



A BRIEF ORIGINAL CONTRIBUTION

Total and Differential Leukocyte Counts as Predictors of Ischemic Heart Disease: The Caerphilly and Speedwell Studies

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A number of studies have shown total leukocyte count to be a risk factor for ischemic heart disease, but there is little information on the role of the individual types of leukocyte, and the role of smoking is controversial. The Caerphilly and Speedwell studies recruited 4,860 men aged 45–63 years between 1979 and 1983 in South Wales and the West of England, respectively. At the 10-year follow-up, the total leukocyte count predicted ischemic heart disease events after adjusting for the classical risk factors, including smoking. Five-year follow-up results were available for differential white cell counts. The main contributor to the increase in total count in the men who developed disease was the neutrophil count. There was also a statistically significant increase in the eosinophil count. *Am J Epidemiol* 1997;145:416–21.

eosinophils; leukocyte count; myocardial ischemia; neutrophils; smoking

A number of prospective epidemiologic studies (1–6) have shown that the leukocyte count is a good predictor of ischemic heart disease. Smoking is a powerful determinant of leukocyte count (7) such that cigarette smokers have counts that average 30–40 percent higher than those for men who have never smoked (8). Most of these prospective studies concluded that part of the association between leukocyte count and ischemic heart disease remained after adjusting for smoking habit, suggesting that the leukocyte count was an independent risk factor for ischemic heart disease. This view was strengthened when three large prospective studies showed that the relation existed among both current and lifetime nonsmokers (9).

If leukocyte count is an independent risk factor for ischemic heart disease, then it is important to consider the role of the various cell types (6). Only two studies have done so. Prentice et al. (3) concluded that the neutrophil and eosinophil counts, and possibly the monocyte count, were associated with the disease. Olivares et al. (10) found only the monocyte count to be predictive.

We have previously reported (5) the relation between leukocyte count and the incidence of ischemic heart disease at the first follow-up of the 4,860 men of the Caerphilly (South Wales) and Speedwell (Bristol, England) studies. Those first follow-ups occurred after 5.1 years in Caerphilly and 3.2 years in Speedwell. We now report that relation after 10.0 years of follow-up in Caerphilly and 9.3 years in Speedwell. One of the advantages of this extended follow-up is that the large increase in the number of events permits us to compare the relation between leukocyte count and incident ischemic heart disease for groups with different smoking habits.

In addition, we also report 5-year incidence of ischemic heart disease in relation to differential counts from the Caerphilly cohort.

MATERIALS AND METHODS

Study population and survey methods

These have been described in detail elsewhere (5). Briefly, in both areas men were recruited between 1979 and 1983, from geographically defined populations. The Caerphilly men were aged 45–59 years, and the Speedwell men were 45–63 years old. A total of 4,860 men were recruited, 91 percent of those eligible.

The two studies had a common core protocol. At an afternoon clinic, a standard medical and smoking history was obtained, the London School of Hygiene and Tropical Medicine chest pain questionnaire (11) was administered, and a 12-lead electrocardiogram was

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Abbreviation: ICD-9, *International Classification of Diseases, Ninth Revision*

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recorded. The men returned, after an overnight fast, to an early morning clinic where a blood sample was taken with minimal venous stasis. The leukocyte count was made by an automated cell counter (Coulter model S-plus; Coulter Electronics, Inc., Hialeah, Florida) and was available for 4,615 men. Differential counts were made only at the first reexamination of the Caerphilly cohort, 5 years after recruitment. They were made by a different laboratory, again using an automated counter (Technicon model H6000; Technicon Instruments Corporation, Tarrytown, New York), and are available for 2,163 men.

Follow-up procedure and definition of incident ischemic heart disease

The results refer to the second follow-up in Caerphilly and to the third in Speedwell. These were at nearly constant intervals of 120 months in Caerphilly and 112 months in Speedwell. At each follow-up, the London School of Hygiene and Tropical Medicine chest pain questionnaire was readministered, and another electrocardiogram was recorded. These, together with notifications from all local hospitals, were used as the basis for a search of hospital notes for events that satisfied World Health Organization (12) criteria for definite acute myocardial infarction. Copies of death certificates were received from the National Health Service Central Registry and coded by one of us (J. W. G. Y.) to the *International Classification of Diseases*, Ninth Revision (ICD-9). From this information, incident ischemic heart disease was defined as death from ischemic heart disease (ICD-9 codes 410–414) or clinical nonfatal myocardial infarction or electrocardiographic myocardial infarction (the appearance of major or moderate Q waves, Minnesota codes 1-1-1 to 1-2-5 or 1-2-7 on any follow-up electrocardiogram when there were no Q waves, 1-1-any, 1-2-any, or 1-3-any on the recruitment electrocardiogram).

Statistical methods

Age-adjusted mean differences between men who developed incident ischemic heart disease and those who did not were obtained by analysis of covariance. The remainder of the analysis used multiple logistic regression with the occurrence, or not, of any of the three categories of incident ischemic heart disease as the dependent variable. In the logistic regressions, leukocyte counts were either continuous variables or divided into five equally sized groups using each area's own quintiles. For the former, results are presented as either predicted probabilities of incident ischemic heart disease or standardized relative odds, defined as the odds associated with a 1 standard deviation increase in leukocyte count. For the latter, the results are presented as relative odds.

Evidence of ischemic heart disease at recruitment was assessed from the London School of Hygiene and Tropical Medicine chest pain questionnaire and the electrocardiogram. Three categories of preexistent disease, namely, angina, history of prolonged severe chest pain, and electrocardiographic ischemia, were defined (13). Among the 4,860 men, 1,122 (23 percent) had some evidence of ischemic heart disease at recruitment, a prevalence similar to that reported by the British Regional Heart Study (14). For reasons given in detail elsewhere (5), these men were not excluded from the analysis. Instead, like the Regional Heart Study (15), we adjust for preexistent disease status by including three covariates in the logistic regressions.

RESULTS

Among the 4,615 men with a fasting leukocyte count, there were 565 (12.2 percent) incident ischemic heart disease events. Table 1 shows the mean leukocyte count according to whether the man developed ischemic heart disease over the 10-year period and

TABLE 1. Mean leukocyte count at recruitment between 1979 and 1983 and 10-year incidence of ischemic heart disease (IHD) in the Caerphilly (South Wales) and Speedwell (West of England) studies

| | No of men | Leukocyte count ($\times 10^9/\text{liter}$) | | Age-adjusted difference from men with no incident IHD | | p value |
|------------------------|-----------|--|------|---|-----------|---------|
| | | Mean | SD* | Mean | 95% CI* | |
| No incident IHD | 4,050 | 6.97 | 1.98 | | | |
| All incident IHD | 565 | 7.72 | 2.26 | 0.75 | 0.57–0.92 | <0.0001 |
| Interval to first IHD† | | | | | | |
| 0–39 months | 164 | 7.84 | 2.30 | 0.87 | 0.55–1.18 | <0.0001 |
| 40–79 months | 147 | 7.83 | 2.07 | 0.86 | 0.52–1.19 | <0.0001 |
| ≥80 months | 161 | 7.65 | 2.53 | 0.68 | 0.36–1.00 | <0.0001 |

* SD, standard deviation; CI, confidence interval.

† No interval to first IHD is available for those events defined purely from sequential electrocardiograms.

according to the interval between recruitment and first incident ischemic heart disease. Overall, the age-adjusted mean leukocyte count was higher by $0.75 \times 10^9/\text{liter}$ ($p < 0.0001$) among the men who developed ischemic heart disease. There was a slight decrease in the magnitude of the difference with increasing interval to first ischemic heart disease, but the mean difference was still $0.68 \times 10^9/\text{liter}$ ($p < 0.0001$) for events occurring more than 80 months after recruitment.

Figure 1 shows the relative odds of incident ischemic heart disease by "fifths" of leukocyte count. Age-adjusted relative odds increase steadily to 2.84 in the 20 percent of men with the highest count. Adjusting additionally for smoking, preexistent ischemic heart disease, diastolic blood pressure, body mass index, and cholesterol reduces the relative odds, but they still increase steadily to 2.10 (95 percent confidence interval 1.51–2.92). The corresponding standardized relative odds adjusted only for age were 1.38, decreasing to 1.29 ($p < 0.0001$) on adjustment for the same additional factors. There was no evidence of any interaction between leukocyte count and smoking habit (chi-square with 2 df = 0.73, $p > 0.6$). Separate regression lines were fitted for current smokers, ex-smokers, and men who had never smoked. Figure 2 shows for each smoking category how the probability of ischemic heart disease increases with leukocyte count. The slope of the increase is similar for all three groups.

Differential counts were made only at the first re-examination of the Caerphilly cohort, 5 years after

recruitment. Table 2 shows mean leukocyte and differential counts by 5-year incidence of ischemic heart disease for the 2,163 men who were reexamined then. These leukocyte counts, from a different laboratory, are higher than those reported in table 1. However, the age-adjusted mean difference in total count between men who developed ischemic heart disease and those who did not was, at $0.69 \times 10^9/\text{liter}$, similar to that reported in table 1. This difference in total count is explained mainly by an increase of $0.52 \times 10^9/\text{liter}$ ($p < 0.0001$) in the neutrophil count. The increase of $0.025 \times 10^9/\text{liter}$ in the eosinophil count was also statistically significant ($p = 0.035$). The increases in the monocyte and basophil counts both approached the conventional 5 percent level of statistical significance.

Table 3 shows the results of fitting five separate multiple logistic regression models, one for each of the different cell types. The relative odds of incident ischemic heart disease by "fifths" of the level of each cell type are given together with the standardized relative odds and a test for trend. The relative odds are adjusted for age, smoking habit, preexistent disease status, total cholesterol, diastolic blood pressure, and body mass index. There is a highly significant ($p = 0.003$) trend for the incidence of ischemic heart disease to increase with increasing neutrophil count. The fully adjusted, standardized relative odds of 1.30 are similar to the odds for the total leukocyte count. The relative odds of ischemic heart disease increase fairly steadily to 3.54 in the 20 percent of men with the highest neutrophil counts. There is also a significant trend ($p = 0.05$) with eosinophil count, the relative

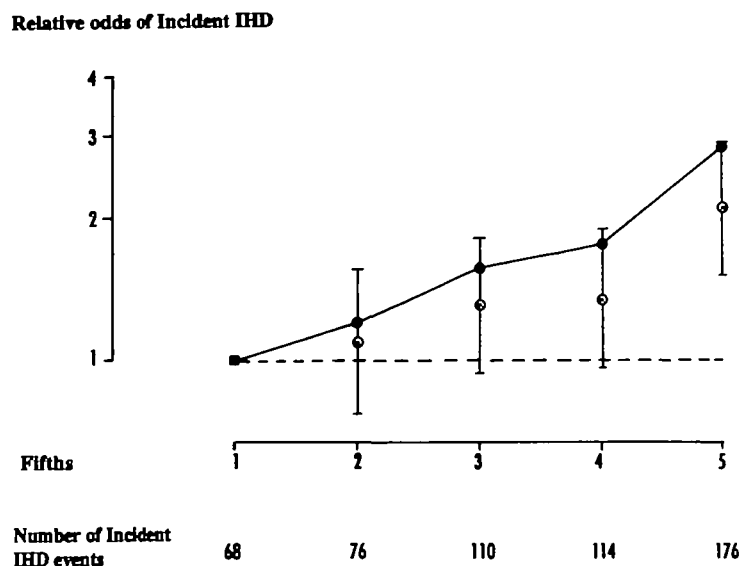


FIGURE 1. Relative odds of major incident ischemic heart disease (IHD) over 10 years (from 1979–1983 to 1989–1993) by "fifths" of total leukocyte count, Caerphilly (South Wales) and Speedwell (West of England) studies. ■, base group for calculation of relative odds; ●, relative odds adjusted for age, ○, relative odds adjusted for age, smoking habit (never, ex-, current), preexistent disease status, total cholesterol, diastolic blood pressure, and body mass index. Bars, 95% confidence interval.

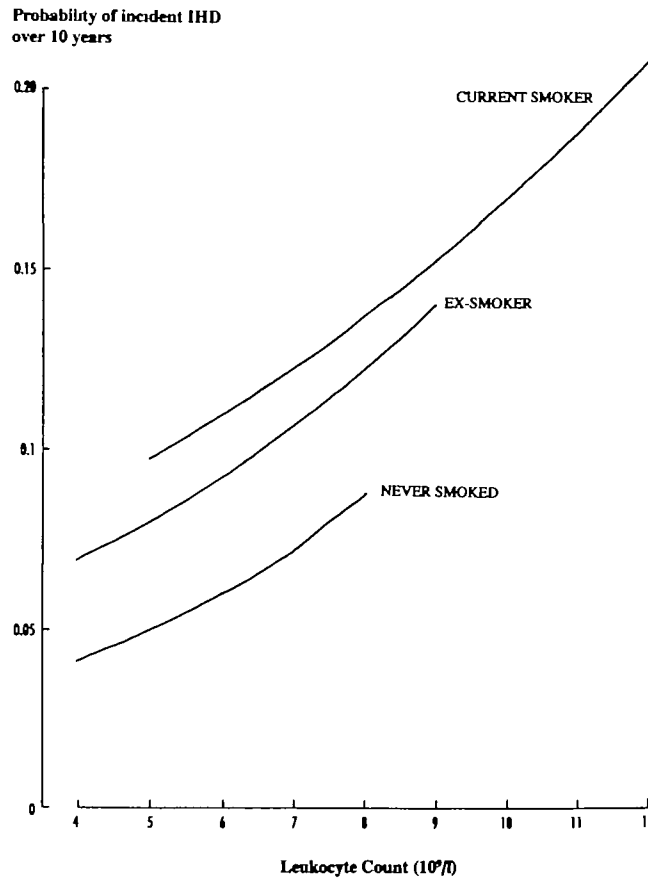


FIGURE 2. Predicted probability, for a 52-year-old man, of major incident ischemic heart disease (IHD) over 10 years (from 1979–1983 to 1989–1993) according to his smoking habit, Caerphilly (South Wales) and Speedwell (West of England) studies. Predicted probabilities obtained from separate logistic regression models for each smoking category, the models including just age and total leukocyte count.

TABLE 2. Total and differential leukocyte counts at first reexamination between 1984 and 1988 and 5-year incidence of ischemic heart disease (IHD) in the Caerphilly (South Wales) study

| | Incident IHD | | Age-adjusted absolute differences | | p value |
|------------------|-------------------|------------------|-----------------------------------|-----------------|---------|
| | No (n = 2,020) | Yes (n = 143) | Mean | 95% CI* | |
| Total leukocytes | 7.48† (2.12)‡ | 8.18 (2.10) | 0.69 | 0.33 to 1.05 | <0.0001 |
| Neutrophils | 4.20 (1.44) | 4.72 (1.47) | 0.516 | 0.273 to 0.759 | <0.0001 |
| Lymphocytes | 2.49 (1.02) | 2.59 (0.88) | 0.108 | -0.064 to 0.280 | 0.22 |
| Monocytes | 0.44 (0.15) | 0.47 (0.13) | 0.025 | -0.002 to 0.052 | 0.064 |
| Eosinophils | 0.23 (0.13) | 0.25 (0.15) | 0.025 | 0.002 to 0.047 | 0.035 |
| Basophils | 0.06 (0.03) | 0.07 (0.03) | 0.005 | -0.001 to 0.011 | 0.084 |

* CI, confidence interval.

† Mean values, as 10^9 cells/liter.

‡ Numbers in parentheses, standard deviation.

odds increasing, less steadily, to 2.15 in the 20 percent of men with the highest levels.

DISCUSSION

We have shown that the leukocyte count is a strong, long-term, predictor of ischemic heart disease. The

standardized relative odds, the odds associated with a 1 standard deviation increase in count, were 1.38 on adjustment for age alone. They decreased to 1.29 (95 percent confidence interval 1.17–1.41) on further adjusting for the main coronary risk factors. This size of effect is similar to that reported from Framingham for

TABLE 3. Five-year incidence of ischemic heart disease (IHD) by "fifth" of the distribution of each leukocyte cell type in the Caerphilly (South Wales) study

| "Fifth" of distribution of cell type* | Relative odds of major incident IHD | | | | | Standardized relative odds† | Test for trend | |
|---------------------------------------|-------------------------------------|------|------|------|------|-----------------------------|----------------|-------|
| | 1 | 2 | 3 | 4 | 5 | | t | p |
| Neutrophils | 1.0 | 2.11 | 1.88 | 2.90 | 3.54 | 1.30 | 3.03 | 0.003 |
| Lymphocytes | 1.0 | 0.63 | 0.83 | 0.86 | 1.08 | 1.11 | 1.26 | 0.21 |
| Monocytes | 1.0 | 0.86 | 1.14 | 1.42 | 1.34 | 1.12 | 1.24 | 0.22 |
| Eosinophils | 1.0 | 1.85 | 1.72 | 1.26 | 2.15 | 1.17 | 1.96 | 0.05 |
| Basophils | 1.0 | 0.81 | 1.54 | 1.50 | 1.31 | 1.08 | 0.91 | 0.36 |

* Five separate multiple logistic models were fitted, one for each cell type. In each model, the relative odds and the standardized relative odds are adjusted for age, smoking habit, preexistent disease status, total cholesterol, diastolic blood pressure, and body mass index.

† The standardized relative odds are the odds associated with a 1 standard deviation increase in the leukocyte count.

fibrinogen (16) and for leukocyte count (6) among nonsmokers.

Most of the reduction in the standardized relative odds on adjustment for the main cardiovascular risk factors was due to the adjustment for smoking habit. However, the adjusted standardized relative odds remained highly statistically significant ($p < 0.0001$), suggesting strongly that the association between leukocytes and ischemic heart disease doesn't simply arise because both are associated with smoking habit. Zolotar et al. (2) found the association between leukocyte count and ischemic heart disease only among smokers who inhale, while Kannel et al. (6) found the association confined to nonsmokers among men. We find no evidence that the association differs within smoking habit. A test for an interaction didn't approach statistical significance, and the standardized relative odds adjusted for age were 1.29 ($p = 0.09$), 1.30 ($p = 0.0002$), and 1.31 ($p < 0.0001$) among never, ex-, and current smokers, respectively.

The association between leukocyte count and ischemic heart disease is almost entirely independent of the other main cardiovascular risk factors and of acute phase reactants, such as fibrinogen (5). In particular, as Phillips et al. (9) found, it is independent of preexistent ischemic heart disease. In the current study, when we excluded the 1,122 men who had any evidence of preexisting disease, the age-adjusted mean difference in leukocyte count between men who developed ischemic heart disease and those who did not increased to 0.88×10^9 /liter.

Mechanisms by which leukocytes may promote atherosclerosis and thrombosis include pressure-dependent plugging of microvessels and rheologic properties, such as reduced deformability (17). Recent work (18) has suggested a role for chronic infection (with associated leukocytosis) with agents such as *Chlamydia* or *Helicobacter*. This hypothesis is cur-

rently being investigated in a number of prospective studies, including the Caerphilly Study. Neutrophils particularly possess the ability to aggregate and, when activated, release potentially damaging substances, such as free radicals and proteolytic enzymes (17, 19). Studies of postinfarction patients also suggest that neutrophils are involved in the genesis and propagation of myocardial ischemia (19). Differential counts were introduced at the first reexamination of the Caerphilly cohort, and we have only 5 years of follow-up. The age-adjusted mean difference in the total leukocyte count between the men who developed ischemic heart disease and those who did not was 0.69×10^9 /liter, very similar to that for the 10-year follow-up of the total cohort. The major contributor to that difference was a highly significant ($p < 0.0001$) increase of 0.52×10^9 /liter of neutrophils. The only other cell type that showed a statistically significant increase in count was the eosinophils ($p = 0.035$). There were significant trends for the incidence of ischemic heart disease to increase with increasing levels of neutrophils ($p = 0.003$) and eosinophils ($p = 0.05$) after adjustment for the main cardiovascular risk factors. Like neutrophils, eosinophils appear to be capable of phagocytosis, but the overall effect of their activity is to dampen down the inflammatory response and reduce granulocyte migration (20). Prentice et al. (3) also found significant relations to coronary heart disease incidence only for neutrophil and eosinophil counts. The other study (10) to report the incidence of ischemic heart disease in relation to differential counts found that only the monocyte count predicted the premature occurrence of coronary events. However, this study was based on a young working population aged 30–50 years, and the total number of incident ischemic heart disease events was 50, of which 26 were angina pectoris. Direct comparison with the current study is restricted therefore.

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