Prediction of calculated future cardiovascular disease by monocyte count in an asymptomatic population

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Introduction: Although atherogenesis is clearly entwined with systemic inflammation, the risk-predictive relationship between preclinical and overt cardiovascular disease (CVD) and systemic white blood cell (WBC) subtypes remains unclear. Implication of an association would greatly facilitate cardiovascular risk prediction, assessment and monitoring.

Methods: 1383 asymptomatic individuals (795 men, 588 women) attending for executive health screening were examined clinically as well as with phlebotomy and exercise stress testing to determine their ten-year risk of developing overt cardiovascular disease (as estimated by both Framingham and SCORE calculations). The significance of their association with overall WBC and subtypes were determined using both univariate and multiple regression modeling.

Results: Of all WBC subtypes, monocyte count was found to have the strongest, independent relationship with overall CVD risk by backwards linear regression modeling (Framingham: \( \beta = 0.057; p = 0.03 \); SCORE: \( \beta = 0.128; p = <0.0005 \)). Independent associations with BMI (\( \beta = 5.214; p = <0.0005 \)), waist circumference (\( \beta = 21.866; p = <0.0005 \)), systolic blood pressure (\( \beta = 10.738; p = 0.003 \)), HDL cholesterol (\( \beta = 0.639; p = <0.0005 \)) and triglyceride concentrations (\( \beta = 0.787; p = <0.0005 \)) were also evident. Overall WBC along with neutrophils, lymphocytes and basophil subfractions were variably (but less strongly) associated with such dependents and outcome measures.

Conclusions: In conclusion, monocyte count, a simple inexpensive test, may provide useful predictive cardiovascular risk information in asymptomatic individuals to inform and guide attempts at interrupting CVD development at a preclinical stage.

Keywords: leukocyte, white cell count, monocyte, cardiovascular risk, asymptomatic population

Introduction

Inflammation plays a key role in the pathogenesis of atherosclerosis and in the development of clinically apparent cardiovascular disease (CVD) (Yarnell et al 1991; Ross 1993, 1999; Libby 1995; Falk et al 1995; Tracey 1998; Ridker 1998). Elevated levels of systemic inflammatory markers have been shown to be associated with an increased risk of symptomatic coronary heart disease (CHD) (Folsom et al 1995, 1997; Danesh et al 2000), while total white blood cell (WBC) count has itself been shown to be an independent risk factor for both CHD development (Danesh et al 1998) and CVD-related morbidity and mortality (Kannel et al 1992; Horne et al 2005). Although a role as a biomarker of cardiovascular risk has been suggested for total WBC (Grimm et al 1985; Kuller et al 1996; Pepys and Berger 2001; Pearson et al 2003), the relative ability of specific WBC subtypes to predict cardiovascular risk in asymptomatic individuals remains largely unexamined. Such an analysis may provide greater insight into the natural history of actual cardiovascular risk. While neutrophil levels have
proven useful in reflecting overt ischemic events post-hoc, a preclinical indicator of impending atherosclerotic crisis could provide greater therapeutic opportunity.

Thus, in this prospective observational study, the predictive ability of total and specific WBC subtypes on predicted cardiovascular risk in a cohort of patients without preexisting symptomatic CVD was evaluated both alone and in comparison to conventional risk factors.

**Methods**

**Study sample**
The study population consisted of consecutive asymptomatic males and females without prior history of clinically apparent cardiovascular disease and aged between 33 and 75 years who attended an executive cardiovascular health examination in the Department of Preventative Medicine, Blackrock Clinic, Dublin, Ireland, between December 2003 and March 2005. All enrolled participants were self-referred and underwent their evaluation by a physician in an outpatient setting. The study was approved by the Ethics Committee of Blackrock Clinic.

Initially, all participants completed a detailed health questionnaire to confirm the presence or absence of symptoms of heart disease (chest pain, dyspnoea, palpitations at rest or with exercise) as well as to detail known risk factors for CVD (hypertension, hypercholesterolemia, diabetes mellitus, cigarette smoking, family history of CVD) and medication usage (aspirin, statins, antihypertensives, diabetic medication). Additionally, individuals were questioned regarding symptoms and signs suggestive of acute infection (fevers, cough, sputum production, etc).

Exclusion criteria included the presence of known heart disease (including previous myocardial infarction [MI]) or symptoms suggestive of cardiac disease, peripheral vascular disease, history of stroke or symptomatic cerebral ischemia as well as the presence of symptoms consistent with current infection. Furthermore, all those taking aspirin, statin and/or anti-hypertensive medications were excluded.

**Cardiovascular risk assessment**
Cardiovascular assessment was performed on all included study subjects and comprised complete physical examination and fasting phlebotomy. Cardiovascular risk was determined by use of both Framingham and SCORE calculation on all individuals.

**Clinical examination**
Physical examination was performed on all subjects and included sphygmomanometry (mmHg) along with measurement of waist circumference and body mass index (BMI) calculation (calculated as weight in kilograms divided by the square of height in meters; kg/m²).

**Hematological and biochemical assessment**
Early morning, blood samples for serum measurement of WBC with differential as well as glucose, total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein (HDL) and triglyceride levels were drawn from an antecubital vein of participants resting in a supine position after an overnight fasting period of a minimum of ten hours. Once drawn, all samples were put on ice and were processed within 30 minutes. Total and peripheral differential WBC counts (neutrophils, lymphocytes, monocytes and basophils) were performed using a Sysmex NE-8000 hematology analyzer (TOA Medical Electronics, Kobe, Japan). Intra-assay and inter-assay coefficients of variation were <10%. Enzymatic colorimetric methods using a Cobras Integra 800 analyser (Roche, Basle, Switzerland) were used to determine the concentrations of fasting cholesterol, triglycerides and glucose (sensitivity <0.003 mmol/l, 0.4 mmol/l, 0.03 mmol/l, respectively). Appropriate standardization of the assays was performed at time intervals throughout the study period in compliance with quality-control measures.

**Framingham heart risk score and SCORE estimation**
10-year risk of CHD (whether fatal or nonfatal) and fatal CVD (including both coronary and cerebrovascular deaths) was calculated for all patients on the basis of the Framingham Heart Risk Score (Wilson et al 1998) and SCORE project (Conroy et al 2003) formulae respectively. Both these estimations are widely used and well validated means of ascribing gender-specific cardiovascular risk. The Framingham Risk Score, derived from the Framingham Heart Study Cohort, predicts gender-specific 10-year risk of CHD development by assigning a weighting to each individual’s age, sex, smoking status, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, and the presence of diabetes. The European SCORE is a risk stratification model developed by the European Society of Cardiology that allows direct estimation of 10-year risk of fatal CVD at any site for use in primary prevention programs. This means of risk stratification again assigns numerical weighting to each individual’s age, sex, total cholesterol, systolic blood pressure, and smoking status.

**Statistical analysis**
Statistical analysis was performed using the SPSS Version 11 (SPSS Inc 1989–2001, Chicago, Illinois, USA). Univariate regression models were fit for each cardiovascular risk
parameter to examine the independent relationships between differential WBC and cardiovascular risk parameters. Log transformation of variables was performed where appropriate to correct for skew (glucose, total cholesterol, HDL, LDL, and triglycerides). Subsequently, backward multiple linear regression models were fitted in order to determine whether independent associations exist between differential WBC and cardiovascular risk, as quantified by the Framingham and SCORE risk stratification models. A relationship was considered statistically significant if \( p < 0.05 \).

**Results**

Of 1716 subjects (1026 men, 690 women) attending for screening over the 15-month study period, 333 were excluded by the criteria detailed above. Fifty one had either known heart disease or a history of cerebrovascular accident or had symptoms suggestive of cardiac disease, peripheral vascular disease, symptomatic cerebral ischemia or current infection and were thus excluded from further evaluation. A further 282 were taking aspirin and/or a statin and/or anti-hypertensive medication on attendance. Therefore, the study group comprised 1383 participants (795 men, 588 women) who met the inclusion criteria. The baseline characteristics for our study population and the distribution of potential risk factors for CVD are displayed in Table 1.

Univariate regression models were fitted in order to quantify the association between total and differential WBC counts and standard risk parameters for the development of CVD (see Tables 2a and 2b). Significant relationships between total WBC count and risk parameters for cardiovascular disease development were demonstrated with WBC found to be significantly related to BMI, waist circumference, systolic blood pressure, HDL cholesterol, and triglyceride levels. Additionally, total WBC count was associated with SCORE but not to Framingham risk estimation. Of the leukocyte subtypes, monocyte count showed the most compelling evidence of a significant relationship with cardiovascular risk and risk factors. Significant relationships were demonstrated between monocyte count and BMI, waist circumference, systolic blood pressure, HDL cholesterol, and triglyceride concentrations. Furthermore, significant relationships between monocyte count and risk of CVD development using both Framingham and SCORE cardiovascular risk prediction models were evident. Univariate regression models also showed neutrophil count to be significantly associated with BMI, HDL cholesterol, and triglyceride measurements. Neutrophil count was, in addition, significantly associated with overall risk of having a fatal cardiovascular event in the next ten years, as estimated by the SCORE cardiovascular risk prediction model. Eosinophil count demonstrated significant associations with BMI, waist circumference, HDL,
Table 2 Statistical analysis using univariate regression models to examine the relationship between total and differential WBC counts and the following cardiovascular risk parameters (a) Framingham and SCORE risk estimates, BMI, waist circumference, and systolic BP; (b) Total cholesterol, LDL, HDL, and triglycerides

(a) Leukocyte count Framingham SCORE BMI (kg/m²) Waist circumference (cm) Systolic BP (mmHg)

| Leukocyte count | β     | SE    | p    | β     | SE    | p    | β     | SE    | p    | β     | SE    | p    |
|-----------------|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|------|
| Total WBC count | 0.054 | 0.042 | 0.196 | 0.129 | 0.052 | 0.013 | 0.365 | 0.064 | <0.0005 | 1.518 | 0.283 | <0.0005 | 0.570 | 0.291 | 0.05 |
| Monocytes      | 1.091 | 0.522 | 0.037 | 3.095 | 0.633 | <0.0005 | 5.214 | 0.785 | <0.0005 | 21.866 | 2.596 | <0.0005 | 10.738 | 3.576 | 0.003 |
| Neutrophils    | 0.085 | 0.054 | 0.116 | 0.175 | 0.066 | 0.008 | 0.33  | 0.83  | <0.0005 | -0.016 | 0.38  | 0.681  | 0.590 | 0.373 | 0.114 |
| Lymphocytes    | 0.015 | 0.125 | 0.904 | -0.052 | 0.153 | 0.730 | 0.908 | 0.19  | <0.0005 | 2.559 | 0.636 | <0.0005 | 1.603 | 0.857 | 0.062 |
| Eosinophils    | 0.104 | 0.548 | 0.849 | 1.883 | 0.668 | 0.005 | 2.461 | 0.834 | 0.003 | 0.1372 | 0.137 | 0.645 | 1.734 | 3.762 | 0.645 |
| Basophils      | 1.987 | 1.859 | 0.285 | 7.582 | 2.262 | 0.001 | 10.552 | 2.82  | <0.0005 | 38.142 | 9.417 | <0.0005 | 27.363 | 12.736 | 0.032 |

(b) Leukocyte count Total cholesterol (mg/dl) HDL (mmol/dl) LDL (mmol/dl) Triglycerides (mg/dl)

| Leukocyte count | Total cholesterol (mg/dl) | HDL (mmol/dl) | LDL (mmol/dl) | Triglycerides (mg/dl) |
|-----------------|---------------------------|---------------|---------------|----------------------|
| β    | SE   | p    | β    | SE   | p    | β    | SE   | p    |
|-------|------|------|-------|------|------|-------|------|------|
| Total WBC count | 0.009 | 0.016 | 0.568 | -0.048 | 0.008 | <0.0005 | 0.016 | 0.015 | 0.289 | 0.091 | 0.017 | <0.0005 |
| Monocytes | -0.208 | 0.203 | 0.168 | -0.639 | 0.094 | <0.0005 | -0.110 | 0.188 | 0.559 | 0.787 | 0.205 | <0.0005 |
| Neutrophils | -0.005 | 0.021 | 0.788 | -0.049 | 0.01 | <0.0005 | 0.009 | 0.02 | 0.645 | 0.091 | 0.021 | <0.0005 |
| Lymphocytes | 0.141 | 0.048 | 0.004 | -0.082 | 0.023 | <0.0005 | 0.091 | 0.045 | 0.042 | 0.222 | 0.049 | <0.0005 |
| Eosinophils | -0.005 | 0.213 | 0.785 | -0.251 | 0.100 | 0.005 | -0.028 | 0.198 | 0.884 | 0.430 | 0.216 | 0.046 |
| Basophils | 0.434 | 0.722 | 0.547 | -0.898 | 0.340 | 0.008 | 0.437 | 0.670 | 0.514 | 2.449 | 0.730 | 0.001 |

Note: *Significant relationship ie, p < 0.05.
Abbreviations: BMI, body mass index; BP, blood pressure; β, regression coefficient; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SE, standard error of the mean; WBC, white blood cell.
Table 3: Statistical analysis using multivariable linear regression modeling to determine whether independent associations exist between (a) differential white cell count and Framingham Risk and (b) differential white cell count and SCORE. Variables entered into the stepwise regression model comprise total white cell count, neutrophils, lymphocytes, basophils, monocytes, and eosinophils.

| WBC subtype | β   | Standard error | p value |
|-------------|-----|----------------|---------|
| Monocytes   | 0.057 | 2.12           | 0.03    |

(b)

| WBC subtype | β   | Standard error | p value |
|-------------|-----|----------------|---------|
| Monocytes   | 0.128 | 4.436          | <0.0005 |
| Lymphocytes | −0.082 | −2.807         | 0.005   |
| Basophils   | 0.067 | 2.366          | 0.02    |

Abbreviations: β, regression coefficient; Standard Error, standard error of regression coefficient; WBC, white blood cells.

choler, and triglyceride concentrations as well as with 10 year risk of having a fatal cardiovascular event, as described by SCORE. Basophil count was significantly associated with BMI, waist circumference, systolic blood pressure, HDL cholesterol, and triglyceride concentrations as well as with SCORE-estimated risk. Lymphocyte count demonstrated significant associations with BMI, waist circumference, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride concentrations. However, despite the significant statistical significance of the relationship between lymphocyte count and risk parameters for CVD development, the association did not translate into associated increased 10 year risk of either CVD development or risk of having a fatal cardiovascular event.

On backward stepwise linear regression model analysis, monocyte count was the only WBC component found to be independently associated with both Framingham and SCORE cardiovascular risk stratification models (see Table 3a). While lymphocyte and basophil counts were also shown by this means to be independently associated with SCORE (see Table 3b), the relationship is not as compelling. Therefore, having demonstrated monocyte count to be an independent predictor of future cardiovascular risk, plots showing median monocyte count versus individual components of the FRS and SCORE were constructed (Figures 1–5). Furthermore, Figure 6 demonstrates the monocyte level increment with increasing calculated Framingham and SCORE coronary risk categories. The beta coefficient already calculated by the multivariate analyses indicated that an increment of monocyte count by 0.057 and 0.128 was associated with a 1% increase in Framingham and SCORE risk estimation, respectively.

Finally, the predictive ability of monocyte count was compared to that of BMI and waist circumference for the strength of an association with both Framingham and SCORE predictive risk of cardiovascular events (see Table 4). Additional parameters such as LDL, smoking status, age, and systolic blood pressure were not examined in this fashion as they themselves are components of both scores. Thus it can be appreciated that, while monocyte count is a predictor of future cardiovascular events, its predictive ability is exceeded by both BMI and waist circumference.

Discussion

WBC count provides a widely available and rapidly performed means of assessing systemic inflammatory status that is both well-standardized and inexpensive (Hoffman et al. 2004). However despite prior studies showing WBC count to be an independent risk factor and prognostic indicator of future cardiovascular outcome that may hold true regardless of actual current clinical CVD status (Prentice et al. 1982a; Zalokar 1983; Kannel et al. 1992; Weiss et al. 1995; Danesh et al. 1998; James et al. 1999; Lee et al. 2001; Barron et al. 2001; Haim et al. 2004; Gillum et al. 2005; Margolis et al. 2005; Roy et al. 2006), the exact clinical relevance of the association remains to be fully elucidated (Madjid et al. 2004). Of WBC sub-fractions, neutrophil counts have, to date, shown the strongest association with CVD incidence and mortality (Prentice et al. 1982b; Zalokar 1983; Olives et al. 1993; Kirtane et al. 2004; Wheeler et al. 2004; Margolis et al. 2005; Gillum et al. 2005) although the majority of studies have concentrated on either patients with acute symptoms or on those at highest risk of overt CVD. Recent findings that neutrophil/lymphocyte ratio provides the greatest risk prediction in patients undergoing angiographic studies for suspected CHD symptoms seem to confirm that this subtype (like C-reactive protein [CRP]) may represent either an adaptive or maladaptive response of reparative intent towards atherosclerotic plaque rupture and clinical significant ischemia (Horne et al. 2005; Dragu et al. 2006). Little information however is available concerning the independent predictive ability of specific WBC subtypes to predict CVD risk, independent of standard risk.
factors, in individuals without overt symptoms of cardiac disease.

This prospective cohort study in an asymptomatic population demonstrates that, of all WBC subtypes, the most significant relationship with established risk parameters for CVD development exists for monocyte count and that, furthermore, this subfraction alone was independently associated with both Framingham and SCORE risk estimation. Monocyte-macrophages are central mediators in the pathogenesis of atherosclerosis in both the coronary and peripheral arterial circulations (Libby 2002; Hansson 2005). Circulating monocytes become recruited to atherogenic foci where, through

Figure 1 Composite figure showing relationship of median monocyte count to patient variables of (a) Age; (b) Systolic blood pressure; (c) Total cholesterol; (d) HDL cholesterol; and (e) LDL cholesterol as well as with (f) Framingham and (g) SCORE risk estimates.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Figure 2 Comparison of median monocyte count and total cholesterol.

Figure 3 Comparison of median monocyte count and LDL cholesterol. 
Abbreviation: LDL, low-density lipoprotein.
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Figure 4 Comparison of median monocyte count and HDL cholesterol. Abbreviation: HDL, high-density lipoprotein.

Figure 5 Comparison of median monocyte count and age.
Figure 6 Comparison of (a) Framingham and (b) SCORE risk estimates with monocyte count ($10^9$ cells/L).
Table 4 Statistical analysis using multivariable linear regression modeling to determine independent association between cardiovascular risk parameters and both Framingham and SCORE predictive risk estimations. Variables entered into the stepwise regression model included monocyte count, BMI, and waist circumference

| Parameter               | Framingham |          |          | SCORE    |          |          |
|-------------------------|------------|----------|----------|----------|----------|----------|
|                         | β          | t value  | p value  | β        | t value  | p value  |
| Monocytes               | 0.014      | 0.528    | 0.598    | 0.064    | 2.474    | 0.013    |
| BMI                     | –0.073     | –1.734   | 0.083    | –0.181   | –4.491   | <0.0005  |
| Waist circumference     | 0.252      | 5.954    | <0.0005  | 0.436    | 10.709   | <0.0005  |

Abbreviations: β, Beta coefficient; BMI, body mass index; t value, t value for beta coefficient.

differentiation into macrophages, they establish a persistent cellular reaction that underlies disease progression. However, more crucial may be their association with atherosclerotic plaque instability and rupture - the precedent events of arterial thrombosis and occlusion that portend clinically significant ischemia (Falk 2006). Although it is somewhat surprising that few studies to date have directly assessed the association of circulating monocyte levels with clinically relevant cardiovascular end-points, the findings of this study are supported by the conclusions of Nasir and colleagues (2005) who also determined a significant association between monocyte count and atherosclerotic disease in patients without known cardiovascular disease albeit in the peripheral arterial circulation. Furthermore, our findings are particularly intriguing given the recent demonstration by Swirski and colleagues (2007) that certain monocyte subsets dominate hypercholesterolemia-associated monocytosis and give rise specifically to macrophages in atheromata. Finally, the utility of this WBC subtype in risk assessment seems particularly appealing given that it less inherently susceptible to fluctuations due to superimposed acute inflammatory conditions or infections than are overall WBC or neutrophil subfractions or indeed other acute phase reactants such as CRP.

As does any clinical investigation, however, this prospective, observational study has potential limitations. Although a single WBC measurement was found to be significantly associated with currently utilized estimates of future cardiovascular risk; multiple measurements over time and changes in those measurements may provide a more accurate mechanism for predicting future CVD and mortality. If this is so however, the association found by this study would represent an underestimation of the actual relationship between monocyte count and CVD, and, thus, our conclusions may in fact be conservative. Additionally, the Framingham Heart Study may not apply to countries with low risk of heart disease, and there has been some concern over its use when applied to the diabetic population, tending to underestimate an individual’s probability of progressing to CHD (McEwan et al 2004). However, despite evidence that risk estimates based on Framingham generalize well to the population of the Republic of Ireland (Haq et al 1999), and is thus applicable to our study population, there is concern that this risk estimate (and indeed others) (Empana et al 2003) may overestimate absolute risk in European populations (Menotti et al 2000; Pyorala 2000; Thomsen et al 2002; Hense et al 2003). Therefore, to ensure an accurate and reliable estimate of absolute CVD risk in our study population, we additionally stratified each participant according to SCORE criteria, a risk stratification model that has specifically validated in European countries with similar levels of risk as Ireland and previously shown to be useful in ascribing risk in asymptomatic populations similar to our own (Aktas et al 2004). Although the validity of such scores has been questioned by some (Topol and Lauer 2003), they have nonetheless been integrated into primary cardiovascular disease prevention guidelines.

The findings of this study therefore suggest that monocyte count, a simple inexpensive test, may provide important risk information to aid prediction of future CVD development in disease free adults and so may help guide therapeutic intervention aimed at its interruption.

Disclosure
No conflicts of interest exist with regard to this manuscript

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