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Total and Differential White Blood Cell Counts and Their Associations With Circulating Interleukin-6 Levels in Community-Dwelling Older Women

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Background. Interleukin-6 (IL-6) is an inflammatory biomediator, and age-related increases in IL-6 levels are associated with osteoporosis, sarcopenia, disability, and mortality in older adults. Although white blood cells (WBC), or leukocytes, are known to produce IL-6 in vitro, their in vivo relationship with circulating IL-6 levels is not well established.

Methods. In this cross-sectional analysis of data from the Women's Health and Aging Study I, the authors evaluated the relationships of total WBC and WBC differential (neutrophil, monocyte, lymphocyte, eosinophil, and basophil) counts to circulating IL-6 levels in 619 community-dwelling older women. Potential associations of age, race, and cigarette smoking with total and differential WBC counts and IL-6 levels were also assessed.

Results. Except for lymphocyte and basophil counts, significant associations of total WBC, neutrophil, monocyte, and eosinophil counts with IL-6 levels were identified. These associations remained highly significant after adjustment for age, race, and smoking status. Total WBC, neutrophil, monocyte, and eosinophil counts had significant stepwise increases in four escalating quartiles of IL-6 levels. In addition, age, race, and cigarette smoking were differentially associated with total and differential WBC counts and IL-6 levels.

Conclusions. Positive in vivo associations of total WBC and its specific subpopulations were identified, including neutrophils, monocytes, and eosinophils, with circulating IL-6 levels in community-dwelling older women. These findings suggest significant contributions of WBC and its subpopulations to circulating IL-6 levels and potential effects from chronic elevation of IL-6 levels to the function of these circulating immune cells that warrant further investigation.

INTERLEUKIN-6 (IL-6) is a proinflammatory cytokine with increased circulating levels in older adults (1). Age-associated increases in IL-6 levels are associated with several pathophysiologic processes, including atherosclerosis, osteoporosis, and sarcopenia, and with disability, frailty, and all causes of death in older adults (2–10). In addition, increased IL-6 levels are associated with lower muscle mass and strength even in well-functioning older men and women (11). In a longitudinal study, Ferrucci and colleagues (12) recently reported that elevated IL-6 levels at baseline predict a significantly higher risk for the development of physical disability and a steeper decline in muscle strength and walking performance during a follow-up period of 3.5 years in older women living in the community. However, little is known about the cellular basis for and the underlying mechanisms that contribute to increased circulating levels of this inflammatory biomediator in older adults.

Although white blood cells (WBCs) are potent in vitro producers of proinflammatory cytokines including IL-6 (13–15), potential in vivo associations of these circulating immune cells with IL-6 in older adults have not been investigated. We hypothesized that total WBC and specific WBC subpopulations contribute significantly to the elevation of IL-6 levels in older adults. Investigating this hypothesis will help to advance our understanding of the physiologic basis for the elevation of IL-6 levels and the interaction between IL-6 and WBC, given the fact that the total WBC count itself has strong association with adverse health outcomes, including mortality (16,17).

To provide initial insight into this question, we conducted a cross-sectional analysis of data from the Women's Health and Aging Study I (WHAS I), evaluating the relationships of total WBC and WBC differential counts to circulating IL-6 levels in community-dwelling older women. We also assessed potential associations of age, race, and cigarette smoking with total and differential WBC counts and IL-6 levels in this population.

Methods

Study Population

The WHAS I is an epidemiologic study of the causes and course of disability among the one-third most disabled women aged 65 years and older who are living in the community. The design of the WHAS and data collection methods have been described in detail elsewhere (18,19). Blood samples were obtained in 791 participants. Among them, 646 had blood samples sent for total and differential WBC counts and 762 for aliquots of serum that were stored at −80°C for IL-6 measurements, with a total of 635 who

Table 1. Mean, Median, and Percentiles of Total and Differential WBC Counts and IL-6 Levels

Study Variable	Mean (*SD*)	5%	25%	Median	75%	95%
WBC counts						
Total WBC, 10^3/mm^3	6.48 (1.74)	4	5.3	6.3	7.5	9.8
Neutrophils, 1/mm^3	3991 (1406)	2045	2916	3818	4811	6716
Lymphocytes, 1/mm^3	1839 (617)	905	1400	1788	2188	2949
Monocytes, 1/mm^3	450 (182)	186	318	426	550	805
Eosinophils, 1/mm^3	148 (108)	38	77	117	195	352
Basophils 1/mm^3	49 (29)	10	28	42	61	111
IL-6, pg/ml	3.05 (2.29)	0.96	1.55	2.33	3.61	9.35

Note: WBC = white blood cell; IL-6 = interleukin-6; *SD* = standard deviation.

had both total and complete differential WBC counts and IL-6 measurements. To minimize the potential influence of acute bacterial infection, 8 participants with total WBCs more than 12×10^3/mm^3 or total WBCs more than 11×10^3/mm^3 plus neutrophils more than 80% were excluded from the analysis. Eight additional participants with influential outlier counts in either total WBC or any of the five differential counts (due to either hematologic cancer or technical error) were also excluded, yielding a final sample of 619 participants for this study. Compared with the 619 participants included in the analysis, the 383 participants who did not provide blood samples were significantly older and had poorer self-reported health status. Race, education, and smoking status were not significantly different between the two groups.

Measurements of IL-6 Levels and Total and Differential WBC Counts

IL-6 was measured in duplicate by enzyme-linked immunosorbent assay from frozen serum with a commercial kit (High-Sensitivity Quantikine Kit, R&D Systems, Minneapolis, MN), and the average of the two measures was used in the analysis (8). Total and differential WBC counts were obtained using a Coulter counter in a well-standardized commercial laboratory.

Statistical Analysis

In exploratory analyses, graphic displays and frequency distributions were constructed for baseline characteristics of the study population. Bivariate associations between total and differential WBC counts and IL-6 levels were evaluated using cross-tabulations of means and standard deviations of the WBC counts by quartiles of IL-6; analysis of variance was used to compare group differences. Multiple linear regressions were used to study the relationships of total and differential WBC counts with IL-6 levels, with separate regression analyses for IL-6 on each total and differential WBC count, with and without adjustment for age, race, and smoking status. To ensure validity of the regression analyses, IL-6 levels, eosinophil, and basophil counts with highly skewed distributions were transformed using the natural logarithm to approximate normality. The estimated regression coefficients and corresponding confidence intervals for the log-transformed variables were exponentiated such that the effects can be interpreted on their original measurement scale as percentage change in the median of the outcome per unit change in the covariate. We checked the assumptions of all models we fit, including functional form and error properties, by comparing sample and model-based statistics and evaluating residual plots. We used STATA version 7 (STATA Corp., College Station, TX) for model estimation and diagnostics.

Results

The mean age of the study participants was 77.4 years, with a standard deviation of 7.8 years. Seventy-two percent of the study participants were white and 28% were not. Their smoking status was categorized by never smoked (53%), former smoker (36%), and current smoker (11%). Table 1 summarizes the mean, median, and percentile range of all the WBC counts and IL-6 levels for the study population.

The potential effects of age, race, and smoking status on IL-6 levels and on WBC counts were assessed by bivariate regression analyses (Table 2). As expected, IL-6 levels were significantly associated with age and smoking status, but not race. Median IL-6 levels were increased by 13% per 10 years of age, by 23% in former smokers compared with never smokers, and by 61% in current smokers versus never smokers. Mean total WBC counts were significantly lower among participants who were not white and higher among

Table 2. Effects of Age, Race, and Smoking Status on IL-6 Levels, Total WBC Count, and Differential Counts as Shown by Regression Coefficients (95% CI)

	Age (×10 y)		Nonwhite vs White		Former-Smoker vs None		Current-Smoker vs None	
	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI
IL-6 (pg/ml)[†]	1.13*	(1.05, 1.21)	1.08	(0.97, 1.21)	1.23*	(1.11, 1.38)	1.61*	(1.37, 1.91)
Total WBC (10^3/mm^3)	−0.02	(−0.19, 0.16)	−0.74*	(−1.04, −0.44)	0.32*	(0.03, 0.61)	1.07*	(0.62, 1.51)
Neutrophils (1/mm^3)	55.18	(−86.24, 196.6)	−750.73*	(−987.60, 513.85)	368.82*	(137.39, 600.25)	833.75*	(478.77, 1188.73)
Lymphocytes (1/mm^3)	−77.46*	(−141.70, −13.24)	102.49	(−5.06, 210.05)	−67.48	(−172.56, 37.61)	187.94*	(26.76, 349.12)
Monocytes (1/mm^3)	3.16	(−15.88, 22.2)	−66.21*	(−98.1, −34.31)	16.07	(−15.08, 47.23)	39.52	(−8.27, 87.32)
Eosinophils (1/mm^3)[†]	0.98	(0.90, 1.06)	0.88	(0.77, 1.00)	1.01	(0.88, 1.14)	0.97	(0.80, 1.19)
Basophils (1/mm^3)[†]	1.01	(0.94, 1.08)	0.83*	(0.73, 0.94)	1.04	(0.92, 1.17)	1.15	(0.96, 1.39)

Notes: *$p < .05$.

[†]Because log-transformed scores were used in the regression analyses, the numbers presented are exponentiated regression coefficients.

IL-6 = interleukin-6; WBC = white blood cell; CI = confidence interval.

Table 3. Mean (*SD*) of Total and Differential WBC Counts Across IL-6 Quartiles

IL-6 Quartiles (Range in pg/ml)	0%–25% (0–1.6)	26%–50% (1.6–2.3)	51%–75% (2.3–3.6)	76%–100% (3.6–10.1)	*p* Value*
Total WBC, 10^3/mm^3	5.67 (1.43)	6.44 (1.57)	6.56 (1.80)	7.24 (1.79)	<.001
Neutrophils, 1/mm^3	3373.97 (1141.16)	3850.27 (1256.57)	4085.15 (1362.22)	4654.80 (1514.68)	<.001
Lymphocytes, 1/mm^3	1734.44 (557.29)	1941.79 (639.82)	1809.80 (579.82)	1871.65 (669.94)	.21
Monocytes, 1/mm^3	381.07 (161.69)	454.70 (169.92)	460.86 (182.98)	503.18 (193.95)	<.001
Eosinophils, 1/mm^3	134.45 (93.30)	142.63 (95.67)	155.08 (125.17)	159.72 (114.09)	.02
Basophils, 1/mm^3	45.60 (26.59)	53.38 (31.82)	45.54 (27.08)	50.40 (30.12)	.30

Note: *p value is from the trend test of the difference in total or differential white blood cell (WBC) counts across interleukin-6 (IL-6) quartiles.

smokers, but they did not vary significantly with age. Among the WBC differential counts, neutrophil and lymphocyte counts were significantly elevated among smokers. All but lymphocyte and eosinophil counts were significantly lower in participants who were not white. Only lymphocyte counts had an inverse relationship with age.

The relationships between WBC counts and IL-6 levels were evaluated next. First, the means were cross-tabulated with standard deviations of the total WBC and each differential count by quartiles of IL-6 level. We found a stepwise increase in total WBC, neutrophil, monocyte, and eosinophil counts across IL-6 quartiles (Table 3). Across the IL-6 quartiles, the differences in total WBC, neutrophil, monocyte counts (all $p < .001$), and eosinophil counts ($p = .02$) were highly significant. No statistically significant differences were noted in lymphocyte or basophil counts (Table 3).

Multiple linear regression analyses were performed using log IL-6 as the outcome measure and the various WBC counts (total WBC in units of 1000/mm^3 and differential counts in units of 1/mm^3) as predictors (separate models, each with and without adjusting for age, race, and smoking status as confounders). Total WBC, neutrophils, monocytes, and eosinophils showed significant crude and adjusted associations with IL-6 levels (Table 4). These results indicate, for example, an increase in median IL-6 level of 13% (crude) or 12% (adjusted) per each 1000/mm^3 increase in total WBC count. In addition, these results also suggest that confounding factors evaluated had minimal impact on the identified associations of IL-6 levels with total and specific differential WBC counts. Lymphocyte and basophil counts did not have significant associations with IL-6 levels (Table 4).

In addition, using the adjusted multivariate regression model, predicted IL-6 levels were calculated at the median and at 1 or 2 standard deviations of total and differential WBC counts for participants who were 78 years old (the mean age of the study population), not white, and current smokers. As shown in Table 5, counts of total WBC, neutrophils, monocytes, and eosinophils at 1 or 2 standard deviations above their median, but not lymphocytes or basophils, predict significantly higher IL-6 levels. Together, these results show that counts of total WBC, neutrophils, monocytes, and eosinophils predict IL-6 levels (Tables 4 and 5), supporting the associations of total and specific differential WBC counts with circulating IL-6 levels. Figure 1 shows the associations of total WBC with log IL-6 (Figure 1A, partial correlation coefficient [r] = .31, $p < .001$) and neutrophils with log IL-6 (Figure 1B, $r = .34$, $p < .001$), after adjustment for age, race, and smoking status. The smoothed observed and fitted trends largely overlap, indicating a good model fit. Similar associations were seen between monocyte and eosinophil counts and log IL-6 (data not shown).

Discussion

In the current study, we identified positive in vivo associations of total WBC, neutrophil, monocyte, and eosinophil counts with circulating IL-6 levels in community-dwelling older women, with adjustment for age, race, and smoking status. We also observed specific associations of age, race, and cigarette smoking with WBC counts or IL-6 levels.

Although many in vitro studies have established that subpopulations of WBC are potent IL-6 producers (3,13–15,20,21), few studies have evaluated their in vivo relationship with circulating IL-6 levels. A subset study of the Established Populations for Epidemiologic Studies of Elderly demonstrated that these participants in the higher tertiles of IL-6 levels had significantly higher total WBC counts (4). However, no adjustments for potential confounding factors or exclusion for possible acute bacterial infection were made in that study (4). We identified a statistically significant crude association of total WBC with log IL-6 used as a continuous variable. This association remained highly significant after adjustment for age, race, and smoking status (Table 4, Figure 1A). Further analysis showed that the total WBC count had highly significant stepwise increases in four escalating quartiles of circulating IL-6 levels (Table 3).

To date, no published data are available on the in vivo relationships of WBC differential counts with IL-6 levels. We found that specific WBC differential counts including neutrophils, monocytes, and eosinophils, but not lymphocytes or basophils, had significant associations with log IL-6

Table 4. Crude and Adjusted Regression Coefficients (95% CI) of Total and Differential WBC Counts With IL-6 Levels

	Crude		Adjusted*	
	Coefficient	95% CI	Coefficient	95% CI
Total WBC	1.13†	(1.10, 1.16)	1.12†	(1.09, 1.16)
Neutrophils	1.17†	(1.13, 1.21)	1.17†	(1.13, 1.21)
Lymphocytes	1.01	(0.99, 1.01)	1.00	(0.99, 1.01)
Monocytes	1.08†	(1.05, 1.11)	1.08†	(1.05, 1.11)
Eosinophils	1.06†	(1.01, 1.11)	1.07†	(1.02, 1.12)
Basophils	1.09	(0.92, 1.30)	1.07	(0.90, 1.27)

*Adjusted for age, race, and smoking status.
†$p < .001$.
CI = confidence interval; WBC = white blood cell; IL-6 = interleukin-6.

Table 5. IL-6 Levels in pg/ml (95% Prediction Interval) Predicted at the Median or ± 1~2 Standard Deviation (SD) of Total and Differential WBC Counts*

	−2 *SD*	−1 *SD*	Median	+1 *SD*	+2 *SD*
Total WBC, 10^3/mm^3	2.28 (0.70, 7.41)	2.80 (0.86, 9.08)	3.43 (1.06, 11.12)	4.19 (1.29, 13.61)	5.14 (1.58, 16.67)
Neutrophils, 10^3/mm^3	2.26 (0.70, 7.33)	2.82 (0.87, 9.14)	3.51 (1.08, 11.39)	4.38 (1.35, 14.20)	5.46 (1.68, 17.70)
Lymphocytes, 100/mm^3	3.42 (0.99, 11.76)	3.50 (1.02, 12.04)	3.58 (1.04, 12.31)	3.67 (1.07, 12.63)	3.76 (1.09, 12.94)
Monocytes, 100/mm^3	2.68 (0.81, 8.94)	3.11 (0.93, 10.35)	3.60 (1.08, 11.98)	4.16 (1.25, 13.87)	4.82 (1.45, 16.05)
Eosinophils, 100/mm^3	3.32 (0.97, 11.36)	3.35 (0.98, 11.45)	3.59 (1.05, 12.26)	3.84 (1.12, 13.13)	4.11 (1.20, 14.05)
Basophils 100/mm^3	3.51 (1.02, 12.07)	3.54 (1.03, 12.19)	3.61 (1.05, 12.42)	3.68 (1.07, 12.66)	3.75 (1.09, 12.91)

Notes: *Predicted from the adjusted model for participants who were 78 years of age, nonwhite, and current smokers.
IL-6 = interleukin-6; WBC = white blood cell.

both in crude analyses and in multivariate analyses after adjusting for age, race, and smoking status (Table 4). Among the WBC subpopulations, neutrophils, which account for most of the total WBC count, had the strongest association (Table 4, Figure 1B).

The lack of association of lymphocyte count with IL-6 levels is somewhat surprising, because lymphocytes are known to produce IL-6 in vitro (3,22,23). One possible explanation is that lymphocytes are heterogeneous, with many functionally different subpopulations. For example, regulatory T cells, such as $CD4^+CD25^+$ T cells, inhibit cytokine production by $CD8^+$ T cells (24,25), which may suppress IL-6 production by other lymphocyte subsets. Studies to further investigate relationships of specific lymphocyte subpopulations, and their cytokine production and regulation, with circulating IL-6 levels are indicated.

This study has several limitations. Although we vigorously excluded participants with values of total and differential WBC counts above the clinically defined normal range and influential outliers, potential contributions from acute or subacute infections that did not cause WBC count to increase beyond the normal range could not be completely eliminated. In addition, we could not determine causal directionality of the identified associations in this cross-sectional study. Another interpretation of our findings is that elevated IL-6 levels cause the increase in total WBC, neutrophil, monocyte, and eosinophil counts. Further studies, including longitudinal analyses, are needed to determine the causal directionality of these associations. Blood samples were not available for approximately one third of participants in WHAS I, and these participants were older and reported frailer health than did those who provided blood samples. However, the identified associations of IL-6 with total and differential WBC counts were not appreciably affected by adjustment for self-reported health status (data not shown) or age. Therefore, it seems unlikely that the incomplete assessment of the entire WHAS I cohort qualitatively invalidates our findings. Finally, we could not directly address the physiologic source of circulating IL-6 in this epidemiologic study. Although WBC and its specific subpopulations are known potent IL-6 producers, other cell types, including adipocytes and endothelial cells, also produce IL-6. Despite

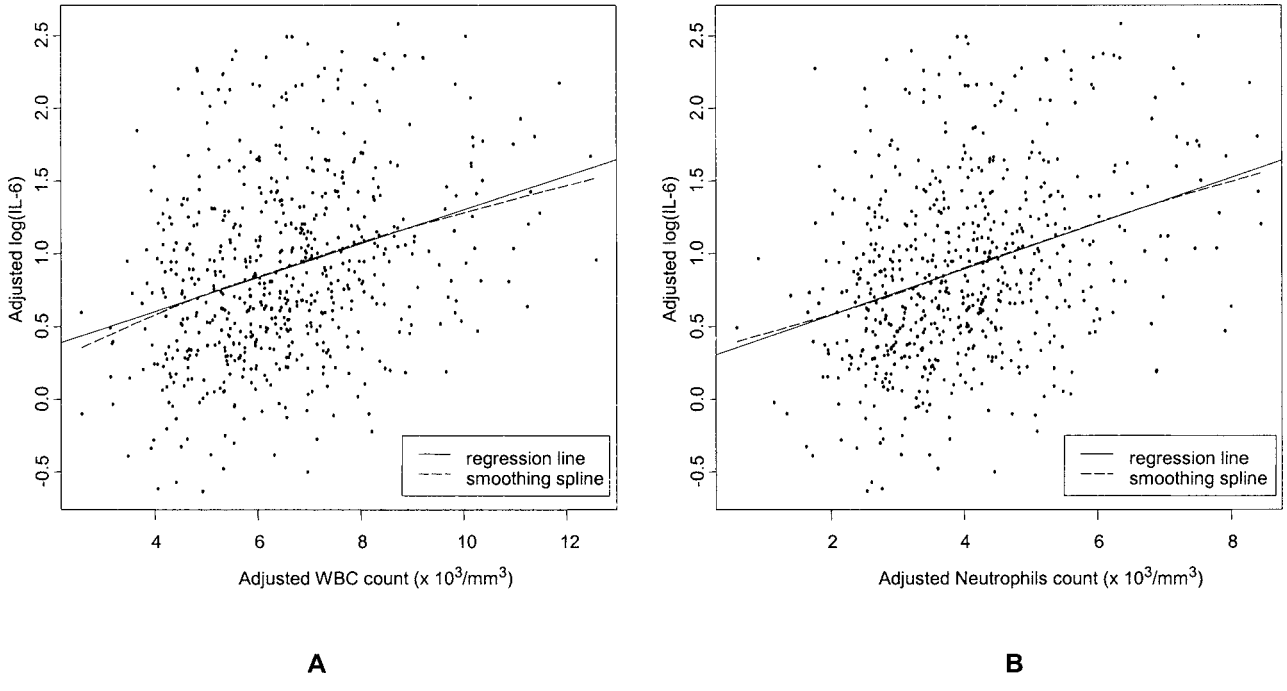

Figure 1. A scatterplot with smooth splines and regression lines shows, respectively, the observed and fitted relationship of **(A)** total white blood cells (WBC) or **(B)** neutrophil counts with log IL-6, adjusting for age, race, and smoking status, from The Women's Health and Aging Study I.

these limitations, results from this study do support our original hypothesis and suggest a potential role for WBC and its specific subpopulations in the production and regulation of IL-6 in older adults. This initial evidence provides a basis for further investigation of the contributions of WBC and its subpopulations to circulating IL-6 levels and potential effect from chronic elevation of IL-6 levels to the function of these circulating immune cells.

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