Range and Determinants of White Blood Cell Count in a Large Survey of Israelis without Inflammation

Arie Steinvil MD[1], Shlomo Berliner MD PhD[1], Yair Herishanu MD[2], Yael Paran MD[1], Dan Justo MD[1], Michael Cohen MD[1], Itzhak Shapira MD[1] and Ori Rogowski MD[1]

[1]Departments of Internal Medicine D and E, and [2]Department of Hematology, Tel Aviv Sourasky Medical Center, Tel Aviv, and Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

ABSTRACT: **Background:** The white blood cell count and its differential are established inflammation-sensitive biomarkers with a proven prognostic value in the field of atherothrombosis. However, the WBCC count range and determinants have not been explored in the absence of a significant inflammatory response.

Objective: To analyze the WBCC range and determinants in a large Israeli sample of individuals, excluding patients with a significant inflammatory response.

Methods: WBCC and differential count reference ranges were determined in a large sample of apparently healthy participants with high sensitivity C-reactive protein concentrations below 10 mg/L. Linear regression models were used to identify the determinants of the WBCC. The central 95% areas under the distribution curves were established for each gender.

Results: The study population comprised 8247 individuals (5391 males and 2856 females). The main laboratory and clinical variables found to affect the WBCC were gender, hemoglobin level, smoking status, triglycerides, and body mass index (all $P < 0.001$). Similar results were obtained for the differential count. The reference ranges for men and women were 3.6–9.9 and 3.4–10.0 x10^3/µl, respectively. The reference ranges for currently smoking men and women were 3.6–11.5 and 3.6–11.2 x10^3/µl, respectively, and were significantly higher compared with those of the never smokers and past smokers.

Conclusions: We have demonstrated new limits of normal values for the WBCC in apparently healthy individuals who lack a significant "background" acute-phase response in a large Israeli sample. Our data might be useful for the risk stratification of apparently healthy individuals in the field of atherothrombosis.

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KEY WORDS: white blood cell count, reference values, C-reactive protein

Atherothrombotic disease is a leading cause for morbidity and mortality in the western world and is associated with a low grade subclinical inflammation (so-called micro-inflammation) [1,2]. The white blood cell count [3-8] and its differential [9-11] are established inflammation-sensitive biomarkers with a prognostic value in the field of atherothrombosis. Until now, normal values for the WBCC were derived from studies in unselected apparently healthy individuals and were not necessarily relevant for individuals who did not harbor a significant background inflammatory disease [12-14].

In this study we explored the range of the WBCC in a large sample of apparently healthy individuals in whom the presence of low grade inflammation was excluded by using a high sensitivity C-reactive protein cutoff of 10 mg/L [15].

PATIENTS AND METHODS

We analyzed the data collected by the Tel Aviv Medical Center Inflammation Survey, a registered data bank of the Israeli Ministry of Justice [16-18]. This is a relatively large survey of apparently healthy individuals attending the center for periodic health examinations.

In our study, patients attending for a routine health examination between September 2002 and November 2007 were invited to participate in the survey. All enrolled individuals gave their written consent in accordance with the guidelines of the institutional ethics committee. A total of 12,251 subjects gave their informed consent (7702 males, 4549 females).

We initially excluded 2204 subjects due to active malignancy or immunosuppressive therapy, known inflammatory diseases (arthritis, inflammatory bowel disease, psoriasis, etc.), pregnancy, steroidal or non-steroidal treatment (except for aspirin at a dose of ≤ 325 mg/day), and acute infection or invasive procedures (surgery, catheterization, etc.) during the preceding 6 months. We later excluded 404 subjects from the analysis due to missing WBCC measurements and 191 individuals in whom smoking status was not ascertained. We further excluded 749 Jewish Yemenites due to their known lower normal range reference values [18]. Finally, in order to

WBCC = white blood cell count

Table 1. Population characteristics according to gender

	Men (N = 5391)		Women (N = 2856)		
	Mean	(SD)	Mean	(SD)	*P* value
Age (yrs)	44	(11)	45	(11)	0.001
BMI (kg/m^2)	27	(5)	25	(4)	< 0.001
Systolic BP (mmHg)	125	(14)	117	(15)	< 0.001
Diastolic BP (mmHg)	78	(8)	74	(8)	< 0.001
Glucose (mg/dl)	95	(18)	91	(16)	< 0.001
LDL-cholesterol (mg/dl)	122	(32)	119	(33)	0.001
HDL-cholesterol (mg/dl)	50	(10)	65	(15)	< 0.001
Triglycerides (mg/dl)	130	(83)	102	(56)	< 0.001
Hs-CRP (mg/L)	1.3	(2.6)	1.4	(2.9)	0.010
WBCC (10^3 cells/mm^3)	6.7	(1.6)	6.7	(1.7)	0.165
PMN (10^3 cells/mm^3)	4.0	(1.2)	4.0	(1.3)	0.808
Lymphocytes (10^3 cells/mm^3)	2.00	(0.61)	2.04	(0.59)	0.035
Platelets (10^3 cells/mm^3)	242	(55)	260	(60)	< 0.001
Physical activity (hr/week)	2.5	(3.0)	1.9	(2.8)	< 0.001
Alcohol consumption (glasses/week)	1.3	(2.4)	0.6	(1.5)	< 0.001

BMI = body mass index, BP = blood pressure, LDL = low density lipoprotein, HDL = high density lipoprotein, hs-CRP = high sensitivity C-reactive protein, WBCC = white blood cell count, PMN = polymorphonuclear count.

Table 2. Medication and cardiovascular risk factors: frequencies according to gender

	Men (N = 5391)		Women (N = 2856)		
	N	(%)	N	(%)	*P* value
Risk factors					
History of atherothrombotic event	244	(4.5)	84	(2.9)	< 0.001
Diabetes mellitus	276	(5.1)	102	(3.6)	0.001
Hypertension	1,347	(25.0)	442	(15.5)	< 0.001
Dyslipidemia	1,874	(34.8)	707	(24.8)	< 0.001
Family history of CHD	832	(15.4)	549	(19.2)	< 0.001
Current smoker	904	(16.8)	565	(19.8)	< 0.001
Past smoker	1,498	(27.8)	592	(20.7)	
Medications					
Aspirin	461	(8.6)	104	(3.6)	< 0.001
Beta-blockers	275	(5.1)	128	(4.5)	0.215
Calcium channel blockers	142	(2.6)	51	(1.8)	0.015
ACE inhibitors	231	(4.3)	72	(2.5)	< 0.001
ARBs	60	(1.1)	20	(0.7)	0.069
Statins	565	(10.5)	204	(7.1)	< 0.001
Fibrates	72	(1.3)	16	(0.6)	0.001
Oral hypoglycemic agents	116	(2.2)	33	(1.2)	0.001
Oral contraceptives			341	(11.9)	
Hormonal replacement therapy			294	(10.3)	

CHD = coronary heart disease, ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor blocker.

exclude subjects with hidden inflammation and/or infection we excluded 456 subjects with hs-CRP concentrations above 10 mg/L [15]. Following these exclusions this sample comprised 8247 individuals (5391 males and 2856 females).

LABORATORY METHODS

Analysis of the complete blood count was performed using the Coulter STKS (Beckman Coulter, Nyon, Switzerland) electronic cell analyzer. The hs-CRP was measured using a Behring BN II Nephelometer (DADE Boehring, Marburg, Germany) [19].

DEFINITIONS OF ATHEROTHROMBOTIC RISK FACTORS

Results of the routine health checkup were assessed using certain definitions in order to recognize atherothrombotic risk factors. These included diabetes mellitus, which was defined as individuals displaying fasting plasma glucose of ≥ 126 mg/dl (7.0 mmol/L) or the intake of insulin or oral hypoglycemic medications. Hypertension was defined as blood pressure of ≥ 140/90 mmHg on two separate measurements or the intake of antihypertensive medications. Dyslipidemia was defined as the need for lipid-lowering medication, or the presence of low density lipoprotein or non-high density lipoprotein-cholesterol concentrations in individuals with elevated triglyceride concentrations of ≥ 200 mg/dl (2.26 mmol/L) [20]. Smokers were defined as individuals who smoked at least five cigarettes per day, while those who had quit smoking for at least 30 days prior to the examination were considered past smokers. Alcohol consumption was documented as the self-reported total number of alcoholic beverage glasses drunk per week. Sport activity was noted as the self-reported total number of weekly sessions of moderate or higher exercise activity. Premature family history of coronary heart disease was defined as having a first-degree relative with coronary heart disease at an age younger than 55 years for males or younger than 65 for females.

STATISTICAL ANALYSIS

All data were summarized and displayed as mean ± standard deviation for continuous variables and number of patients and percentage in each group for categorical variables. For all categorical variables the chi-square test was used to assess statistical significance between the two genders, while the independent Student's *t*-test was used for continuous variables. The age-adjusted comparison of continuous variables between the two genders was performed using ANCOVA with a general linear model.

In order to assess which variables contributed to the variability of the WBCC, we performed a linear regression with WBCC as the dependent variable and many known and possible confounding parameters as the independent variables. These variables included age; body mass index; alcohol consumption and sport activity; use of medication including aspirin, beta-blockers, calcium channel blockers, angiotensin-

converting enzyme inhibitors, angiotensin II receptor blockers, statins, fibrates, oral contraceptives and hormonal replacement therapy; cardiovascular risk factors including systolic and diastolic blood pressure measurements; family history of coronary heart disease as well as menopause status; lipid profile including HDL, LDL and triglyceride concentrations; fasting plasma glucose; and hemoglobin concentrations.

In addition to the linear regression model, we calculated the crude estimated mean WBCC and range in each gender group plus the overall significance between these groups, using ANOVA. Normal ranges for the WBCC were expressed as the mean ± 1.96 x SD. All the above analyses were considered significant at $P < 0.05$ (two-tailed). The SPSS statistical package was used to perform all statistical evaluations (SSPS Inc., Chicago, IL, USA).

RESULTS

We analyzed the data of 8247 individuals, of whom 5391 were males with a mean age of 44 years (median 45, range 18–83), and 2856 were females with a mean age of 45 years (median 46, range 19–77). The anthropometric values, blood pressure measurements, relevant laboratory values, inflammation-sensitive biomarkers including the WBCC, as well as physical activity and alcohol consumption in the two genders are described in Table 1. Clearly evident are significant differences between the genders in most of the parameters. Table 2 shows the respective cardiovascular risk factors as well as frequency of medication in the two genders. It is worth noting that the prevalence of cardiovascular risk factors as well as the levels of the measurements given in Table 1 are similar to those of previous studies in the Israeli population [21-24]. In order to assess which variables best explain the variability in the WBCC, we performed linear regression; Table 3 shows the 10 most significant variables that entered the model. It can be seen that smoking status, gender and hemoglobin level were the major determinants. Analysis of the differential/neutrophil counts showed similar results. Gender and smoking status had a significant effect on the WBCC and neutrophil count. Hence, the crude mean WBCC and neutrophil count and the expected normal range limits (mean ± 1.96 times the SD) were stratified by gender and smoking status [Table 4].

HDL = high density lipoprotein
LDL = low density lipoprotein

Table 3. Determinants in the white blood cell count

	Beta	*P* value	Partial correlation
Current smoker	0.746	< 0.001	0.173
Hemoglobin	0.237	< 0.001	0.146
Female gender	0.686	< 0.001	0.131
Triglycerides	0.003	< 0.001	0.124
Pack year	0.011	< 0.001	0.096
Body mass index	0.028	< 0.001	0.082
Calcium channel blockers	0.779	< 0.001	0.076
Oral contraceptives	0.550	< 0.001	0.065
Menopause	-0.374	< 0.001	-0.064
HDL-cholesterol	-0.008	< 0.001	-0.058

Table 4. Crude mean and 95% range (normal range) of WBCC (upper part) and polymorphonuclear count (lower part) in men and women by smoking status

WBCC	All		Men		Women	
All	6.7	(3.5–10.0)	6.7	(3.6–9.9)	6.7	(3.4–10.0)
Never smokers	6.5	(3.6–9.5)	6.6	(3.6–9.5)	6.5	(3.4–9.6)
Past smokers	6.6	(3.7–9.5)	6.6	(3.8–9.5)	6.5	(3.5–9.5)
Current smoker	7.5	(3.6–11.4)	7.5	(3.6–11.5)	7.4	(3.6–11.2)
Polymorphonuclears	**All**		**Men**		**Women**	
All	4.0	(1.5–6.5)	4.0	(1.4–6.6)	4.0	(1.6–6.4)
Never smokers	3.9	(1.6–6.1)	3.9	(1.7–6.0)	3.9	(1.5–6.3)
Past smokers	3.9	(1.7–6.1)	3.9	(1.7–6.2)	3.9	(1.5–6.2)
Current smoker	4.5	(1.4–7.6)	4.5	(1.3–7.7)	4.5	(1.5–7.4)
Lymphocytes	**All**		**Men**		**Women**	
All	2.0	(0.8–3.2)	2.0	(0.8–3.2)	2.0	(0.9–3.2)
Never smokers	2.0	(0.8–3.2)	2.0	(0.8–3.2)	2.0	(0.9–3.1)
Past smokers	2.0	(0.9–3.1)	2.0	(0.9–3.0)	2.0	(0.9–3.1)
Current smoker	2.2	(1.0–3.5)	2.2	(1.0–3.5)	2.2	(1.0–3.5)

DISCUSSION

To the best of our knowledge, this is the first study to document the determinants of the white blood cell count in a relatively large group of apparently healthy individuals and those with atherothrombotic risk factors in whom a significant underlying acute-phase response was excluded using an hs-CRP cutoff value of 10 mg/L [15]. The main outcome of the study is the definition of new limits of normal values for the WBCC in apparently healthy individuals who lack a significant "background" acute-phase response. In addition, we found that the determinants of the WBCC in these carefully selected individuals were similar to those in previous publications [12-14].

Both the WBCC and CRP are inflammation-sensitive biomarkers that have documented prognostic values in patients with atherothrombotic disease [1-3]. Various studies have shown that elevated WBCC and CRP concentrations are

independent risk factors for coronary heart disease, for future cardiovascular events in individuals apparently without cardiovascular disease, and as prognostic biomarkers for future events in patients who already have cardiovascular disease [5]. Most studies did not, however, differentiate the prognostic value of the inflammatory response [3-6,10,11]. This prognostic value attributed to the baseline WBCC was first demonstrated by Sabatine et al. [25] in patients with an acute coronary syndrome. Patients with high hs-CRP concentrations (> 15 mg/L) had a higher risk of death at 6 months, which was similar across all WBCC strata. In contrast, for patients with low hs-CRP concentrations (< 15 mg/L) the 6 month mortality rate correlated with the baseline WBCC. Thus, it has been shown that baseline WBCC can be used to sub-stratify mortality risk among patients with a low CRP level.

Although the population described in the present study resembles previous publications from Israel regarding the level of some anthropometric measurements, blood pressure and lipid profile levels as well as the prevalence of cardiovascular risk factors [21-24], we acknowledge a shortcoming that may limit its use for the entire population. The study was conducted as part of a health survey that recruited mainly healthy working individuals, and it might therefore incorporate a healthy worker selection bias effect. Consequently, the results cannot be extrapolated to the general population. The CRP cutoff value was chosen based on recommendations of the U.S. Centers for Disease Control and Prevention and the American Heart Association [15], according to which an hs-CRP level ≥ 10 mg/L should be discarded and a search initiated for an obvious source of infection or inflammation.

Our study is unique in that it presents new normal value ranges for the WBCC in apparently healthy individuals who lack a significant "background" acute-phase response. The data presented here might be useful for the risk stratification of apparently healthy individuals in the detection of subclinical atherothrombosis.

Correspondence:
Dr. D. Justo
Dept. Internal Medicine D, Tel Aviv Sourasky Medical Center, 6 Weizmann Street; Tel Aviv 64239, Israel
Phone: (972-52) 266-739
Fax: (972-9) 740-8575
email: justo1@bezeqint.net

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