

Monocytosis is an Independent Risk Marker for Coronary Artery Disease

Abrahão Afiune Neto, Antonio de Pádua Mansur, Solange Desirée Avakian, Everly P. S. G. Gomes, José Antonio F. Ramires
Instituto do Coração do Hospital das Clínicas – FMUSP - São Paulo, SP - Brazil

OBJECTIVES

Inflammation and activation of immune system cells play an important role in the pathogenesis of atherosclerosis. This study analyzes the white blood count, including neutrophils, eosinophils, lymphocytes, monocytes and basophils, of patients with chronic coronary artery disease (CAD) and acute myocardial infarction (AMI).

METHODS

The white blood cell count was analyzed in 232 patients without diabetes between the ages of 15 and 88. One hundred and forty-two patients were angiographically diagnosed with CAD (57 with stable CAD and 85 with AMI) and compared to 90 control individuals. The control and CAD groups were similar in respect to age, body mass index, family history, smoking habits, hypertension, HDL and LDL (all variables with $p > 0.25$).

RESULTS

The univariate analysis revealed a higher prevalence of leukocytosis in the CAD group, which in turn was higher in the AMI patients than the stable CAD patients. The same trend was observed for monocytes. However, the distribution of all other cells in the complete blood count (CBC) was similar. Multivariate analysis using the logistic regression method with the stepwise (all variables) and backward models ($p < 0.25$), showed that monocytosis was an independent variable for CAD and AMI.

CONCLUSION

The number of monocytes, one of the most important components of the inflammatory process in the atherosclerosis plaque was an independent risk marker for CAD and AMI.

KEY WORDS

Atherosclerosis, coronary artery disease, acute myocardial infarction, hemogram, leukocytosis, monocytosis, inflammation.

Cardiovascular disease is the leading cause of death in Brazil¹. The main etiopathogenic mechanism is the atherosclerosis process. Inflammation and activation of immune system cells play an important role in the pathogenesis of atherosclerosis². Various biochemical markers have been suggested for coronary artery disease (CAD). Currently the most popular markers include C-reactive protein, homocysteine, uric acid, fibrinogen etc. However, other more traditional inflammatory markers are frequently relegated to the sidelines. Among these is the white blood cell count.

Friedman et al were the pioneers in the detection of a high white blood cell count as a predictor for acute myocardial infarction (AMI)³. Later, multi-center studies showed that an increased number of white blood cells was associated with higher mortality, more serious atherosclerosis and a lower response to fibrinolytic treatment⁴⁻⁶. Despite the important role of leukocytes in atherosclerosis, particularly acute coronary syndrome, little is known about the prevalence of the white blood cell count elements in CAD, particularly monocytes. It is known that monocytes are the main elements in the progression of atherosclerosis, inducing atherogenesis and thrombogenesis. This study analyzed the white blood cell count and its elements in the blood of patients with chronic CAD and in the acute phase of AMI.

METHODS

The white blood cell distribution was analyzed in 232 patients without diabetes with ages between 15 and 88 years. One hundred and forty-two patients were diagnosed with CAD (57 with stable CAD and 85 with AMI) by means of an angiography, compared to 90 control individuals. Inclusion criteria were: 1) Control Group: comprised of individuals referred to a secondary health care facility, with a low possibility of developing coronary artery disease, that is, asymptomatic individuals with no major CAD risk factors and a normal electrocardiogram at rest and a normal cardiac stress test; 2) Stable CAD Group: coronary disease verified by an angiography for asymptomatic patients or patients with angina pectoris typical for strenuous (class I) or moderate exertions (class II of the Canadian Cardiovascular Society)⁷, that had no history of myocardial infarction or any infarction indicators on the electrocardiogram; 3) AMI Group: comprised of acute myocardial infarction patients⁸.

The AMI diagnosis was based on the presence of at least two of the following criteria: typical pain with a duration of more than 20 minutes; increased CKMB activity (creatinase kinase MB fraction), or increase in the CK mass or troponin I levels; an elevation of the ST segment ≥ 1 mm for at least two frontal leads or ≥ 2 mm for at least two precordial leads on the electrocardiogram at rest; appearance of new Q waves on the electrocardiogram at rest. The control and CAD groups were similar in

respect to age, body mass index, family history, smoking habits, hypertension, HDL and LDL (all variables with $p > 0.25$).

The main risk factors and their definitions for CAD are as follows. Smoker: person who smokes more than five cigarettes per day or quit smoking within the past six months. Ex-smoker: person who previously smoked more than five cigarettes per day and quit smoking more than six months before the clinical assessment. Dyslipidemia: triglyceride serum levels ≥ 200 mg/dl, and/or total cholesterol ≥ 200 mg/dl, and/or HDL-cholesterol ≤ 40 mg/dl, and/or LDL-cholesterol ≥ 130 mg/dl⁹. Diabetes Mellitus: blood glucose levels ≥ 126 mg/dl after nocturnal fasting for twelve hours¹⁰. The hypertension diagnosis was based on a diastolic blood pressure measurement that was ≥ 90 mmHg¹¹. Family History: parents or siblings with a history of coronary disease under the age of 55 for men and 65 for women.

Exclusion criteria were: patients with a previous history of diabetes mellitus (fasting blood glucose level ≥ 126 mg/dl), chronic kidney failure (creatinine serum ≥ 2.0 mg/dl), liver failure and clinically significant endocrine, hematologic, respiratory or metabolic diseases.

Laboratory tests - Blood was collected in the morning after twelve hours of fasting and the samples were analyzed using the following methods: 1) Automated electronic red blood cell count (normal, in millions/mm³, from 4.2 to 5.2 for women and from 4.6 to 6.2 for men), hemoglobin (normal, in g%, from 12 to 16 for women and from 14 to 17 for men), hematocrit (normal, in %, from 37 to 47 for women and from 40 to 54 for men), platelets (normal from 150 to 350 mil/mm³), and white blood cells (normal from 5,000 to 10,000/mm³). 2) HDL-cholesterol levels (normal above 40 mg/dl) were determined using the enzymatic calorimetric method and LDL-cholesterol levels (normal up to 130 mg/dl) using the Friedewald formula: $LDL = CT - [HDL + (TG/5)]$ ¹³, considering triglyceride levels lower than 400 mg/dl for the calculation. 3) Apolipoprotein AI (normal, in g/l, from 1.15 to 1.90 for men and from 1.15 to 2.20 for women) and apolipoprotein B (normal, in g/l, from 0.70 to 1.60 for men and from 0.60 to 1.50 for women) levels were obtained using the automated immunoturbidimetry method (Cobas Integra, 700 Roche Ltd., Diagnostics Division, Basilea, Switzerland).

Cardiac catheterization - Cardiac catheterization was conducted using the Sones and Shirey technique¹⁴. The subepicardial coronaries were classified as normal, single, double or triple artery or left coronary trunk according to the number of subepicardial coronary arteries that had or did not have obstructions caused by atherosclerosis with more than 50% reduction of the vascular lumen in comparison with the nearest normal segment.

Statistical analysis - The computer program SAS (SAS Institute Inc, 1996, version 6.12) was used for the statistical analysis. The Student's t-test was used for

the univariate analysis of the continuous variables and the χ^2 tests. The Mann-Witney and Kruskal-Wallis tests were used for the univariate analysis of the categorical variables. Statistical significance was established as $p < 0.05$. Multivariate analysis was conducted afterwards using the logistic regression method and the stepwise model for all variables and the backward model for variables with values of $p < 0.25$.

RESULTS

The clinical characteristics of the patients are found in table 1. The main risk factors, lipid profile including the apolipoproteins, and glucose blood levels were similar between the control and CAD groups. The control group had more women ($p = 0.031$).

In relation to the white blood cell count, univariate analysis revealed a higher prevalence of leukocytosis in the CAD groups, which was higher in the AMI patients than the stable CAD patients. The same trend was seen

for monocytes. The distribution of the remaining elements of the CBC was similar for both the control and CAD patients (tab. 2). However, a comparison between the control, stable CAD and AMI groups shows a gradual increase in the white blood cells, band cells, segmented cells and monocytes (tab. 3). The seriousness of CAD in single (41% versus 44%), double (21% versus 19%) or triple artery (35% versus 37%), was similar between the stable CAD and AMI groups. Two patients in the stable CAD group and one in the AMI group had a lesion on the left coronary trunk.

Multivariate analysis using the logistic regression method with the stepwise (all variables) and backward stepwise ($p < 0.25$) models showed that gender and monocytosis are independent variables for CAD (tab. 4). Monocytosis was also an independent variable for AMI.

DISCUSSION

In our study, the monocytes, one of the most

Table 1 – Clinical Characteristics

	Control	CAD
n (232)	90 (39)	142 (61)
Age (years)	58.66 ± 11.48	57.34 ± 13.14
Gender (male/female)	53(59) / 37(41)	103(72) / 39(28)*
Smokers	23 (26)	39 (28)
Systemic Hypertention	23 (26)	56 (39)
Family History	26 (29)	41 (29)
Acute Myocardial Infarction		85 (60)
Body Mass Index (Kg/m ²)	26.73 ± 4.68	26.75 ± 3.78
Systolic Blood Pressure (mmHg)	141.61 ± 24.69	140.39 ± 25.91
Diastolic Blood Pressure (mmHg)	85.24 ± 13.62	87.52 ± 15.84
Triglycerides (mg/dl)	153.89 ± 76.17	166.24 ± 120.45
Total Cholesterol (mg/dl)	205.98 ± 50.06	207.86 ± 59.86
HDL-cholesterol (mg/dl)	41.29 ± 14.59	40.24 ± 13.93
LDL-cholesterol (mg/dl)	125.34 ± 47.74	128.37 ± 52.41
Apolipoprotein AI (g/L)	1.48 ± 0.62	1.30 ± 0.49
Apolipoprotein B (g/L)	1.20 ± 0.49	1.30 ± 0.49
Glucose (mg/dl)	100.26 ± 35.48	101.14 ± 33.86

* $p = 0.031$; Numbers shown are mean values ± SD and percentages are shown in brackets; CAD = coronary artery disease

Table 2 – Complete Blood Count

	Control	CAD	p*
n (232)	90 (39)	142 (61)	0.436
White blood cells	6,444 ± 1,715	7,942 ± 3,355	< 0.0001
Band Cells	68.5 ± 155.7	139.5 ± 250.6	0.077
Segmented	1,317 ± 2,111	2,258 ± 3,125	0.077
Neutrophils	2,419 ± 1,889	2,371 ± 2,297	0.934
Eosinophils	248 ± 256	327 ± 385	0.156
Basophils	40.7 ± 37.5	39.3 ± 45	0.801
Lymphocytes	1,824 ± 603	2,107 ± 1,780	0.157
Monocytes	517 ± 203	602 ± 228	0.0002

*Mann-Witney U-Test; CAD = coronary artery disease

Table 3 – Complete Blood Count for the ischemic syndromes

	Control	Stable CAD	AMI	p*
n (232)	90 (38)	57 (25)	85 (37)	
White Blood Cells	6,444 ± 1,715	6,982 ± 1,756	8,585 ± 3,974	< 0.0001
Band Cells	68.5 ± 155.7	80.4 ± 172	179 ± 285	0.023
Segmented	1,317 ± 2,111	1,391 ± 2,295	2,836 ± 3,468	0.012
Neutrophils	2,419 ± 1,889	2,677 ± 2,119	2,166 ± 2,398	0.584
Eosinophils	248 ± 256	320 ± 370	331 ± 396	0.366
Basophils	40.7 ± 37.5	37.7 ± 40.7	40.3 ± 47.9	0.951
Lymphocytes	1,824 ± 603	1,932 ± 567	2,225 ± 2,252	0.365
Monocytes	517 ± 203	542 ± 167	672 ± 248	< 0.0001

*Kruskal-Wallis test; CAD = coronary artery disease; AMI = acute myocardial infarction

Table 4 – Multivariate analysis using the logistic regression method. (backward stepwise elimination procedure)

Variable	p	Odds Ratio (Confidence Interval 95%)
Intercepter	0.7548	
Age	0.1456	0.977 (0.946-1.008)
Body Mass Index	0.4447	1.040 (0.941-1.148)
Systolic Blood Pressure	0.4209	0.990 (0.966-1.015)
Diastolic Blood Pressure	0.2390	1.028 (0.982-1.078)
Smoking	0.6309	0.814 (0.353-1.881)
Monocytes	0.0083	1.003 (1.001-1.005)
Triglycerides	0.1566	1.005 (0.998-1.012)
Total Cholesterol	0.0729	0.985 (0.969-1.001)
HDL-cholesterol	0.3424	0.982 (0.947-1.019)
LDL-cholesterol	0.1695	1.011 (0.995-1.027)
Glucose	0.0597	1.015 (0.999-1.031)
Apolipoprotein AI	0.3663	1.682 (0.545-5.193)
Apolipoprotein B	0.8583	1.120 (0.324-3.875)

important components of the inflammatory process in atherosclerosis plaque, were an independent marker for the prognosis of stable CAD and AMI. A gradual increase of monocytes was noted in a comparison between the control group and the stable CAD and AMI groups. Studies have shown that this gradual increase is related to the seriousness of atherosclerosis, the coronary blood flow (TIMI score) and higher mortality for patients with acute coronary syndrome¹⁵. Similarly, another study showed a higher incidence of congestive heart failure and higher intra-hospital mortality for both men and women with leukocytosis ($\geq 10,000/\text{mm}^3$)¹⁶.

Various other studies have analyzed the relation between more severe leukocytosis in patients with acute coronary syndrome however few have analyzed leukocytosis in patients with stable CAD. Recent studies have observed: leukocytosis is more severe in patients with stable CAD that have had a prior AMI than those

with just stable CAD¹⁷; patients with some type of ischemic heart disease (chronic or acute) have more severe neutrophilia¹⁸; in the CAPRIE study that compared the effects of clopidogrel with aspirin, neutrophilia was also an independent risk variable for cardiovascular disease¹⁹. An interesting issue in the CAPRIE study is that mononucleosis was an independent variable for ischemic stroke and a limit variable for the recurrence of ischemic events [OR = 1.03, IC(1.00 – 1.05); p = 0.062]. Leukocytosis also enhances the effects of CAD risk factors.

Smokers who have more than nine thousand white blood cells are four times more likely to have a myocardial infarction than smokers with a white blood cell count less than six thousand²⁰. Generally speaking leukocytosis and in some studies monocytes are independent variables for cardiovascular disease. However from all the medical literature consulted, this study was the first to show that monocyte concentration is directly related to the clinical stage of coronary atherosclerosis, accounting for the slightly higher values for the AMI group in comparison with the stable CAD group and the stable CAD group in comparison with the control group.

These alterations suggest different degrees of white blood cell activation and reflects the severity of the existing inflammatory process which is responsible for the instability of the atherosclerosis plaque, which can lead to coronary artery spasms²¹ or contribute to posterior coronary stent occlusion²². Various substances can participate in this increase/recruitment/activation process of the monocytes. For example, MCP-1 (monocyte chemoattractant protein-1) is a cytokine that promotes monocyte recruitment in the atherosclerotic plaque and is an independent risk marker for CAD²³. Consequently, the white blood cell count is an important risk marker that could be a guide in the diagnosis and treatment for individuals with circulatory diseases. Nevertheless, further studies are required to define the role of these substances in daily clinical practice.

REFERENCES

- Mansur AP, Favarato D, Souza MFM, et al. Tendência da Mortalidade por Doenças Circulatórias no Brasil de 1979 a 1996. *Arq Bras Cardiol* 2001; 76: 497-503.
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999; 340: 115-26.
- Friedman GD, Klatsky AL, Siegel AB. The leukocyte count as a predictor of myocardial infarction. *N Engl J Med* 1974; 290: 1275-8.
- Barron HV, Cannon CP, Murphy SA, Braunwald E, Gibson CM. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction. *Circulation* 2000; 102: 2329-34.
- Cannon CP, McCabe CH, Wilcox RG, Bentley JH, Braunwald E, for the OPUS-TIMI 16 Investigators. Association of White Blood Cell Count With Increased Mortality in Acute Myocardial Infarction and Unstable Angina Pectoris. *Am J Cardiol* 2001; 87: 636-9.
- Sabatine MS, Morrow DA, Cannon CP et al. Relationship between baseline white blood cell count and degree of coronary artery disease and mortality in patients with acute coronary syndromes: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy- Thrombolysis in Myocardial Infarction 18 trial) substudy. *J Am Coll Cardiol* 2002; 40: 1761-8.
- Campeau L. Grading of angina pectoris (letter). *Circulation* 1976; 54: 522-3.
- Willerson JT, Cohen LS, Maseri A. Pathophysiology and clinical recognition. In: Willerson JT, Cohen JN, ed. *Cardiovascular Medicine*. New York, Churchill Livingstone, 1995, 335.
- III Diretrizes Brasileiras Sobre Dislipidemias e Diretriz de Prevenção da Aterosclerose do Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. *Arq Bras Cardiol* 2001; 77(supl III): 4-8.
- Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997; 20: 183-97.
- Chobanian AV, Bakris GL, Black HR, et al. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; 42: 1206-52.
- Warnick GR, Benderson JM, Albers JJ. Dextran sulfate precipitation procedure for quantification of high density lipoprotein. *Clin Chem* 1982; 28: 1379-88.
- Freidewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
- Sones FM, Shirey EK. Cinecoronary arteriography. *Mod. Concepts Cardiovasc Dis* 1962; 31: 735-8.
- Barron HV, Harr SD, Radford MJ, Wang Y, Krumholz HM. The association between white blood cell count and acute myocardial infarction mortality in patients > or = 65 years of age: findings from the cooperative cardiovascular project. *J Am Coll Cardiol* 2001; 38: 1654-61.
- Furman MI, Gore JM, Anderson FA et al. GRACE Investigators. Elevated leukocyte count and adverse hospital events in patients with acute coronary syndromes: findings from the Global Registry of Acute Coronary Events (GRACE). *Am Heart J* 2004; 147: 42-8.
- Sposito AC, Lemos PA, Maranhão RC, Mansur AP, Cesar LA, Ramires JAF. The pre-existence of an acute coronary event predicts differences in biological parameters and clinical evolution among patients with longstanding stable angina. *Int J Cardiol* 2003; 91: 193-200.
- Wheeler JG, Mussolino ME, Gillum RF, Danesh J. Associations between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30,374 individuals. *Eur Heart J* 2004; 25: 1287-92.
- Grau AJ, Boddy AW, Dukovic DA et al. CAPRIE Investigators. Leukocyte count as an independent predictor of recurrent ischemic events. *Stroke* 2004; 35: 1147-52.
- Zalokar JB, Richard JL, Claude JR. Leukocyte count, smoking, and myocardial infarction. *N Engl J Med* 1981; 304: 465-8.
- Hung MJ, Kuo LT, Cheng CW, Chang CP, Cheng WJ. Comparison of peripheral monocyte counts in patients with and without coronary spasm and without fixed coronary narrowing. *Am J Cardiol* 2004; 93: 620-4.
- Fukuda D, Shimada K, Tanaka A et al. Circulating monocytes and instant neointima after coronary stent implantation. *J Am Coll Cardiol* 2004; 43: 18-23.
- Deo R, Khera A, McGuire DK et al. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *J Am Coll Cardiol* 2004; 44: 1812-8.