White blood cell subsets are associated with carotid intima-media thickness and pulse wave velocity in an older Chinese population: the Guangzhou Biobank Cohort Study

Short title: Cell counts, Carotid IMT and PWV

Anna C. Phillips[1], Chao Qiang Jiang[2], G Neil Thomas[3]*, Jie Ming Lin[2], Xiao Jun Yue[2], Kar Keung Cheng[3], Ya Li Jin[2], Wei Sen Zhang[2], Tai Hing Lam[4]

[1] School of Sport & Exercise Sciences, University of Birmingham, UK

[2] Guangzhou No.12 Hospital, Guangzhou, China

[3] Public Health, Epidemiology, and Biostatistics, University of Birmingham, Birmingham, UK

[4] Department of Community Medicine, School of Public Health, University of Hong Kong, Hong Kong

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*Corresponding author: Anna C. Phillips, School of Sport & Exercise Sciences, University of Birmingham, Birmingham, UK, Tel: 44 121 414 4398, Fax: 44 121 414 4121; Email: A.C.Phillips@bham.ac.uk

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Abstract

Cross-sectional associations between white blood cell count, lymphocyte and granulocyte numbers, and carotid intima-media thickness and brachial-ankle pulse wave velocity were examined in a novel older Chinese community sample. 817 men and 760 women from a sub-study of the Guangzhou Biobank Cohort Study had a full blood count measured by an automated hematology analyzer, carotid intimal-medial thickness (IMT) by B-mode ultrasonography, and brachial-ankle pulse wave velocity (PWV) by a non-invasive automatic waveform analyzer. Following adjustment for confounders, white blood cell count ($\beta = 0.07$, $p < 0.001$) and granulocyte ($\beta = 0.07$, $p < 0.001$) number were significantly positively related to PWV, but not lymphocyte number. Similarly, white blood cell count ($\beta = 0.08$, $p = 0.03$), lymphocyte ($\beta = 0.08$, $p = 0.002$), and granulocyte ($\beta = 0.03$, $p = 0.04$) number were significantly positively associated with carotid IMT, but only the association with lymphocyte count survived correction for other cardiovascular risk factors. In conclusion, higher white blood cell, particularly lymphocyte and granulocyte count could be used, respectively, as markers of cardiovascular disease risk, measured through indicators of atherosclerosis and arterial stiffness. The associations for white blood cell count previously observed by others were likely driven by higher granulocytes an index of systemic inflammation.

Keywords: carotid intima-media thickness; granulocytes; lymphocytes; pulse wave velocity;

Introduction

Atherosclerosis is a risk factor for coronary artery disease [1]. Given this association, research has concentrated on risk factors for or markers of the risk of atherosclerosis itself. For example, metabolic syndrome, a constellation of biological and anthropometric indicators including high blood pressure, glucose, triglycerides, abdominal obesity, and low HDL-cholesterol is strongly predictive of atherosclerosis risk [2]. One method of diagnosing atherosclerosis is to measure the thickness of the arterial wall. High resolution ultrasonographic imaging allows measurement of carotid intima-media thickness (IMT) sensitively and non-invasively [3] and is now a standard measure of atherosclerotic burden and a strong predictor of subsequent cardiovascular events [3]. Arterial stiffness is also recognised as a risk marker for cardiovascular disease and mortality [4], and can be measured via pulse pressure, but it is thought that pulse wave velocity (PWV) is a more descriptive non-invasive measure [5]. Pulse wave velocity is often measured as the time in which the pulse wave can travel from between the brachial artery to the ankle, as this is quicker and just as reliable as carotid to femoral artery pulse wave time [6] or carotid-ankle pulse wave velocity which has been shown to indicate large arterial stiffness in a Chinese population [7]. Brachial-ankle PWV has also been shown to correlate positively with other indicators of atherosclerosis in a Chinese population, including carotid intima-media thickness [8]. It is also a good predictor of cardiovascular disease risk [9], although reports have often been restricted to populations with existing hypertension or end-stage renal disease [10].

Studies have attributed the pathogenesis and perpetuation of atherosclerosis to inflammation [11], and links have been shown in epidemiological studies between white blood cell count and risk of

cardiovascular disease [12]. During an acute coronary event, white blood cell number in circulation becomes elevated and plays a central role in repair and replacement of necrotic tissue [12]. Thus, cell number indicates the intensity of the peri-infarction inflammatory response, and elevated counts are also associated with adverse outcomes following an event [12], carotid atherosclerosis plaque number [13], and risk of ischemic cardiovascular disease [14]. A few studies have examined the use of white blood cell count (WBC) as a predictor of carotid intima thickness. One group showed that WBC was independently positively associated with carotid IMT even after adjustment for the components of metabolic syndrome, in non-diabetic normotensive offspring of type 2 diabetic patients [15], and individuals at risk for developing diabetes [16]. Further, carotid atherosclerosis patients had higher white blood cell counts than controls and also higher IMT than controls [17]. One large scale longitudinal study demonstrated that the change in IMT over nine years was significantly associated with baseline WBC [18] and another showed a positive relationship between WBC and maximal internal carotid plaque thickness [19] which was particularly strong in Hispanics. In contrast, in a large Japanese study, WBC did not correlate with IMT [20]. The consensus of evidence suggests a positive association between WBC and IMT, although this may differ by population studied. However, less is known about the predictive ability of the individual subsets of white blood cells such as lymphocytes and granulocytes, although one study has shown an association between memory T-lymphocytes and IMT in patients with primary antiphospholipid syndrome [21]. Similarly, only a few studies have examined the associations between WBC and pulse wave velocity. One study used WBC as a marker of systemic inflammation and showed that age-adjusted PWV increased across quartiles of WBC in 788 Korean adults [22]. In contrast, in a sample of women with systemic lupus erythematosus,

higher aortic stiffness, measured by carotid-femoral PWV was associated with a lower white blood cell count [23]. No studies have examined this relationship in an older Chinese population, and, as far as we are aware, no studies have examined associations between sub-types of leukocyte and PWV. In Chinese populations, atherosclerosis is thought to be highly prevalent and is associated with cardiovascular disease risk factors including blood pressure, plasma glucose and low density lipoproteins cholesterol [24]. Similarly, thoracic aortic calcification was positively related to carotid IMT in Chinese as in other ethnic groups [25], but Chinese had higher prevalence of aortic wall calcification than blacks and Hispanics, with prevalence similar to whites [26]. However, the multiethnic study of atherosclerosis which showed these previous associations has also reported lower multi-site presence of atherosclerosis in Chinese in comparison to Caucasians, African Americans, and Hispanics [27]. Further, Chinese Americans showed the lowest carotid artery IMT among these ethnic groups [27], which has also been shown by others [28]. Given this lower prevalence of IMT, it was not known whether white blood cell counts would be predictive in a Chinese population.

Hence, we examined the associations between white blood cell, lymphocyte, and granulocyte count, and both intima-media thickness and pulse wave velocity in a novel large sample of older Chinese men and women.

Materials and Methods

Participants and Procedure

The Guangzhou Biobank Cohort Study (GBCS) is a three way collaboration between the Guangzhou People's No. 12 Hospital, Guangzhou, China and the Universities of Hong Kong, Hong Kong; and Birmingham, UK. The study aims to examine environmental and genetic determinants of chronic diseases has recruited about 30,000 older (>50 years) subjects from Guangzhou in southern China. The study has received ethical approval from the Guangzhou Medical Ethics Committee of Chinese Medical Association, Guangzhou, China.

In a more detailed sub-study on cardiovascular disease and diabetes, 1996 subjects were randomly selected from a list of eligible subjects [29]. A standardized questionnaire was used to collect information on age, sex, education, and lifestyle, including smoking, drinking status and physical activity according to the International Physical Activity Questionnaire (IPAQ) [30]. Height and weight were measured using standardized procedures described elsewhere [29]. Body mass index (BMI) was calculated as weight (kg) / height $(m)^2$. Metabolic equivalents (METS), as an index of physical fitness/activity, was calculated from the IPAQ measure based on frequency of a number of vigorous, moderate, and mild physical activities. A fasting blood sample was also taken for measurement of cholesterol, glucose, and C-reactive protein (CRP). Details of the participants' anthropometric measurements and questionnaire validation have been described elsewhere [31].

Complete blood count was performed in an automated hematology analyzer (KX-21, SYSMEX, Japan) including a WBC differential. The total white blood cell count, counts and differential

proportions of leukocytes including lymphocyte and neutrophils were automatically calculated by the hematology analyzer. Intima-media thickness was measured in the common carotid artery on both the right and left sides via carotid B-mode color ultrasonographic examination using ALT HDI 3000 mainframe enhanced, linear array scanner (medium frequency 7.5MHz) by a specialist physician. The operators were registered ultrasound doctors who had a professional certificate for color Doppler ultrasound measurement awarded by the Ministry of Health of China. All scans were performed following a predetermined, standardized scanning protocol for the right and left carotid arteries using images of the far wall of the distal 10mm of the common carotid arteries. Three scanning angles, with the image focused on the posterior wall, were recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. The physician who performed the scanning work was blinded to participant's information. Details on the procedure used in this study and its reproducibility has been published elsewhere [29]. A thicker IMT was diagnosed if the IMT was ≥1.0mm[32]. Data on IMT were available for 1577 participants. Brachial-ankle pulse wave velocity (PWV) was measured in the supine position after 5 minutes of bed rest using an automatic waveform analyzer (BP-203RPE; Colin Medical Technology, Komaki, Japan). This device stored data of the waveforms of both brachium and ankles for a sampling time. The time interval between the wave front of the brachial waveforms and that of the waveforms of ankle was automatically measured, which was defined as T. The path length from the suprasternal notch to the elbow (La) and also from the suprasternal notch to the ankle (Lb) were automatically calculated based on the patient's height. Then, baPWV was calculated using the following equation: baPWV (cm/s) = (Lb-La)/T, and the

averaged left and right baPWV was obtained for the data analysis. Greater values of PWC indicate greater arterial stiffness. Data were available on PWV for 1518 participants.

Statistical Analysis

All analyses were conducted using SPSS version 17. As the left and right IMT measurements did not differ significantly, mean IMT was computed from the right and left common carotid artery measurements. The right and left PWV were significantly different ($p < 0.001$), so separate analyses were run for these variables and their arithmetic mean. As the data were skewed, the blood counts, IMT, and PWV were subject to natural log transformation. Associations between IMT and PWV, and total white blood cells, lymphocytes, and neutrophils were examined using linear regressions, with IMT or PWV as the dependent variable in each model. Significant models were then repeated adjusting for age, sex, and health behaviours (smoking, alcohol consumption, METS). Finally, a set of models were run additionally adjusting for CVD risk factors including body mass index (BMI), fasting glucose, total cholesterol, triglycerides, systolic blood pressure (SBP), and C-reactive protein (CRP). Change in R-squared is used to indicate effect size.

Results

In the present analyses, the analytic sample consisted of 1577 participants including 760 women (48%). The descriptive statistics for the characteristics of the sample are shown in Table 1.

[Insert Table 1 about here]

Those with a higher WBC count were slightly older, lower in education level, more likely to smoke, more likely to have consumed alcohol in the past year, less physically active, and had a higher BMI, fasting glucose level, triglycerides, and SBP (all $p < 0.001$). Those who had higher lymphocyte numbers were more likely to smoke and have a higher total cholesterol and BMI ($p \leq 0.01$). Participants who had higher granulocyte numbers were older, lower in education level, more likely to smoke, more likely to have consumed alcohol in the past year, less physically active, and had a greater triglycerides and BMI (all $p < 0.05$).

The mean (SD) overall IMT was 0.076 (0.031) mm. For the right and left sides, the mean (SD) IMT was 0.08 (0.05) and 0.08 (0.04), respectively. Those with greater IMT were older, more likely to smoke, and had higher fasting glucose and SBP (all $p < 0.05$). The mean (SD) overall PWV was 1526 (309) ms. For the right and left sides, the mean (SD) PWV was 1518 (313) and 1533 (309), respectively. Those with greater PWV were older, lower in education level, and less physically fit, and had higher fasting glucose, total cholesterol, triglycerides, and SBP (all $p < 0.05$).

Intima-media thickness

White blood cell count, $\beta = 0.13$, $p < 0.001$, $\Delta R^2 = 0.016$, lymphocyte number, $\beta = 0.07$, $p = 0.002$, $\Delta R^2 = 0.005$, and granulocyte number, $\beta = 0.11$, $p < 0.001$, $\Delta R^2 = 0.012$, were all significantly positively associated with mean IMT. Following adjustment for age, sex, smoking, alcohol consumption, education, and physical activity, WBC remained associated with mean IMT, $\beta = 0.08$, $p = 0.03$, $\Delta R^2 = 0.003$, as did lymphocyte count, $\beta = 0.08$, $p = 0.002$, $\Delta R^2 = 0.006$. The association between granulocyte number was similarly only marginally attenuated, $\beta = 0.05$, $p = 0.04$, $\Delta R^2 = 0.003$. However, when the CVD risk factors were added to this model, the original associations were no longer significant, with the exception of lymphocyte count, which was slightly attenuated, $\beta = 0.06$, $p = 0.03$, $\Delta R^2 = 0.003$. The covariates responsible for this attenuation were BMI, triglycerides, and SBP, in each case (see Table 2).

[Insert Table 2 about here]

Pulse wave velocity

For the mean PWV, significant positive associations emerged for WBC, $\beta = 0.23$, $p < 0.001$, $\Delta R^2 = 0.054$, lymphocyte, $\beta = 0.05$, $p = 0.05$, $\Delta R^2 = 0.003$, and granulocyte count, $\beta = 0.25$, $p < 0.001$, $\Delta R^2 = 0.060$, such that those with greater pulse wave velocity (i.e. greater arterial stiffness had higher WBC, lymphocyte and granulocyte counts). For the right PWV, similar associations were observed for white blood cell count, lymphocytes and granulocytes, $\beta = 0.23$, $p < 0.001$, $\Delta R^2 = 0.053$, $\beta = 0.05$, $p = 0.04$, $\Delta R^2 = 0.003$, $\beta = 0.24$, $p < 0.001$, $\Delta R^2 = 0.059$, respectively. Similarly, for left PWV, $\beta = 0.23$, $p < 0.001$, $\Delta R^2 = 0.053$, $\beta = 0.05$, $p = 0.05$, $\Delta R^2 = 0.003$, $\beta = 0.24$, $p <$

0.001, $\Delta R^2 = 0.059$. Consequently, further analyses with potential confounding variables were run for mean PWV only. Following adjustment for age, sex, smoking, alcohol consumption, education, and physical fitness, WBC remained associated with mean PWV, $\beta = 0.20$, $p < 0.001$, $\Delta R^2 = 0.037$, as did lymphocyte count, $\beta = 0.08$, $p < 0.001$, $\Delta R^2 = 0.006$, and granulocyte count, $\beta = 0.20$, $p < 0.001$, $\Delta R^2 = 0.038$. However, when the CVD risk factors were additionally adjusted for, WBC, $\beta = 0.04$, $p = 0.03$, $\Delta R^2 = 0.002$, and granulocyte, $\beta = 0.04$, $p = 0.03$, $\Delta R^2 = 0.001$, count remained associated with PWV, but lymphocyte count showed no association. SBP, glucose, triglycerides, CRP, and BMI were responsible for this attenuation (see Table 3).

[Insert Table 3 about here]

Sex differences

Fully-adjusted analyses were then repeated individually for each gender. In these analyses, lymphocyte count significantly predicted IMT in men, $\beta = 0.10$, $p = 0.004$, $\Delta R^2 = 0.009$, but not women, and there was a non-significant trend for a positive association between WBC and IMT in men only, $\beta = 0.07$, $p = 0.06$, $\Delta R^2 = 0.004$. For PWV, in fully adjusted analyses, WBC was a significant predictor for women, $\beta = 0.12$, $p < 0.001$, $\Delta R^2 = 0.011$, but not men, as was lymphocyte count, $\beta = 0.09$, $p < 0.001$, $\Delta R^2 = 0.008$. Granulocyte count significantly predicted PWV in both men, $\beta = 0.06$, $p = 0.04$, $\Delta R^2 = 0.003$, and women, $\beta = 0.08$, $p = 0.003$, $\Delta R^2 = 0.006$.

Sensitivity Analyses

When the significant fully adjusted models were rerun using quartiles of cell count (see Table 4) rather than continuous logged values, much the same associations emerged, although the relationship between lymphocyte count and IMT was slightly attenuated, $\beta = 0.05$, $p = 0.07$, $\Delta R^2 = 0.002$. The associations between WBC, $\beta = 0.06$, $p = 0.004$, $\Delta R^2 = 0.003$, and granulocyte count, $\beta = 0.04$, $p = 0.03$, $\Delta R^2 = 0.001$, and PWV remained the same.

[Insert Table 4 about here]

Finally, the multivariate analyses with continuous forms of cell counts were repeated with further adjustment for diagnoses of diabetes (N = 45) or coronary heart disease (N = 33), as these diseases and their associated medications might influence the relationships between cell count, IMT and PWV. In each case, the results remained the same as reported above, all $p < 0.03$.

Discussion

White blood cell, lymphocyte, and granulocyte count were all positively associated with greater faster pulse wave velocity (ie. greater arterial stiffness) and carotid intima-media thickness. The associations between WBC and granulocyte number remained significantly related to PWV following full adjustment for other cardiovascular risk factors. However, only the association between lymphocyte count remained significantly predictive of IMT following full adjustment for confounders including age, sex, smoking status, alcohol intake, physical fitness, BMI, fasting glucose, total cholesterol, triglycerides, SBP, and CRP.

The positive relationship between WBC number and pulse wave velocity is in line with previous studies [22], although not all [23]. However, it is possible that the opposite counterintuitive direction of the association in the previous contradictory study reflected the sample from which participants were drawn, who were women with systemic lupus erythematosus, making comparison with our study difficult. Our WBC count finding appeared to be accounted for mainly by granulocyte number, supported by our positive association between granulocyte number and PWV. Further, our study is unique in considering WBC subsets in terms of PWV as a risk factor, given that most usually measure WBC alone. It is likely that granulocytes are driving the association between white blood cell count and pulse wave velocity, because this group mainly consists of neutrophils, which, in higher numbers in circulation, are considered an indication of systemic inflammation[33], also reflecting inflammation in the arterial vessels contributing to atherosclerosis. Indeed, it has been proposed that neutrophils may be one of the key factors contributing to microvasculature changes and inflammation, via cysteinyl leukotrienes formed when neutrophils adhere to the endothelial cell wall[34]. Higher levels of neutrophils may also indicate greater CVD risk via their ability to disrupt plaques and aggregate with platelets to plug the microvasculature, thereby promoting cardiovascular events [35]. That lymphocyte numbers were not significantly related to PWV in this study, but do relate to IMT in the present data and others [21] may suggest that they have their impact on atherosclerosis via different mechanisms. It also further confirms our hypothesis that granulocyte numbers are driving the association between WBC and PWV.

The present findings for white blood cell count and intima-media thickness mirrors those reported previously in participants at risk of diabetes[15, 16], and patients with carotid atherosclerosis[17] and other population studies[19, 20]. It appeared to be the increase in lymphocyte number which was mainly responsible for the overall association with white blood cells in the present study, as this was the only predictor to remain significant following full adjustment. This underlines the role of lymphocytes in cardiovascular disease, for example, by stimulating the production of procoagulants and collagen-degrading proteinases[36] and release of proinflammatory cytokines[37]. However, this finding contrasts with those of a previous study which showed that a lower lymphocyte count was associated with increased risk of myocardial infarction or mortality, although other white blood cell counts were positively associated with risk, which even the authors reported as not intuitively obvious [38]. This contradictory finding may be accounted for by the fact that the participants in this previous study were patients with or at high risk for CHD, whereas the present study was based on a mainly healthy community sample. Further, the participants in the present study were also considerably younger than those in the Utah study, and previous studies were also primarily in participants at primary risk of CHD. It is possible that in older sicker populations at higher risk or with existing CHD, it is a lower lymphocyte count which indicates increased risk due to their reparative role in inflammatory illness [38], rather than the higher counts associated with risk observed here. That our findings for lymphocyte count remained significant following adjustment for diagnosis of CHD provides some evidence that the negative direction of relationship is more likely to be observed among much higher disease risk samples. Further evidence can be found in a study on patients with primary antiphospholipid syndrome, where lymphocyte subsets, particularly memory T-cell, were positively associated

with IMT, suggesting a mechanism for atherogenesis [21], and in a Japanese sample of elderly men where increased circulating lymphocyte subpopulations, particularly memory cells, related positively to mean IMT following adjustment for other risk factors [39].

That granulocyte counts were not associated with IMT also contrasts with previous studies, given that other studies have shown a positive relationship with cardiovascular mortality and disease progression [38, 40-42]. However, again it is possible that these relationships differ somewhat between relatively healthy populations such as the present sample and those with existing high disease risk. Further, given that lymphocyte count was associated with IMT, but granulocyte count with PWV, may indicate different mechanisms of effect on atherosclerosis aetiology and prognosis, although the present data do not allow for a proper investigation of such mechanisms.

The associations described above suggest that aspects of white blood cell count could be used differentially as markers of the risk of atherosclerosis and arterial stiffness, independently of other cardiovascular risk factors and prior to the diagnosis of cardiovascular disease, where more time-consuming, invasive measures, or those requiring specialist equipment are not available, such as the GP surgery. Others have suggested that such other measures of atherosclerosis should be adjusted for cell counts rather than vice versa [38]. That the associations with IMT were more evident for men than women, while the opposite was true for PWV, suggests that the mechanisms underlying cardiovascular disease risk may be different for men and women. Previous research has also demonstrated that the predictors of IMT or PWV or the strength of

associations with predictors are somewhat different for men and women. For example, the metabolic syndrome predicted IMT more strongly in women than men in Taiwan[43], and ageing predicted PWV more strongly in women than men[44]. This emphasises that gender differences should be taken into account when examining predictors of cardiovascular disease risk.

The present study has several limitations. First, the data for these analyses were collected cross-sectionally, so the direction of causation is uncertain. Nonetheless, that high cell counts contribute to greater IMT and faster PWV is a parsimonious explanation, rather than vice versa, given that these cells are involved in the initial inflammatory processes leading to the formation of atherosclerotic plaques[45] and increases in arterial stiffness[46]. Nevertheless, it cannot be fully discounted that increased numbers of immune cells in circulation may be a consequence of atherosclerosis and the inflammatory processes it involves. Second, our sample is all Chinese, however, other studies of white blood cell count associations in different populations suggest that our findings are likely to be generalisable across ethnicities[15-18]. Third, it is possible that our findings are biased through the inclusion of individual with a variety of disease diagnoses. However, that our findings remained significant following adjustment for diagnoses of coronary heart disease and diabetes lends confidence to these results. Fourth, our data are unable to shed any light on the potential mechanisms underlying the associations between cell counts and atherosclerosis risk markers, and this would need to be explored separately. Finally, at present, longitudinal data is not available on the diagnosis of confirmed cardiovascular disease and its progression in these individuals; such longitudinal studies of diseases and these individual blood cell counts are warranted.

In conclusion, the present study showed, in a large older Chinese community sample, positive associations between white blood cell, lymphocyte, and granulocyte counts with carotid intima-media thickness and pulse wave velocity. However, lymphocyte count was the main independent predictor of IMT, while it was granulocyte count that was most strongly associated with PWV. This is the first time these relationships have been studied in a Chinese older relatively healthy population suggesting that such associations are generalisable beyond previously studied Western samples. These simple blood counts could potentially be used as a risk marker for future development of atherosclerosis and cardiovascular disease. Future studies could also consider the longitudinal associations between these markers and the diagnosis and prognosis of cardiovascular disease.

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Conflict of interest

We declare that we have no conflict of interest.

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Summary Table

What is known about the topic	White blood cell count is implicated in atherosclerosis. WBC is associated with IMT and PWV in some populations.
What this study adds	Which subsets of WBC are particularly predictive of IMT and PWV. Associations between WBC and IMT and PWV in a large Chinese cohort.

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Table 1: Characteristics of the sample

Characteristic		Mean/N	SD/%
Age, years		59.3	6.76
Sex (% male)		817	51.8
Education:	≤primary	436	27.6
	middle	941	59.7
	≥college	200	12.7
Smoking status (current/occasional)		347	22
Alcohol consumption in last yr (yes)		1090	69.1
METS		4464	3713
BMI kg/m^2		23.8	2.99
Glucose mmol/l		5.6	1.42
Total Cholesterol mmol/l		5.8	1.09
Triglycerides mmol/l		1.8	1.44
SBP mmHg		126.9	20.65
CRP mmol/l		2.4	2.80
White blood cell count × 10^9 cells/L		6.4	1.55
Lymphocyte count × 10^9 cells/L		2.2	0.60
Granulocyte count × 10^9 cells/L		3.8	1.22

BMI = Body Mass Index, CRP = C-reactive protein, METS = metabolic equivalents, SBP = Systolic Blood Pressure

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Table 2: Fully adjusted multiple regression analyses predicting carotid intima-media thickness individually by white blood cell, lymphocyte, and granulocyte count.

	β	*p*	ΔR^2
Step 1:			
Age, years	.18	< .001	
Sex (0 = male, 1 = female)	-.11	<.001	
Education (≤primary, middle, ≥college)	.02	.33	
Smoking status (current/occasional vs. non)	.06	.04	
Alcohol consumption in last yr (yes/no)	.04	.13	
METS	-.03	.26	
BMI kg/m^2	.07	.01	
Glucose mmol/l	.03	.26	
Cholesterol mmol/l	.02	.45	
Triglycerides mmol/l	-.07	.009	
SBP mmHg	.17	<.001	
CRP mmol/l	.03	.20	.137
Step 2:			
White blood cell count	.02	.40	<.001
Lymphocyte count	.06	.03	.003
Granulocyte count	-.01	.83	<.001

BMI = Body Mass Index, CRP = C-reactive protein, METS = metabolic equivalents, SBP = Systolic Blood Pressure

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Table 3: Fully adjusted multiple regression analyses predicting mean pulse wave velocity individually by white blood cell, lymphocyte, and granulocyte count.

	β	*p*	ΔR^2
Step 1:			
Age, years	.34	<.001	
Sex (0 = male, 1 = female)	.01	.69	
Education (≤primary, middle, ≥college)	-.02	.40	
Smoking status (current/occasional vs. non)	-.002	.91	
Alcohol consumption in last yr (yes/no)	.02	.41	
METS	-.02	.18	
BMI kg/m^2	-.07	<.001	
Glucose mmol/l	.07	<.001	
Cholesterol mmol/l	.01	.56	
Triglycerides mmol/l	.07	<.001	
SBP mmHg	.54	<.001	
CRP mmol/l	.10	<.001	.554
Step 2:			
White blood cell count	.04	.03	.002
Lymphocyte count	.02	.38	<.001
Granulocyte count	.04	.03	.001

BMI = Body Mass Index, CRP = C-reactive protein, METS = metabolic equivalents, SBP = Systolic Blood Pressure

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Table 4: Descriptive statistics for quartiles of cell counts

	Mean (SD)			
	1	2	3	4
WBC	4.7 (0.50)	5.8 (0.26)	6.8 (0.31)	8.5 (1.10)
Lymphocyte	1.5 (0.19)	2.0 (0.11)	2.3 (0.11)	3.0 (0.43)
Granulocyte	2.6 (0.37)	3.3 (0.17)	4.0 (0.23)	5.5 (1.05)

WBC = White Blood Cell Count