

STATE-OF-THE-ART PAPER

Leukocyte Count and Coronary Heart Disease

Implications for Risk Assessment

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Inflammation is a key feature of atherosclerosis and its clinical manifestations. The leukocyte count is a marker of inflammation that is widely available in clinical practice. This paper reviews the available epidemiologic evidence for a relationship between the leukocyte count and coronary heart disease (CHD). Numerous epidemiologic and clinical studies have shown leukocytosis to be an independent predictor of future cardiovascular events, both in healthy individuals free of CHD at baseline and in patients with stable angina, unstable angina, or a history of myocardial infarction. This relationship has been observed in prospective and retrospective cohort studies, as well as in case-control studies. It is strong, consistent, temporal, dose-dependent, and biologically plausible. The relationship persists after adjustment for multiple CHD risk factors, including smoking. Elevated differential cell counts, including eosinophil, neutrophil, and monocyte counts, also predict the future incidence of CHD. Leukocytosis affects CHD through multiple pathologic mechanisms that mediate inflammation, cause proteolytic and oxidative damage to the endothelial cells, plug the microvasculature, induce hypercoagulability, and promote infarct expansion. In summary, leukocytosis has been consistently shown to be an independent risk factor and prognostic indicator of future cardiovascular outcomes, regardless of disease status. The leukocyte count is inexpensive, reliable, easy to interpret, and ordered routinely in inpatient and outpatient settings. However, its diagnostic and prognostic utility in CHD is widely unappreciated. Further studies are needed to assess the true impact of leukocytosis on CHD, compare it with other inflammatory markers such as C-reactive protein and lipoprotein phospholipase A₂ levels, and promote its use in CHD prediction. (J Am Coll Cardiol 2004;44:1945-56)

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Cardiovascular disease (CVD) is the leading cause of death in the Western world (1). One of its most insidious forms is coronary heart disease (CHD) due to atherosclerosis (2). Although many risk factors for CHD have been identified, they do not fully account for all cases of the disease. Thus, the search is underway for additional biologic markers and especially inflammatory markers. The interest in inflammatory markers is warranted for several reasons. Various types of inflammatory cells, including monocytes, lymphocytes, eosinophils, and neutrophils, have been implicated in CHD (3,4). Numerous epidemiologic and clinical studies (reviewed in Tables 1 to 3) have shown the leukocyte count to be an independent risk factor for CHD, a risk factor for future cardiovascular events in individuals apparently without CVD, and a prognostic marker of future events in patients who already have CVD (5). This review discusses the relationship between leukocyte count and CHD in prospective, retrospective, and case-control studies, as well as the implications for clinical risk assessment and prognosis. We searched for the relevant studies using online

databases (with major emphasis on PubMed), reference sections of major textbooks, and personal communication with experts in the field. We also searched through the references of all the studies found from previous sources to locate additional references that may be useful for this review. To avoid a selection bias, we made every effort to find and discuss studies with negative findings. To ensure the quality of our data, we limited our review to the studies published as full text in peer-reviewed journals.

INFLAMMATION AND ATHEROSCLEROSIS

Correlation of the leukocyte count with CHD and investigations into the utility of the leukocyte count as a risk factor and prognostic indicator in patients with CHD are consistent with the current concept that atherosclerosis is an inflammatory disease (2). According to this concept, monocytes are recruited from the peripheral blood into the vessel wall after endothelial injury. The recruited monocytes differentiate into macrophages that phagocytose lipids and secrete metalloproteinase enzymes, such as elastase and collagenase, within the atherosclerotic lesion (2,6,7). In addition, neutrophils and mast cells that also secrete or induce degradative proteases begin to accumulate in the plaque (8-10). Over time, the recruitment and accumulation of inflammatory cells increase the lipid and inflammatory cell content of the plaque and cause extensive neovas-

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Abbreviations and Acronyms

BMI	=	body mass index
CAD	=	coronary artery disease
CHD	=	coronary heart disease
CK	=	creatinine kinase
CRP	=	C-reactive protein
CVD	=	cardiovascular disease
IL	=	interleukin
MI	=	myocardial infarction
UA	=	unstable angina
WBC	=	white blood cell

cularization of involved adventitia and intima (11). The atherosclerotic plaque becomes more vulnerable to rupture, leading to cardiovascular events. When plaque rupture does occur, it is usually followed immediately by mural or occlusive coronary thrombosis and dynamic vasoconstriction on exposed intimal tissue in or near areas of luminal inflammation (12,13).

TOTAL LEUKOCYTE COUNT AS A RISK FACTOR IN SUBJECTS FREE OF CHD

The leukocyte count has been correlated with CHD since the 1920s (14). Over the last several decades, an increasing number of prospective studies conducted in CHD-free populations have shown a clear and positive correlation between the leukocyte count and risk of CHD (Table 1) (15-19). The correlation appears to persist even after adjustment for other risk factors (20-22). It also appears to be especially strong in individuals who smoke, despite some evidence to the contrary from a study done in elderly Netherlanders (23). The Evaluation of c7E3 Fab in the Prevention of Ischemic Complications (EPIC) trial (24) revealed a correlation between baseline leukocyte count and the incidence of myocardial infarction (MI) after elective percutaneous coronary intervention.

Two case-control studies in disease-free subjects (25,26) confirmed the correlation between leukocyte count and CHD. One of these studies (25), which evaluated gender-, age-, and risk-matched subjects, found that the mean leukocyte count was higher in patients than in control subjects (8,000/mm³ vs. 7,500/mm³). The other study, which used a cohort of dyslipidemic men from the Helsinki Heart Study of coronary atherosclerosis primary prevention, found leukocyte counts to be higher in patients than in controls at baseline before admission. In addition, smokers with elevated white blood cell (WBC) counts had a higher relative risk for disease than non-smokers. As discussed later, the WBC count is usually higher in smokers; however, their effect is mostly additive, as WBC is an independent risk factor for CHD.

Retrospective studies in disease-free subjects have also correlated the leukocyte count and CHD. A large, retrospective five-year cohort study (27) showed not only that high leukocyte counts at baseline correlated with the devel-

opment of acute coronary syndromes, but also that baseline leukocyte counts were higher in diseased patients than in patients who had no significant CHD or stable angina.

TOTAL LEUKOCYTE COUNT AS A PROGNOSTIC INDICATOR IN SUBJECTS WITH CHD

Stable CHD. Several studies have shown a strong link between leukocyte count and prognosis in patients who have stable CHD after a previous MI. The leukocyte count was strongly associated with the prognosis of patients who have had an MI within the previous three months (28). In the first Persantin-Aspirin Re-Infarction Study (PARIS-1) (29), the baseline leukocyte count was strongly associated with coronary event recurrence and total mortality 2 to 60 months after MI, even after adjustment for other variables, including smoking. In a study of patients who had angiographically documented coronary artery stenosis (30), both the leukocyte count and neutrophil count at baseline correlated with the number and extent, severity, and location (Gensini index) of coronary stenoses and survival. Another study (31) found that patients with angiographically documented coronary artery disease (CAD) had higher leukocyte counts than patients without it. The same study also indicated that, after adjustment for age, gender, cholesterol, triglyceride level, and smoking, the leukocyte count was an independent marker of CHD severity (i.e., diameter stenosis). In a study of patients who have had an acute MI within the previous six months (32), high leukocyte counts were associated with an increased risk of re-infarction or death, even after adjusting for confounding risk factors.

Acute MI or unstable angina (UA). In a study that correlated hemostatic variables with mortality in patients with acute MI (33), leukocyte counts at presentation were significantly higher in patients with acute MI, as opposed to UA, in patients with transmural infarcts, as opposed to subendocardial infarcts, and in patients with radiographically documented left ventricular failure, as opposed to no heart failure. In addition, leukocyte counts strongly correlated with increased mortality at six weeks and one year. In a study of the relationship between leukocyte count and in-hospital mortality after MI (34), the case-fatality rate increased markedly with each incremental (1 SD) increase in leukocyte count. In one study of patients with acute MI or high-risk UA pectoris (35), mortality was especially higher among patients who had leukocyte counts >10,000. Barron et al. (36) utilized the Cooperative Cardiovascular Project database and stratified patients with acute MI into quintiles by leukocyte count and found that patients in the highest quintile were three times more likely to die early than patients in the lowest quintile. In the Treat Angina with Aggrastat Plus Determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis In Myocardial Infarction-18 (TACTIS-TIMI-18) trial of patients with UA or non-ST-segment elevation MI (37), high baseline leukocyte counts were associated with poorer reper-

Table 1. Studies Correlating Leukocyte Count and Coronary Heart Disease in Subjects Free of Disease at Baseline

Study Type	Study (Ref.)	Sample Size	Follow-Up (yrs)	Clinical End Points	Risk Estimate*	95% CI	p Value	Setting	Gender	Years of Enrollment	Leukocyte Count Cutoff Values
P	Zalokar et al. (15)	7,206	6.5	Fatal/nonfatal MI	4.5	2.5-7.8	<0.01	France	M	1967-1972	<6,000 vs. ≥9,000
P	Grimm et al. (16)	6,222	7	CHD death	1.98	1.2-3.4	0.001	U.S.	M	1973-1982	≤6,000 vs. >7,700
P	Grimm et al. (16)	6,222	7	CHD death/nonfatal MI	1.53		0.001				
				All-cause mortality	1.49		0.02				
P	Philips et al. (17)	7,344	8	CHD incidence	1.32 (RO)†	1.18-1.47	<0.0001	UK/W/SC	M	1978-1980	NA
		12,845	7	CHD death	1.15 (RO)‡	1.07-1.23	0.0001	U.S.	M	1973-1976	NA
		7,992	12		1.14 (RO)§	1.05-1.25	0.003	U.S.	M		NA
P	Kannel et al. (18)	1,393 (M) 1,401 (F)	12	CHD events	>3 (M)¶ >2 (F)¶		<0.001 NS	U.S.	M/F	NA	NA
P	Gillum et al. (19)¶	6,196 (CHD mortality) 5,293 (CHD incidence)	13.9	CHD incidence CHD death CVD death All-cause mortality	1.31 for CHD incidence	1.07-1.61 (M) 1.05-1.63 (F)		U.S.	M/F	1971-1975	<6,100 vs. >8,100
P	Weijenberg et al. (23)	884	5	CHD incidence CHD death	1.14 1.32	0.98-1.32 1.13-1.53		Netherlands	M	1985	7 × 10 ⁹ /l vs. 6.6 × 10 ⁹ /l
P	Folsom et al. (20) [§]	14,477	5.2	CHD incidence	1.13 (M) 1.45 (F)	0.98-1.31 (M) 0.86-2.44 (F)		U.S.	M/F	1987-1989	6,200 vs. 6,900
P	Lee et al. (21)	13,555	8	CHD incidence	1.8 (overall) 1.69 (whites) 1.91 (blacks)	1.32-2.43 1.13-2.53 1.19-3.09	<0.001 0.001 0.009	U.S.	M/F	1987-1989	<4,800 vs. ≥7,000
P	Lee et al. (21)	13,555		CHD mortality	2.26 (blacks) 2.22 (whites)	1.38-3.72 1.13-4.37	<0.001 0.001				
P	Brown et al. (22)	8,914	17	CVD mortality	1.4	1.1-1.8	1.1-1.8	U.S.	M/F	1976-1980	7.7 × 10 ⁹ /l vs. 18.4 × 10 ⁹ /l
P	Aronow et al. (24)	880	NA	MI incidence	1.136 (OR)	1.049-1.231	0.002	U.S.	NA	NA	NA
CC	Friedman et al. (25)	464	1.4	MI incidence	1.6		<0.001	U.S.	M/F	1964-1970	<6,300 vs. >8,800
CC	Manttari et al. (26)	420	5	CHD incidence	1.13 (OR) 3.07 (nonsmokers) 1.86 (smokers)	1.00-1.27 2.23-8.19 0.81-4.28	0.03	Finland	M/F	NA	NA
R	Kostis et al. (31)	573	NA	CAD incidence	NA	NA	0.0005	U.S.	M/F	NA	7,280 vs. 6,664
R	Takeda et al. (27)	6,021	NA	ACS incidence	2.049	1.042-4.016	0.038	Japan	M/F	1994-1999	9,209 vs. 6,205
MA	Danesh et al. (5)#	7,229	8	MI incidence + CHD mortality	1.5	1.4-1.6	NA	NA	M/F	NA	8.4 × 10 ⁹ /l vs. 5.6 × 10 ⁹ /l

*Risk estimate is given in terms of relative risk, except where the relative odds (RO) or odds ratio (OR) is indicated. †British Regional Heart Study (BRHS). ‡Multiple Risk Factor Interventional Trial I (MRFIT I). §Multiple Risk Factor Interventional Trial II (MRFIT II). ¶Relative risk of coronary heart disease (CHD) incidence determined by comparing lowest leukocyte count tertile with highest leukocyte count tertile. ||For each 1,000/ μ l increase in leukocyte count. #Meta-analysis by Danesh et al. yielding overall relative risk and 95% confidence interval (CI) in all prospective and prognostic studies. √Values for CHD death and all-cause mortality have not been mentioned. §Values after an increase of 1 SD in leukocyte count.

ACS = Acute coronary syndrome; CAD = coronary artery disease; CC = case-control; CVD = cardiovascular disease; MA = meta-analysis; MI = myocardial infarction; NA = not applicable; NS = not significant; P = prospective; R = retrospective; S = Scotland; W = Wales.

Table 2. Studies Correlating Leukocyte Count and Coronary Heart Disease in Subjects With Acute Myocardial Infarction or Unstable Angina

Study Type	Study (Ref.)	Sample Size	Follow-Up (yrs)	Clinical End Points	Risk†	95% CI	p Value	Setting	Gender	Years of Enrollment	Leukocyte Count Cutoff Values
P	Amaro et al. (30)	152	3.15	Gensini index CHD death			<0.05	Spain	M	1985–1990	8,309 vs. 7,548
P	Hajj-Ali et al. (32)	1,294	2.1	RI/CHD death	1.18	1.00–1.40	0.05	U.S./Canada	M/F	1983–1986	52,000 vs. 10,600
P	Hajj-Ali et al. (32)	1,294	2.1	AMI/death	1.26	1.08–1.47	0.003	U.S./Canada	M/F	1983–1986	52,000 vs. 10,600
P	Furman et al. (34)	2,863	4	Post-AMI mortality	1.65‡	1.09–2.49		U.S.	M	1986, 1988, 1990–1991	≤7,900 vs. ≥14,200
P	Cannon et al. (35)	10,288	0.8	AMI, recurrent ischemia	2.38 (30 days)§ 1.85 (10 months)§		<0.001	U.S./UK U.S./UK	F	1997–1998	≤6,000 vs. >10,000
P	Cannon et al. (35)	10,288	0.8	CHD death	5.2 (30 days)¶ 3.5 (10 months)¶		<0.001				
P	Barron et al. (36)	153,213 2,208	NA 0.5	CHD death Death, nonfatal MI	2.37 (OR) 4.3 (HR)	2.25–2.49	0.0001	U.S.	M/F	1994–1995	<6,000 vs. >12,000
P	Sabatine et al. (37)			ACS			0.049	U.S.	M	NA	NA
P	Mueller et al. (38)	1,391	1.4	CHD death	3.3 (HR)	1.94–5.58	<0.001	Switzerland	M/F	1996–1999	<6,800 vs. >10,000
CC	Hung et al. (39)	36	2.3	CHD death	NA	NA	0.005	Taiwan	M/F	1998–2002	<6,650 vs. >10,100
R	Barron et al. (40)	975	NA	Death/CHF/shock*	0.24 (death) 0.24 (CHF/shock) 0.19 (death/CHF/shock)	0.07–0.75 0.08–0.74 0.07–0.47	0.014 0.01 <0.001	U.S.	M/F	NA	NA
P	Zouridakis et al. (43)	71	1.1	UA/MI/CHD death	5.9 (OR)	1.03–33.65	0.041	UK	M/F	1996–1997	2.40 × 10 ⁹ /l
R	Ommen et al. (47)	211	3.75	CHD mortality	1.8#		0.04	U.S.	M/F	1981–1985	NA

*All values are for the highest leukocyte count tertile. †Risk is given in terms of relative risk, except where the RO, OR, or hazard ratio (HR) is indicated. ‡OR for the highest leukocyte quintile. §Leukocyte count >10,000. ¶Leukocyte count >15,000. ||Ratio with 10-U decrease of lymphocyte count. #Odds ratio for individuals with lymphocyte counts lower than the cut-off value.

AMI = acute myocardial infarction; CHF = congestive heart failure; RI = reinfarction; UA = unstable angina. Other abbreviations as in Table 1.

Table 3. Studies Correlating Differential Leukocyte Counts and Coronary Heart Disease in Subjects Free of Disease

Study Type	Study (Ref.)	Sample Size	Follow-Up (yrs)	Clinical End Points	Risk*	95% CI	p Value	Setting	Gender	Years of Enrollment	Leukocyte Count Cutoff Values
P	Prentice et al. (3)	15,909	16	Angina, MI, CHD death	1.82†		0.01	U.S.	M/F	NA	<4,000 vs. >10,000
P	Olivares et al. (4)	3,779	5.5	Stable angina, UA, MI, CHD death	1.04 (TLC) 1.15 (monocytes)	0.95-1.14 1.07-1.24	0.4 <0.001	France	M	1980-1985	NA
P	Sweetnam et al. (42)	4,615	10 in C, 9.3 in S	CHD incidence	2.1 (RO)	1.51-2.92		UK	M	1979-1983	NA

*Risk is given in terms of relative risk, except where RO, OR, or HR is indicated. †OR for the highest leukocyte quintile.
C = Caerphilly; S = Speedwell; TLC = total leukocyte count. Other abbreviations as in Tables 1 and 2.

fusion, more extensive CAD, and higher six-month mortality. In a study that stratified patients with non-ST-segment elevation acute coronary syndrome and Braunwald class IIB or IIIB UA into quartiles by leukocyte count (38), those patients in the highest quartile were 3.3 times more likely to die of cardiac causes than those in the lowest quartile. Moreover, in a case-control study of patients with acute MI (39), leukocyte counts were significantly higher in patients who died of cardiac causes than in those who did not.

A number of retrospective studies have been conducted in patients with acute MI or unstable angina. The TIMI-10A and -10B trials (40) found that relatively high leukocyte counts were associated not only with new-onset congestive heart failure or shock but also with higher mortality rates. In a study assessing the sensitivity and specificity of leukocyte counts for disease in patients whose echocardiograms were non-diagnostic (41), baseline total creatine kinase (CK), CK-MB fraction, leukocyte count, and absolute neutrophil count were relatively insensitive (39%, 73%, 35%, and 36%, respectively) but relatively specific (94%, 93%, 85%, and 86%, respectively) as prognostic markers. In a logistic regression model, leukocytosis was an independent predictor of acute MI. Moreover, a combined decision rule of either elevated CK-MB or elevated leukocyte count was 88% sensitive and 79% specific, whereas a combined decision rule of both elevated CK-MB and leukocyte count was 20% sensitive and 99% specific. Table 2 lists these studies.

DIFFERENTIAL LEUKOCYTE COUNT AS A RISK FACTOR AND PROGNOSTIC INDICATOR

The utility of differential leukocyte counts as a risk marker has been evaluated in a number of studies (Table 3). In the Hiroshima and Nagasaki Adult Health Study (3), the total leukocyte count correlated positively with the incidence of CHD in a large population of individuals free of disease at baseline. When differential cell counts were considered, a correlation was found between moderately elevated eosinophil count and increased risk of disease, as well as between neutrophil, eosinophil, and monocyte (but not lymphocyte) counts and the incidence of disease. The Paris Prospective Study II (4) revealed that, after adjustment for other variables, the risk of CHD increased 1.15 times for each increase of 100 cells/mm³ in monocyte count. In two studies from the United Kingdom (42), the age-adjusted relative odds of CHD were highest for those men with the highest leukocyte counts; in fact, these odds were more than twice those for neutrophils, eosinophils, lymphocytes, monocytes, or basophils. In addition, a positive correlation was seen between neutrophil and eosinophil counts and disease incidence, but not between lymphocyte, monocyte, or basophil counts and disease incidence.

Prospective studies have shown that the inflammatory cell activity and leukocyte count may be useful prognostic indicators in patients with acute MI or UA. In a one-year

follow-up study of patients with UA (43), patients who had the highest leukocyte counts were roughly eight times more likely to have a major cardiovascular event than patients with the lowest counts and were five times more likely to have one than all other patients. In a large prospective study of patients who underwent coronary artery bypass grafting and were stratified by preoperative leukocyte count (44), total mortality was 2.6% (294 of 11,270); the adjusted mortality, however, was almost three times higher for patients with the highest versus lowest leukocyte quintiles ($\geq 12.0 \times 10^9/l$ vs. $< 6.0 \times 10^9/l$). Other evidence comes from the study by Erdogan et al. (45) in which the authors were able to find elevated levels of serum immunoglobulin E ($p = 0.002$), basophils ($p = 0.02$ and $p = 0.012$ for acute MI and UA patients, respectively) and eosinophils ($p = 0.005$) in the patients diagnosed with acute MI, UA pectoris, and stable angina pectoris, compared with healthy control subjects.

Several retrospective and case-control studies have examined the relationship between total and differential leukocyte counts and CAD. In a retrospective study of patients who underwent coronary angiography (46), both the total leukocyte count and band neutrophil count correlated significantly with coronary atherosclerosis, although the correlation with total leukocyte count lost significance after adjustment for other risk factors. One retrospective study of patients with CAD (47) showed that five-year survival was significantly better for patients who had a normal as compared with a low ($< 20.3\%/l$) relative lymphocyte count (92% vs. 83%). Several studies of patients with congestive heart failure have identified a low lymphocyte count or low percentage of lymphocyte as an adverse prognostic factor (see the subsequent text). In a small case-control study comparing patients with vasospastic angina pectoris, stable angina, and no angina (controls) (48), eosinophil counts were significantly higher in those who had severe vasospastic angina pectoris than in those who had mild vasospastic angina pectoris, stable angina, or no angina. After treatment with anti-anginal drugs, however, the eosinophil counts decreased to control levels. Maseri et al. (49) have reported that the number of neutrophils was much higher in the patients with acute MI than in those with UA or stable angina.

POSSIBLE MECHANISMS OF ACTION OF LEUKOCYTES IN CHD

The possible biologic mechanisms by which leukocytes might influence the development of CHD are protean (Table 4). They include biochemical, biomechanical, hematologic, and electrical mechanisms of action and are subsequently discussed in more detail.

Proteolytic and oxidative vascular damage. Leukocytes may influence the development of CHD through their ability to cause proteolytic and oxidative damage to coronary arteries. Stimulated neutrophils are known to: 1) secrete

Table 4. Possible Mechanisms of Action of Leukocytes in Coronary Heart Disease

Endothelial cell injury caused by proteolytic enzymes (50-54,56)
Vessel plugging (61)
Decreased perfusion (40)
Abnormal leukocyte aggregation (51,63)
Effects on blood flow (57,58)
Increased expression of monocyte tissue factors (80)
Activation of coagulation system (77,82)
Association with atherosclerotic risk factors (77,86-88)
Electrical instability (85)
Increased thrombus formation in CHD (81)
Involvement in hematologic stress syndrome (66)
Increased leukocyte adhesion in CAD (62)

CAD = coronary artery disease; CHD = coronary heart disease.

proteolytic neutral proteases that promote the detachment of endothelial cells from vessel walls and the adherence of platelets to subendothelial collagen and fibronectin (50); 2) release large amounts of the chemotactic agent leukotriene B₄ in patients with stable angina (51); 3) secrete large amounts of inflammatory mediators (52); and 4) release superoxide anions in hyperlipidemic patients (53). Some researchers have observed increased neutrophil aggregation and oxidase activity in the coronary sinuses of patients with angiographically documented CAD (54). Even observations from cancer research argue for this mechanism: the endothelial injury caused by leukostasis in patients with leukemia is apparently due to an overabundance of leukemic cells that activate adhesion molecules and to the migration of these cells into perivascular spaces (55). Oxygen-free radicals play an important role in the atherosclerotic process, but their destructive effects can be prevented, at least in theory, by antioxidant enzymes such as superoxide dismutase and catalase (56). Proteolytic enzymes are another likely source of arterial damage.

Vessel plugging. Leukocytes may influence the development of CHD through their ability to affect blood flow through the cardiac microvasculature. Because they are stiffer and larger than either red blood cells or platelets, leukocytes may obstruct small nutrient vessels (57,58). In patients with acute MI, the leukocytosis that follows necrotic injury usually (although not always [59]) renders leukocytes less deformable and less able to pass through the microvasculature, thus aggravating ischemia, extending the infarct area, and leading to further complications (60,61). Adhesion molecules on leukocytes become up- or down-regulated in atherosclerosis, hence increasing chemotaxis of monocytes beneath the endothelium in early stages of atherosclerosis (62).

Abnormal leukocyte aggregation. Abnormal leukocyte aggregation may play a role in CHD. Similar to platelets, granulocytes aggregate when stimulated and so may theoretically block microvessels (61,63). As shown in patients with peripheral vascular disease, increased platelet number and activity, neutrophil count, and acute-phase reactant protein levels correlate with increased plasma, serum, and blood viscosity (64), and mononuclear leukocytes become

less filterable during periods of ischemic pain (65). Abnormal leukocyte aggregation and high leukocyte counts may even be seen as a manifestation of hematologic stress syndrome (66), which some have argued represents a non-specific response to atherosclerosis (64).

Leukocytes and infarct expansion. Leukocytes may influence the development of CHD by causing infarct expansion (67,68). During reperfusion of ischemic myocardium, neutrophils and platelets can plug capillaries in the coronary microcirculation, resulting in the no-reflow phenomenon, ventricular arrhythmia, loss of coronary vascular reserve, infarct extension, and even organ dysfunction (69–71). The vascular obstruction may be partly due to the binding of neutrophils to ischemic endothelia via integrins such as CD18B and adhesion molecules such as intercellular adhesion molecule (ICAM)-1. Support for this idea comes from the observation that treatment with CD18 antibodies can reduce infarct size (72,73), as well as the observation that a deficiency in either CD18 or ICAM-1 can markedly reduce neutrophil accumulation and myocardial necrosis after MI-reperfusion injury (74).

The mechanism of action underlying neutrophil-induced infarct expansion remains unclear. Perhaps reperfusion further inflames areas that are already ischemic, resulting in the accumulation of neutrophils that then worsen the existing myocardial damage by unleashing more destructive oxygen-derived free radicals, proteases, and leukotrienes. This view is borne out by experimental studies showing the cardioprotective effects of neutrophil inhibition (75) and the interference of monoclonal antibodies in interactions between leukocytes and endothelium (76).

Leukocytes and hypercoagulability. Another way that leukocytes may influence the development of CHD is by inducing a state of hypercoagulability in response to acute MI and subsequent reperfusion. Indeed, the leukocyte count correlates positively with coagulation factors, including fibrinogen and factors VII and VIII (77). It is also known that the systemic inflammatory response that usually follows successful reperfusion involves the expression of cytokines (interleukin [IL]-1-beta, IL-8, and IL-6) and adhesion molecules (macrophage adhesion molecule [MAC]-1) on circulating monocytes, which in turn leads to increased monocyte procoagulant activity (78–81). Both IL-6 and -8 exert their procoagulant effects in a time- and dose-dependent fashion and at concentrations found in the peripheral blood of patients with acute MI (80). MAC-1—a beta₂ integrin that mediates the adhesion of leukocytes to vessel walls, catalyzes the conversion of factor X to factor Xa and binds fibrinogen (82)—may mediate the adherence of activated platelets to neutrophils and any thrombosis that may follow.

Leukocytes and reperfusion. The leukocyte count immediately after a coronary event may influence the chances of successful reperfusion. One study of patients undergoing thrombolytic therapy after an acute MI (83) found that those with higher non-neutrophil counts at presentation

Table 5. Studies Reporting a Significant Association Between Leukocyte Count and Smoking

Study (Ref.)	p Value
Zalokar et al. (15)	<0.01
Kannel et al. (18)	<0.01
Kostis et al. (31)	0.035
Freidman et al. (25)*	NA

*One-third of cases were due to risk factors independent of smoking. NA indicates that such data were not mentioned in the original report.

achieved successful reperfusion (i.e., TIMI flow grade 3) more often. However, this observation may be due to the fact that a longer time from symptom onset to treatment was associated with a higher neutrophil count and a lower non-neutrophil count, and this delay in treatment has affected the response to reperfusion. In general, an increased leukocyte count immediately after acute MI has been associated with poorer myocardial reperfusion, higher mortality, increased risk of new-onset CHF or shock, thromboresistance, and greater thrombus burden (40). The increased thromboresistance may be due to the enhanced generation of thrombin at sites of vascular injury by circulating monocytes and neutrophils that have been recruited to such sites (84). The enhanced thrombin production is mediated by interactions between P-selectin glycoprotein ligand-1 expressed by leukocytes and P-selectin expressed by activated platelets and by the monocytes themselves. These interactions make the membrane surface available for the assembly and function of coagulation complexes involved in tissue factor-initiated thrombin production.

Leukocytes and electrical instability. Leukocyte counts may affect the electrical stability of the heart. At least one study has shown that a high leukocyte count is a significant predictor of ventricular fibrillation in patients who have suffered an acute MI (85).

Leukocytes and other risk factors of CHD. The associations between leukocyte counts and other risk factors for CHD, especially smoking, have been evaluated in a number of studies. The leukocyte count has been shown to correlate positively with cigarette smoking, serum total cholesterol, serum triglycerides, clotting factors, hematocrit, fasting glucose levels, and diastolic blood pressure (77,86–88) and inversely with serum high-density lipoprotein cholesterol, forced expiratory volume, forced vital capacity, and height (77,86,87). The interdependence of leukocyte count and smoking remains unclear, as the WBC count is usually higher in smokers (Table 5). However, the WBC count has been shown to be an independent predictor of CHD in multivariate analyzes adjusting for other risk factors, including smoking in different population (Table 6).

Leukocytes and body mass index (BMI). There is increasing evidence suggesting a relationship between obesity and inflammation. A higher BMI is associated with higher serum C-reactive protein (CRP) concentrations in both children and adults (89,90). New findings suggest a state of low-grade systemic inflammation in overweight and obese

Table 6. Studies Reporting Leukocyte Count as an Independent Risk Factor

Study (Ref.)	p Value	95% CI
Grimm et al. (16)	0.09	
Phillips et al. (17)		
BRHS	<0.0001	
MRFIT I	0.0001	
MRFIT II	0.003	
Weijenberg et al. (23)		1.15-1.51
Lee et al. (21)		
Whites	0.001	
Blacks	0.009	
Brown et al. (22)		1.1-1.8
Manttari et al. (26)	0.03	
Lowe et al. (29)	<0.001	
Hajj-Ali et al. (32)	0.003	
Amaro et al. (30)	<0.0001	
Sweetnam et al. (42)	<0.0001	

BRHS = British Regional Heart Study; CI = confidence interval; MRFIT = Multiple Risk Factor Intervention Trial.

persons. Brown et al. (22) reported a higher BMI (26.2 vs. 25.3 kg/m²) in persons with a WBC count $>7.6 \times 10^9/l$, as compared with those with a WBC count $<6.1 \times 10^9/l$. Targher et al. (91) found a positive relationship between the WBC count and BMI. Nakanishi et al. (92) reported higher WBC counts in subjects with an increased BMI (6.64 vs. $6.52 \times 10^9/l$ for BMI <25 and ≥ 25 mg/kg², respectively; $p < 0.023$). Kullo et al. (93) showed that persons with a BMI ≥ 30 kg/m² had a higher WBC count than those with a BMI <25 kg/m² (WBC count = 5.86 vs. $5.26 \times 10^9/l$, respectively; $p < 0.05$). Huang et al. (94) reported a positive and significant relationship between the total WBC, neutrophil, lymphocyte, and monocyte counts with smoking and BMI ($p < 0.001$). Schwartz et al. (95), in National Health And Nutrition Examination Survey (NHANES) II, showed that BMI is a significant predictor of elevated WBC counts (beta = 0.53, $p < 0.001$). The Helsinki Heart Study (26) was an exception that did not show a significant relationship between WBC counts and BMI (beta = 0.06, $p = 0.2$).

Leukocytes and heart failure. The leukocyte count appears to be a predictor of heart failure. In the TIMI-10A and -10B thrombolysis trials (40), high leukocyte counts were significantly associated with the development of new CHF or shock, even after adjustment for potential confounding variables in a multivariate model. High leukocyte counts have also been associated with the development of heart failure and one-year mortality in patients with MI (33). However, in patients with chronic CHF, a low lymphocyte count or low percentage of lymphocytes predicts death. This belief is strengthened by the study by Omen et al. (47). They found that one- and four-year survival rates were 90% and 73% in patients with normal lymphocyte counts versus 78% and 34% in patients with lower lymphocyte counts. Acanfora et al. (96) evaluated the relationship between lymphocyte count and end points of total mortality and cardiovascular mortality. The three-year mortality rate

was found to be 64% in persons with a lymphocyte count $\leq 20\%$ versus 40% in those with a lymphocyte count of $\geq 20\%$ ($p = 0.0001$). The multivariate adjusted hazard ratio of mortality was found to be 1.73 (95% confidence interval [CI] 1.21 to 2.48, $p = 0.0026$).

Other blood cell types and CHD. Although our review so far has focused on the relationships between leukocyte count and CHD, other blood cell counts and measurements appear to correlate with risk as well. The erythrocyte count has been shown to correlate with carotid atherosclerosis (97), coronary artery stenosis (31), and disease severity (31). Hematocrit has been shown to correlate with carotid atherosclerosis (97), coronary insufficiency (98), CHD incidence (18), MI incidence (98), and CHD mortality (99,100). Blood viscosity has been shown to correlate with carotid atherosclerosis (97) and disease mortality (99,100). Although the platelet count does not appear to predict cardiovascular outcomes (20,101,102), a larger platelet size may correlate with CAD (101) and MI (103).

Systemic markers of inflammation and atherosclerosis. There are no data that inflammation, as evident from leukocytosis, is the cause or effect of the disease. Future studies are needed to answer this question. Emerging evidence suggests that serum markers of inflammation may be important predictors of CHD. The erythrocyte sedimentation rate has long been known to be an independent predictor of CHD (104). Indeed, baseline plasma levels of CRP strongly predict the risk of future cardiovascular events in apparently healthy subjects (105,106) and independently predict recurrent events and mortality in patients with acute coronary syndrome (107-109). In a long-term study in healthy men, baseline levels of IL-6 predicted the incidence of MI (110). The IL-18 serum levels predict death in cardiovascular patients (111). Serum amyloid A has been shown to be associated with a higher rate of recurrent coronary events in stable patients who have had an acute MI (107,112) and to predict a poor outcome in patients with UA (113). At the moment, one of the most attractive candidate markers of cardiovascular outcome is CRP (114). It appears to be a strong predictor of CHD and is now being used in clinical assessment of CHD risk (115). When combined with the widely available and inexpensive leukocyte count, CRP may yield additional information on the risk and prognosis for patients with UA or MI (37).

CLINICAL IMPLICATIONS

The leukocyte count has been consistently shown to be an independent risk factor and prognostic indicator of future cardiovascular outcomes, regardless of disease status. In patients with a history of CVD, it is an independent predictor of future events. Moreover, the leukocyte count is inexpensive, reliable, easy to interpret, and ordered routinely in inpatient and outpatient settings. However, when we surveyed 100 randomly selected studies (available on PubMed) of CHD risk assessment, diagnosis, or prognosis,

the leukocyte count as a risk factor was mentioned in only two studies. The relative lack of attention in research studies was understandably reflecting the interest of investigators in molecular mechanisms and the industry's interest in new assays.

IMPLICATIONS FOR THE STEM CELL THERAPIES

The increased risk of coronary thrombosis associated with leukocytosis, together with the association of UA with activation of circulating leukocytes, and the well-established risk of leukocyte plugging on reperfusion raise the question of the safety of stem cell therapies that rely on intracoronary infusion of leukocytes or systemic injections of granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor, which raise leukocyte counts. These approaches may increase the risk of thrombosis and may be more problematic than the small arrhythmogenic potential of subendocardial injections.

NEED FOR FURTHER TRIALS

Although the leukocyte count appears to be an independent predictor of cardiovascular events, some of its predictive ability can be explained by its interdependence with smoking. Therefore, further studies are needed to clarify just how prominent a role leukocytes play in the pathogenesis of CHD, as well as the clinical implications. Obviously, there is a need to determine the degree to which leukocyte count is independent of smoking and other risk factors. Existing databases may be useful in this regard.

The most important questions are: 1) Which leukocyte count cutoff points will be most useful for predicting CHD risk in clinical practice? 2) What is the relative predictive value of differential leukocyte counts? Which leukocyte subtype or subtypes (eosinophils, basophils, or neutrophils), if any, will be most useful for predicting risk? 3) Will combining serum markers of inflammation (such as CRP, lipoprotein phospholipase A₂, pregnancy-associated plasma protein-A, and myeloperoxidase) with the leukocyte count increase predictive ability (114,116,117)?

Other questions also warrant investigation. For example, can the leukocyte count be used as a surrogate outcome in studies of anti-inflammatory medications? Also, of great importance would be identification of patient populations that will benefit from the test and also appraisal of the available data to find the optimal timing for doing the test. Such studies should identify the best clinical approach to take once these patients are found to have a high leukocyte count. More aggressive anti-inflammatory and general risk factor modification treatment may be indicated in these patients.

FUTURE THERAPIES

If one or more types of leukocytes prove to have prognostic utility independent of new markers, it would be of interest

if studies of anti-inflammatory drugs for CHD (e.g., nitric oxide, statins, angiotensin-converting enzyme inhibitors, anti-tumor necrosis factor antibodies, sirolimus, peroxisome proliferators-activate receptors agonists, taxanes, and aspirin) include leukocyte counts at baseline and in response to therapies. It is also necessary to investigate if the agents that strictly inhibit inflammation (and specifically those that inhibit leukocyte function), without affecting platelet function or lipid levels, are able to reduce the risk of CHD.

CONCLUSIONS

A high leukocyte count is associated with increased CHD-related morbidity and mortality in various patient populations and clinical settings. It also appears to be an independent risk factor, regardless of atherosclerotic disease status. Thus, it may turn out to be a less expensive and more readily available risk marker than other currently available risk factors, as well as equally informative. Further studies are required, however, to determine the implications of using the leukocyte count to predict clinical risk and outcome.

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