipase A2 (Lp-PLA2),[17] and monocyte chemotactic protein-1 (MCP-1).[18] Jenny et al.[19] also investigated the

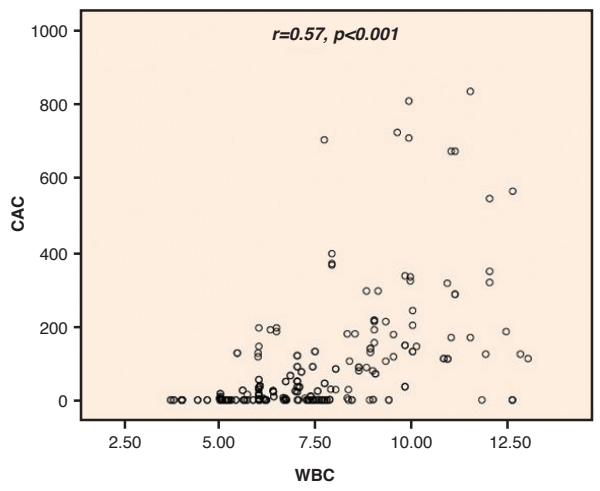

Figure 2. Correlation between CAC and leukocyte count. WBC: White blood cell count.

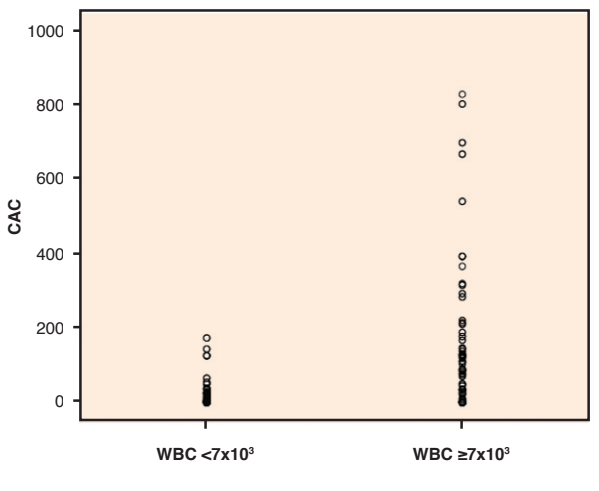

Figure 3. Coronary artery calcification scores for each leucocyte count group. WBC: White blood cell count.

relations between CRP, IL6, fibrinogen, and CAC. Their study demonstrated a weak association between these inflammatory markers and both CAC presence and atherosclerotic burden. However, to the best of our knowledge, no study has examined the relation between leucocytes and coronary calcification score.

Detection of coronary calcification is important, especially in asymptomatic subjects who may be prone to develop fatal and non-fatal cardiovascular events.[20] Coronary calcium detection by CT has been shown to identify atherosclerotic plaque and to quantitatively assess coronary calcium, a surrogate for plaque burden, thus providing powerful prognostic information.[21-24] The CCS can provide individual risk assessment and can reclassify the low and, particularly, the intermediate Framingham risk cohorts into lower- and higher-risk strata.[25]

Our study may contain two clinical suggestions. First of all, it may provide evidence for the role of inflammation, represented by higher leucocyte count, in the evolution of subclinical atherosclerosis. Secondly, it may provide physicians with a simple, readily available method for risk-stratifying patients, one which does not require complex devices or have any adverse effects on patients.

There are several limitations to our study. The sample size was modest and our data were cross-sectional, and thus, cannot be used to determine whether leucocyte count or CAC scores predict event rates. Our population consisted of patients free of clinical CAD. Therefore, our results cannot be generalized to patients with established cardiovascular disease. In addition, our patients were divided into groups based on an arbitrary leucocyte count cut-off of 7 x 10^9 cells/L.

In summary, our study suggests that leukocytes may have an independent role in the early phase of the atherosclerotic process and may be used to detect subclinical atherosclerosis in asymptomatic subjects. The clinical significance of these results requires further research.

Conflict-of-interest issues regarding the authorship or article: None declared

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Key words: Atherosclerosis/blood; biological markers/blood; calcium; coronary artery disease/epidemiology; risk factors.

Anahtar sözcükler: Ateroskleroz/kan; biyolojik belirteç/kan; kalsiyum; koroner arter hastalığı/epidemiyoloji; risk faktörü.