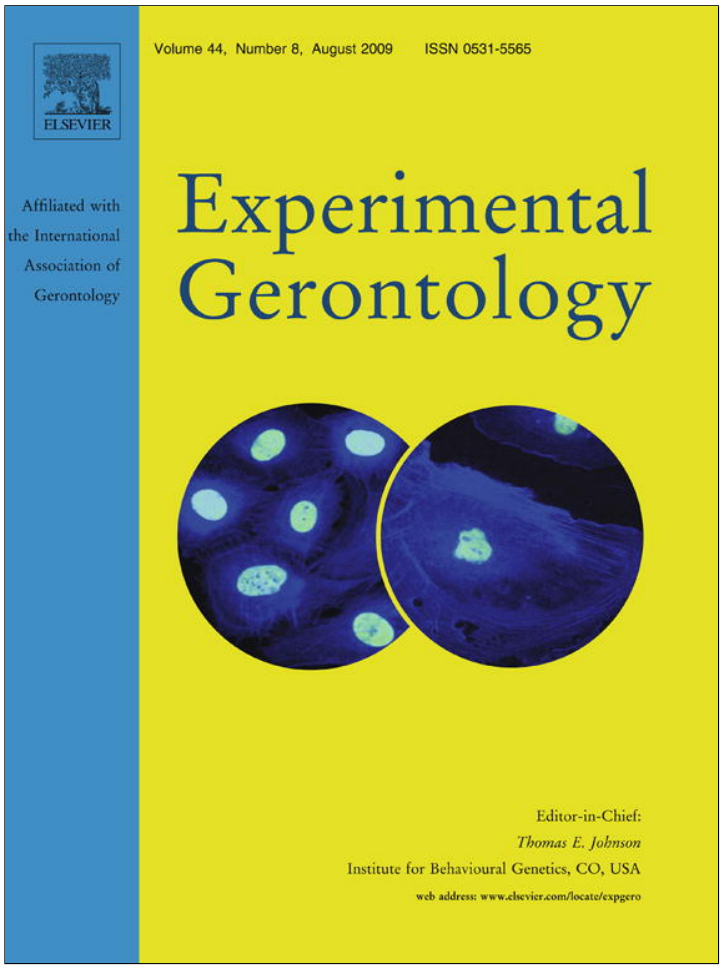

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Associations of neutrophil and monocyte counts with frailty in community-dwelling disabled older women: Results from the Women's Health and Aging Studies I

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ABSTRACT

Frailty is an important geriatric syndrome that predicts disability and mortality. Substantial evidence suggests that inflammation marked by elevated IL-6 levels and total white blood cell (WBC) counts contribute to this syndrome. However, the relationships of WBC subpopulations, the important inflammatory and immune cells, with frailty have not been investigated. To address this important question, we conducted cross-sectional polytomous logistic regression analyses evaluating associations between baseline WBC differential counts and prevalent frailty (defined by the validated Fried's criteria) of 558 disabled women aged 65–101 years and 548 women aged 70–79 living in the community, both from the Women's Health and Aging Studies. The results showed that high neutrophil and monocyte counts were associated with frailty in disabled older women, albeit these associations did not reach statistical significance in women aged 70–79, adjusting for age, race, education, body mass index, smoking, and antibiotic use. In addition, the identified associations were independent of IL-6. No significant associations of lymphocyte, eosinophil, or basophil counts with frailty were observed. These findings provide initial insight into potential roles of neutrophils and monocytes in the pathogenesis of frailty and a basis for further investigation into their function and regulation in frail older women.

1. Introduction

Frailty is an important geriatric syndrome characterized by multisystem dysregulations, decreased physiologic reserve, and increased vulnerability for serious adverse health outcomes including disability, dependency, and mortality (Fried et al., 2001, 2005; Fried and Walston, 2007; Lipsitz, 2002). Substantial evidence suggests that chronic systemic inflammation is a cardinal pathophysiologic feature of the frailty syndrome (Fried et al., 2005; De et al., 2006; Maggio et al., 2006; Leng et al., 2002; Schmaltz et al., 2005; Walston et al., 2002). For example, frailty is associated with elevated levels of interleukin-6 (IL-6) and C-reactive protein (CRP) (Maggio et al., 2006; Leng et al., 2002; Schmaltz et al., 2005; Walston et al., 2002). In addition, studies have shown that circulating IL-6 levels have inverse associations with insulin-like growth factor-1 (IGF-1) levels and hemoglobin concentrations in frail older adults; low IGF-1 levels and low hemoglobin concentrations (and anemia) are each individually associated with frailty, as well (Leng et al., 2002, 2004b; Chaves et al. 2005). These studies and others suggest that chronic systemic inflammation may contribute directly or through other physiologic dysregulations to the pathogenesis of frailty (De et al., 2006; Ferrucci et al., 2002; Paganelli et al., 2006; Barbieri et al., 2003; Payette et al., 2003). However, limited data is available on the cellular basis of this frailty-associated inflammatory state.

White blood cell (WBC) and its subpopulations are important circulating cells of the inflammation and immune systems. It has been shown that increased WBC counts are associated with cardiovascular, cancer, and all-cause mortality (Grimm et al., 1985; de Labry et al., 1990; Ruggiero et al., 2007; Grau et al., 2004). Recently, we have shown that increased total WBC counts, particularly increased neutrophil counts and decreased lymphocyte counts at baseline have significant predictive value for 5-yr mortality in community-dwelling older women (Leng et al., 2005b). In the syndrome of frailty, we have demonstrated that total WBC counts and IL-6 levels have independent associations with this clinical phenotype (Leng et al., 2007). However, relationships of counts of WBC subpopulations (neutrophils, monocytes, lymphocytes, eosinophils, and basophils) with frailty have not been investigated.

The objective of this study was to evaluate our hypothesis that counts of neutrophils and monocytes, the specific WBC subpopulations, would have independent associations with frailty. Address-

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ing this hypothesis will help to advance our knowledge about the cellular basis of frailty-associated inflammatory state. It will also provide the rationale for further investigation into the function and regulation of specific WBC subpopulations in frail older adults. To test this hypothesis, we conducted cross-sectional analyses of data from the Women's Health and Aging Studies I and II, evaluating the associations of each of the WBC differential counts with prevalent frailty, individually as well as in combination with IL-6 levels.

2. Methods

2.1. Study population

Subjects in this study were older women who participated in the WHAS I and II, two population-based studies designed to evaluate the causes and course of physical disability in community-dwelling older women. Details of the study methods and sampling design of the WHAS studies have been described elsewhere (Guralnik et al., 1995; Fried et al., 2000). Participants in WHAS I were 1002 in total, representing community-dwelling women aged 65–101 years with disability in 2 or more domains of the 4 physical function domains (mobility, upper extremity function, higher-functioning household management, and self-care). Participants in WHAS II were 436 in total, representing highly functioning women aged 70–79 years living in the community. The Johns Hopkins Medical Institutions institutional review board approved the research protocols. Written informed consent was obtained from all participants.

A total of 635 WHAS I participants had complete data on total and differential WBC counts, IL-6 level, and frailty measures. Of them, 4 participants with missing data on education (indicator for socioeconomic status, an important potential confounding factor) and 41 with missing body mass index (BMI) were excluded. To minimize potential influence of acute bacterial infection or hematological cancer, 8 participants with a total WBC count of more than $12 \times 10^3/mm^3$ or the percentile of neutrophils more than 80% and 1 participant with an outlier value of monocyte count greater than $15{,}000/mm^3$ were excluded. Women with a diagnosis of hematological malignancy or undergoing chemotherapy ($N = 23$) were also excluded, yielding the final sample size of 558 WHAS I subjects for this study.

To explore if the results from WHAS I participants would apply to older women in a younger age range with a majority of highly functioning individuals, the same analyses were performed in a merged data set from both WHAS I and II cohorts limited to the age range of 70–79 years. The same exclusion criteria were applied, yielding a comparable sample size of 548 subjects (219 subjects from WHAS I and 329 subjects from WHAS II) for the repeated analyses. This merged data set was employed in our previous study (Leng et al., 2007) and other studies (Chaves et al., 2005; Schmaltz et al., 2005).

2.2. Determination of frailty status

Participants were categorized as frail, pre-frail, and non-frail according to validated and widely utilized frailty screening criteria (Fried et al., 2001, 2005; Woods et al., 2005; Strandberg and Pitkala, 2007). These criteria are based on the presence or absence of five measurable characteristics: slowed motor performance (by walking speed), weakness (by grip strength), low physical activity level, weight loss, and self-reported exhaustion (Fried et al., 2001). Individuals with a critical mass of three or more of the five components were defined as frail, those with one or two components as pre-frail, and those with none of the components as non-frail.

2.3. Measurements of IL-6 levels and WBC differential 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 (Leng et al., 2007; Schmaltz et al., 2005). WBC differential counts were obtained using a Coulter counter in a well-standardized commercial laboratory.

2.4. Statistical analysis

Summary statistics were constructed for comparing baseline characteristics of the original WHAS I cohort ($N = 1002$) with a subset of 558 women used in the analyses. Distributions of sociodemographic and health characteristics, IL-6 levels, and WBC total and differential counts were summarized according to frailty status at baseline. Polytomous logistic regression models were used to assess the effects of each of the WBC differential counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) on the risk of being frail or pre-frail versus non-frail cross-sectionally at baseline, with and without adjusting for IL-6 tertiles. For ease of interpretation, each of the WBC differential counts and IL-6 levels were modeled as tertiles in association with frailty. The tertiles were based on the combined WHAS I and WHAS II women aged 70–79. As secondary analyses, we also modeled neutrophil count, monocyte count, and log IL-6 levels as continuous predictors in the regression models. Stata 9.0 was used for model estimation and diagnostics (StataCorp, College Station, TX).

3. Results

Table 1 summarizes the baseline demographic and clinical characteristics of all study participants in the WHAS I cohorts and 558 participants included in this study. Compared to the 444 participants excluded from this study, the 558 participants included in the analysis were younger. There was no significant difference in race, education, cigarette smoking, body mass index (BMI), total number of medical diagnoses, use of antibiotics, self-reported health status, or frailty status between the two groups, suggesting that the participants included in this study is a representative substudy sample of the entire WHAS I cohort.

Table 2 reports study participants' demographic characteristics, WBC differential counts (counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils), and levels of IL-6 and CRP across frailty categories. There were significant or marginally significant differences in mean counts of neutrophils and monocytes and the percentages of their tertiles, with P-values ranging from <.01 to .06 for trend of stepwise increase (mean counts and percentages of top tertiles) or decrease (percentages of bottom tertiles) from non-frail to pre-frail and frail categories. Compared with non-frail participants, the pre-frail and frail participants were significantly older ($P < .01$), had less ideal body weight (either under weight or obese by BMI), and were less educated ($P < .01$). As expected, they also had higher levels of IL-6 ($P < .01$) and CRP ($P = .05$).

Fig. 1 shows that neutrophil counts (a) and monocyte counts (b) had positive cross-sectional associations with being pre-frail and frail. No significant association was identified between counts of lymphocytes, eosinophil, or basophile with prevalent frailty (data not shown).

The associations of neutrophil and monocyte counts with frailty and pre-frailty were examined through evaluating the odds ratios (ORs) of being frail or pre-frail versus non-frail across tertiles of neutrophil or monocyte counts, particularly in relationship with

Table 1
Selected baseline demographic and health characteristics of participants evaluated within the Women's Health and Aging Study I cohort (WHAS I).

	WHAS I (*N* = 1002)	Study Sub-sample (*N* = 558)	*P*-value*
Mean age (years)	78.3 (8.1)+	77.4 (7.8)	.02
Race (% white)	71.2	72.6	.6
Mean years of education	9.6 (5.1)	9.8 (3.7)	.7
Current smoker (%)	11.5	12.0	.8
Mean body mass index (kg/m^2)	28.3 (6.8)	28.8 (6.8)	.2
Mean total # chronic diseases(‡)	4.0 (1.7)	3.9 (1.6)	.9
Use of antibiotics (%)	2.8	3.1	.7
Self-reported health status (%)			0.5
Excellent or very good	18.5	18.1	
Fair or good	64.0	67.9	
Poor	17.4	14.0	
Frailty (%)			0.09
Non-frail	11.0	13.6	
Pre-frail	54.2	56.3	
Frail	34.8	30.1	

* *P* values were based on analysis of variance for continuous variables and χ^2 tests for categorical variables, for comparing the 558 women who were included in this study to those who were not (i.e. 1002 − 558).
+ Numbers in parentheses, standard deviation.
‡ The number of "definite" chronic conditions including coronary artery disease (angina pectoris and/or myocardial infarction), congestive heart failure, degenerative disc disease, spinal stenosis, hip fracture, osteoporosis, osteoarthritis (of knee, hip and hand), rheumatoid arthritis, stroke, Parkinson's disease, pulmonary disease, diabetes mellitus, peripheral arterial disease, and cancer.

Table 2
Baseline demographic characteristics and WBC differential counts of the study subjects across frailty categories in WHAS I.

	Non-frail (*N* = 76)	Pre-frail (*N* = 314)	Frail (*N* = 168)	*P**
Mean age (years)	72.8 (6.0) +	77.0(7.6)	80.0 (7.8)	<.01
Race (% white)	84.2	71.3	69.6	.05
Mean years of education	10.7 (4.0)	10.0 (3.6)	9.1 (3.6)	<.01
Current smoking (%)	11.8	11.8	12.5	.9
Body mass index (kg/m^2) (%)‡	27.2 (4.3)	29.6 (6.8)	27.9 (7.7)	<.01
<21.5	2.6	8.0	20.8	<.01
21.5–24.9	29.0	14.3	14.3	
25.0–29.9	46.1	36.0	26.8	
>30	22.4	41.7	38.1	
Use of antibiotics (%)	6.9	3.3	1.2	.07
Mean neutrophil count (1/mm^3)	3494 (1307)	3989 (1358)	4203 (1444)	<.01
Tertiles (%)				
Bottom (< 3249)	44.7	32.2	28.0	<.01
Middle (3249–4433)	39.5	33.2	33.3	
Top (>4433)	15.8	34.7	38.7	
Mean lymphocyte count (1/mm^3)	1797 (531)	1849 (591)	1851 (668)	.8
Tertiles (%)				
Bottom (<1545)	31.6	28.7	29.2	.7
Middle (1545–2050)	25.0	32.8	34.5	
Top (>2050)	43.4	38.6	36.3	
Mean monocyte count (1/mm^3)	402 (179)	455 (185)	463 (187)	.04
Tertiles (%)				
Bottom (<352)	47.4	30.3	31.7	.06
Middle (352–504)	26.3	31.9	37.9	
Top (>504)	30.4	33.3	36.3	
Mean eosinophil count (1/mm^3)	135 (105)	151 (110)	154 (113)	.4
Tertiles (%)				
Bottom (<90)	52.6	39.2	40.5	.2
Middle (90–162)	30.3	33.8	31.0	
Top (>162)	17.1	27.1	28.6	
Mean basophil count (1/mm^3)	47 (28)	48 (30)	51 (31)	.5
Tertiles (%)				
Lower (<32)	27.6	32.8	25.6	.1
Middle (32–53)	40.8	29.3	38.7	
Top (>53)	31.6	37.9	35.7	
Mean IL-6 level (pg/ml) §	2.8 (2.0)	3.9 (2.0)	4.4 (2.1)	<.01
Mean CRP(pg/ml) §	3.5 (2.0)	4.5 (2.3)	4.7 (2.7)	0.05

* *P*-values were determined using Jonckheere–Terpstra trend test.
+ Numbers in parentheses, standard deviation.
‡ Body mass index was considered a categorical variable as defined and was adjusted in all of the regression models.
§ Geometric means and geometric standard deviations are presented for IL-6 and CRP.

levels of IL-6, an important pro-inflammatory cytokine with known association with frailty (Maggio et al., 2006; Leng et al., 2002, 2007). Women with neutrophil or monocyte counts in the top-tertile were significantly more likely to be pre-frail (OR = 4.13, 95% confidence interval [95% CI] = 1.88–9.17, and OR = 2.60, 95% CI = 1.34–5.05, respectively) or frail (OR = 6.20, 95% CI = 2.59–14.81, and OR = 2.78, 95% CI = 1.31–5.89, respectively) than those with neutrophil or monocyte counts in the bottom tertile, after adjusting for age, race, education, BMI, smoking, and use of antibiotics (Table 3, Model I). As reported previously, women with IL-6 levels in the top-tertile were significantly more likely to be pre-frail (OR = 2.87, 95% CI = 1.37–6.04) or frail (OR = 4.08, 95% CI = 1.78–9.33) than those with IL-6 levels in the bottom tertile, after controlling for the same potential confounders (Table 3, Model II). When neutrophil and IL-6 tertiles were entered in the same model, the ORs for association between top-tertile of neutrophil counts or IL-6 levels with frailty, independently, were 4.66 (95% CI = 1.86–11.62) and 2.40 (95% CI = 1.00–5.76), respectively. The association of the top-tertile of neutrophil counts with pre-frailty remained statistically significant (OR = 3.33, 95% CI = 1.45–7.63) while that of the top-tertile of IL-6 levels was not (Table 3, Model III). Similarly, when monocyte and IL-6 tertiles were entered in the same model, the ORs for association between top-tertile of monocyte counts or IL-6 levels with frailty, independently, were 2.16 (95% CI = 1.00–4.67) and 3.45 (95% CI = 1.48–8.01), respectively. The associations of the top tertiles of both monocyte counts and IL-6 levels with pre-frailty also remained statistically significant (OR = 2.15, 95% CI = 1.08–4.26 and OR = 2.42, 95% CI = 1.13–5.17, respectively, Table 3, Model III). In addition, when tertiles of CRP, another inflammatory marker with known association with frailty (Walston et al., 2002) and available data in the WHAS I dataset, were entered in Model III, the associations of the top tertiles of both neutrophil and monocyte counts with frailty remained statistically significant (OR = 6.45, 95% CI = 2.48–16.76, and OR = 2.50, 95% CI = 1.15–5.45, respectively).

To further assess the strength of these associations using the actual counts of neutrophils and monocytes as continuous predictors, ORs of being frail or pre-frail versus non-frail were evaluated for every one standard deviation unit increase in counts of neutrophils or monocytes. As shown in Table 4, one standard deviation unit increase in neutrophil or monocyte counts was associated with significant risks of being pre-frail (OR = 1.74, 95% CI = 1.26–2.40, or OR = 1.52, 95% CI = 1.12–2.06, respectively) and frail (OR = 2.17, 95% CI = 1.52–3.10, or OR = 1.70, 95% CI = 1.22–2.37, respectively), after adjusting for age, race, education, BMI, smoking, and use of antibiotics (Model I). When neutrophil or monocyte counts were entered in the same model with log IL-6 levels, risks of being pre-frail or frail associated with one standard deviation unit increase in neutrophil or monocyte counts remained significant (pre-frail: OR = 1.60, 95% CI = 1.14–2.26, or OR = 1.42, 95% CI = 1.04–1.93, respectively; frail: OR = 1.90, 95% CI = 1.30–2.77 or OR = 1.54, 95% CI = 1.09–2.16, respectively), after adjusting for the same potential confounders (Model III).

Finally, we explored if the above results would apply to community-dwelling older women in a younger age range and with the majority of highly functioning individuals through conducting the same analyses in 548 participants from the merged WHAS I

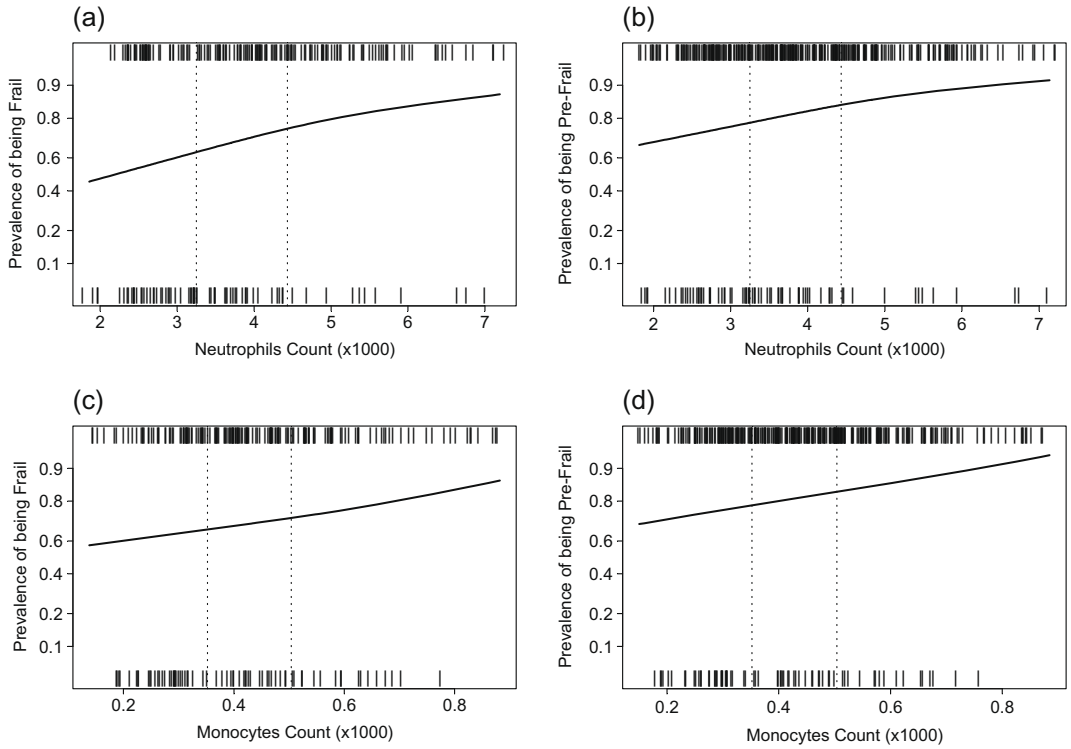

Fig. 1. Cross-sectional associations of counts of neutrophils (a and b) and monocytes (c and d) with prevalence of frailty (a and c) and pre-frailty (b and d) in WHAS I. Each hash mark (|) along the top and bottom axes represents a response of non-frail (bottom) versus pre-frail or frail (top). The solid curve shows the prevalence of being frail or pre-frail versus non-frail. The vertical dotted lines indicate tertiles. The curves were based on smooth splines with three degrees of freedom.

Table 3
Odds of being frail *vs* non-frail or pre-frail *vs* non-frail comparing top and middle tertiles with bottom tertile of counts of neutrophils or monocytes and IL-6 levels in the Women's Health and Aging Study I ($N = 558$)[#].

Analysis		Model I		Model II		Model III	
		Pre-frail	Frail	Pre-frail	Frail	Pre-frail	Frail
Odds ratio (95% confidence interval)							
Neutrophil counts	Mid-tertile	1.17 (0.62, 2.21)	1.42 (0.68, 2.96)			1.12 (0.59, 2.11)	1.33 (0.63, 2.79)
	Top-tertile	4.13[+] (1.88, 9.17)	6.20[+] (2.59, 14.81)			3.33[+] (1.45, 7.63)	4.66[+] (1.86, 11.62)
IL-6 levels	Mid-tertile			1.57 (0.83, 2.95)	2.51[*] (1.21, 5.21)	1.34 (0.70, 2.56)	2.05 (0.97, 4.32)
	Top-tertile			2.87[+] (1.37, 6.04)	4.08[+] (1.78, 9.33)	1.85 (0.84, 4.08)	2.40[*] (1.00, 5.76)
Monocyte counts	Mid-tertile	1.89 (0.97, 3.70)	2.12 (0.99, 4.52)			1.82 (0.92, 3.60)	1.95 (0.90, 4.22)
	Top-tertile	2.60[+] (1.34, 5.05)	2.78[+] (1.31, 5.89)			2.15[*] (1.08, 4.26)	2.16[*] (1.00, 4.67)
IL-6 levels	Mid-tertile			1.57 (0.83, 2.95)	2.51[*] (1.21, 5.21)	1.37 (0.72, 2.63)	2.20 ([*]) (1.04, 4.63)
	Top-tertile			2.87[+] (1.37, 6.04)	4.08[+] (1.78, 9.33)	2.42([*]) (1.13, 5.17)	3.45 ([+]) (1.48, 8.01)

[#] Adjusted for age, race, education, BMI, smoking status, and use of antibiotics.
[*] *P*-value < 0.05.
[+] *P*-value < 0.01.

Table 4
Odds ratios (95% Confidence Interval) of being frail versus non-frail or pre-frail versus non-frail for one standard deviation increase in counts of neutrophils or monocytes or Log IL-6 levels in WHAS I[#,&].

	Model I		Model II		Model III	
	Pre-frail	Frail	Pre-frail	Frail	Pre-frail	Frail
Neutrophil counts	1.74[+] (1.26, 2.40)	2.17[+] (1.52, 3.10)			1.60[+] (1.14, 2.26)	1.90[+] (1.30, 2.77)
Log IL-6 levels			1.48[*] (1.08, 2.03)	1.79[+] (1.27, 2.53)	1.26 (0.90, 1.76)	1.44[*] (1.00, 2.07)
Monocyte counts	1.52[+] (1.12, 2.06)	1.70[+] (1.22, 2.37)			1.42[*] (1.04, 1.93)	1.54[*] (1.09, 2.16)
Log IL-6 levels			1.48[*] (1.08, 2.03)	1.79[+] (1.27, 2.53)	1.37 (0.99, 1.89)	1.63[+] (1.15, 2.31)

[#] Odds ratios presented in the table are for every one standard deviation unit increase in neutrophil or monocyte counts or log IL-6 level.
[&] Adjusted for age, race, education, BMI, smoking status, and use of antibiotics.
[*] *P*-value < 0.05.
[+] *P*-value < 0.01.

and II cohorts limited to the age range of 70–79 years. The results showed that associations of neutrophil, monocyte, or any other WBC differential counts with frailty did not reach statistical significance in this population (data not shown).

4. Discussion

In the present study, we have identified significant associations of neutrophil and monocyte counts with frailty in the WHAS I participants representing community-dwelling older women with a broad range of age and high prevalence of functional disability. The associations remained significant after minimizing potential influence from acute bacterial infection or hematological malignancy and adjusting for age, race, education, BMI, smoking, and antibiotic use. In addition, these associations are independent of IL-6 levels. However, these results may not be applicable to highly functioning women aged 70–79 years.

Frail older adults manifest an altered inflammatory state. We previously reported significant and independent associations of total WBC counts and IL-6 levels with frailty (Leng et al., 2007). Building on these findings, this study evaluated the relationships of individual differential WBC counts with frailty and identified, for the first time, significant associations of neutrophil and monocyte counts with frailty in community-dwelling disabled women. The significance of these results is evident by the fact that neutrophils and monocytes represent distinct WBC subpopulations with critically important functions in the inflammation and immune systems. For example, neutrophils, representing 40–70% of total WBC counts, play an important role in inflammation and innate immunity. Monocytes are critical in both innate and adaptive immunity. They also produce numerous cytokine mediators (also termed monokines) that perpetuate and regulate inflammation, including pro-inflammatory cytokine IL-6.

We have recently reported that counts of WBC and its subpopulations including neutrophils and monocytes are associated with circulating IL-6 levels (Leng et al., 2005a). In addition, monocytes and neutrophils are known to produce IL-6 *in vitro* (Maggio et al., 2006; Leng et al., 2004a). To evaluate whether the association between neutrophil or monocyte counts and frailty is mediated by elevated IL-6 levels, we included these cell counts and IL-6 levels in the same model (Model III, Tables 3 and 4). The observed independent associations between neutrophil or monocyte counts and frailty suggest that other mechanisms in addition to IL-6 may mediate such associations. Potential mechanisms include pro-inflammatory mediators other than IL-6 produced by neutrophils and monocytes that can initiate and perpetuate inflammatory pathways. This is supported, at least in part, by our recent studies demonstrating that purified monocytes had significant upregulation in unstimulated and lipopolysaccharide (LPS)-induced *ex vivo* expression of chemokine CXCL10 and other inflammatory pathway molecules in frailty (Qu et al., 2009a,b). However, due to the complexity of the biology of IL-6 and inflammation, more in-depth investigations including functional laboratory studies of neutrophils and monocytes from frail older adults are required in order to draw a definitive conclusion.

To further explore the applicability of our results from the WHAS I in highly functioning women aged 70–79 years, we performed the same analyses in the merged WHAS I and II dataset of older women aged 70–79 years with the majority being highly functioning individuals. While our previous study reported significant association between total WBC counts and frailty in this dataset (Leng et al., 2007), no significant associations of neutrophil or monocyte counts with frailty were observed in the dataset in the present study. Since the merged WHAS I and II dataset represents women in a narrower and younger age range with the majority being highly functioning individuals, it is possible that major factors (such as catastrophic life events) other than inflammation and its cellular components may contribute to prevalent frailty in this population. The prevalence of frailty in this dataset is much less than that in the WHAS I (55 or 10% *vs* 168 or 30%, respectively). The severity level of frailty (those with 4 or 5 of the frailty criteria *vs* those with only 3) is also less in the merged dataset (data not shown). In addition, the merged dataset has lower prevalence of cardiovascular diseases including angina and/or myocardium infarction (20.8% *vs* 30.5%) and congestive heart failure (4.4% *vs* 10.4%) which are known for their associations with elevated WBC differential counts (Grau et al., 2004; Grimm et al., 1985) and frailty (Newman et al., 2001). These differences can modify the relationships between frailty and WBC differential counts and potentially account for the different results in the merged dataset. Further studies including longitudinal evaluation of factors contributing to the development of frailty in the elderly within a younger age range are warranted.

The following limitations should be considered for this study. First, sample size of the subgroups in the analysis across tertiles of each WBC differential counts and frailty categories is relatively small and provide limited statistical power. Thus, analyses across tertiles, particularly when tertiles of IL-6 levels were added into the model (Table 3, Model III), might be under powered. Secondly, potential contributions from acute or subacute bacterial infections leading to elevated neutrophil or monocyte counts could not be completely eliminated. However, participants with values of total WBC counts or a percentage of neutrophil counts above the normal range were excluded to minimize such potential influence. Reported use of antibiotics was also adjusted in all data analyses. Lastly, as discussed earlier, the WHAS I cohort represents disabled older women living in the community with a broad range of age. As such, the generizability of our findings to older women in a younger age range and highly functioning, as well as to the general older adult population with both men and women included, will need to be further investigated. Despite these limitations, findings from this study do support our original hypothesis and provide initial insight into the potential roles of neutrophils and monocytes in the pathogenesis of frailty. They also provide a basis for further investigations into their function and regulation in frail older women.

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