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Lymphocyte sub-population cell counts are associated with the metabolic syndrome and its components in the Vietnam Experience Study

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Abstract

Objective: The metabolic syndrome (MetS) increases the risk of cardiovascular disease morbidity and mortality. MetS is also associated with increases in the number of circulating white blood cells. Lymphocyte sub-population counts have also been implicated in cardiovascular disease; this analysis will examine whether or not they are associated with MetS. **Methods:** Participants were 4255 Vietnam-era US veterans. From military service files, telephone interviews, and a medical examination, occupational, socio-demographic, and health data were collected. MetS was ascertained from: body mass index; fasting blood glucose or a diabetes medication; blood pressure or a diagnosis of hypertension; HDL cholesterol; and triglyceride levels. Circulating T, T4, T8 and B lymphocytes cell numbers were determined by flow cytometry. **Results:** In fully adjusted logistic regression analyses, high lymphocyte sub-population counts were associated with an increased risk of MetS: T cells, OR = 2.68, 95%CI 1.99 – 3.61, $p < .001$; T4 cells, OR = 2.37, 95%CI 1.78 – 3.15, $p < .001$; T8 cells, OR = 1.79, 95%CI 1.43 – 2.24, $p < .001$; B cells, OR = 1.82, 95%CI 1.51 – 2.19, $p < .001$. High lymphocyte sub-population numbers were also associated with an increased likelihood of possessing each of the MetS components, as well as the number of components possessed. **Conclusions:** These results extend previous research which has largely been confined to total white blood cell or overall lymphocyte counts. If the present associations arise in prospective research, it is possible that simple lymphocyte cell counts could provide an additional prognostic indicator of risk for MetS.

Key words: B lymphocytes, T lymphocytes, Metabolic Syndrome

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Introduction

Numerous prospective studies now attest to an association between higher total white blood cell count, a marker of inflammation, and subsequent cardiovascular disease morbidity and mortality (1-8). This association was confirmed in a meta-analysis of individual-participant data (9). Additionally, we have recently shown that high lymphocyte sub-population counts predict cardiovascular disease mortality (Phillips et al., in submission).

An increased total white blood cell count would also seem to increase the risk of metabolic syndrome (MetS) (10, 11) and its components (12-15). MetS is a diagnosis based on having three or more of the following: obesity, high triglycerides, low HDL cholesterol, raised blood pressure, and high fasting blood glucose or a diagnosis of diabetes) which increases the risk of cardiovascular disease (16-19). Although the link between total white blood cell count and the MetS has been well explored, the association between circulating lymphocytes, and particularly lymphocyte sub-populations, and MetS has received scant attention. Three studies have reported a positive relationship between the total number of circulating lymphocytes and the number of MetS components (12, 14, 15) and one study has found a positive association between overall lymphocyte numbers and a diagnosis of MetS in men but not women (12). However, this latter study was modest in terms of sample size, and only 51 participants were identified as having MetS. Finally, there is also one report of a positive association between T4 cell numbers and the number of MetS components (15).

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Given this paucity of data, the present study examined the association of circulating lymphocyte sub-population counts and MetS. It is now acknowledged that cardiovascular disease is associated with altered cellular immunity (20) and that lymphocyte sub-population numbers can be markers of altered cellular immune status (15). Accordingly, it was hypothesised that higher lymphocyte sub-population numbers would be associated with an increased likelihood of MetS and many of its component symptoms, as well as with an increase in the number of MetS components possessed.

Materials and Methods

Sample

Participants were Vietnam era military veterans. The effective sample size was 4255. Ethical approval for the study was given by various bodies, including the US Centers for Disease Control and participants gave informed consent. Details of sampling at each stage of data collection are described in detail elsewhere (21). Inclusion criteria were: entered military service between January 1, 1965 and December 31, 1971; served only one term of enlistment and at least 16 weeks of active duty; earned a military specialty other than "trainee" or "duty soldier"; had a military pay grade at discharge no higher than sergeant.

Data collection

Information on place of service and ethnicity was extracted from the military archives. From a telephone survey in 1985, socio-economic position was measured using

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household income in midlife and the grade from which participants left school. Alcohol consumption, smoking habits, and marital status were ascertained using standard questions. In 1986, participants underwent a thorough 3-day medical examination. Mean age at medical examination was 38.3 yr. (range: 31.1 to 49.0). Participants fasted from 7 pm on the previous evening until blood was drawn the following morning. From the fasted blood sample, triglycerides and cholesterol fractions were assessed using a Kodak Ektachem 700 autoanalyzer (22, 23). Serum glucose level was determined with an adaptation of the glucose oxidase-peroxidase-chromogen-coupled system (22, 23). Blood pressure was measured twice in the right arm using a sphygmomanometer and an average computed. Height and weight were measured to calculate body mass index (BMI, kg/m^2). Current medication status was also determined at the medical examination.

MetS was defined as having at least three of the following characteristics: BMI > 30 kg/m^2 (in the absence of waist circumference data, BMI at this threshold is regarded by World Health Organization as an acceptable substitute in defining MetS); triglycerides ≥ 1.7 mmol/l (150 mg/dl); HDL cholesterol < 1.036 mmol/l (40 mg/dl); blood pressure ≥ 130/85 mmHg or taking antihypertensive medication; fasting glucose ≥ 6.1 mmol/l (110 mg/dl) or taking diabetes medication. All laboratory assays were assured by using bench and blind repeat controls. In 677 randomly chosen samples repeat sample correlations exceeded 0.98. Bench controls yielded coefficients of variation that were all < 10%.

Peripheral blood mononuclear cells were stained with fluorescent tagged monoclonal antibodies. Antibodies used were: OKT3 for T lymphocytes; OKT4A for T4 lymphocytes; OKT8 for T8 lymphocytes; CCB1 for B lymphocytes. The percentages of

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mononuclear cells that fluoresced were determined by flow cytometry. Absolute counts were calculated from the proportion of mononuclear cells in a given sub-population and the total lymphocytes per ml of blood, as determined by microscopic differential counts performed on whole blood smears. The laboratory assays were assured by using bench and blind repeat controls. As above, the bench controls yielded coefficients of variation that were all < 10%. The blind repeat tests were run for one in 20 randomly chosen samples; the correlations between first and repeat samples for the four lymphocyte subsets ranged from .93 to .97.

Statistical analysis

The lymphocyte sub-population counts were not normally distributed and were, accordingly, subject to natural log-transformation. Demographic, service, health behaviour, metabolic, and haemodynamic variables were compared between those with and without MetS using χ^2 and ANOVAs. Logistic regression was used to examine the relationships between lymphocyte counts and MetS, first in age-adjusted analyses and then in analyses additionally adjusting for place of service, ethnicity, marital status, alcohol consumption, smoking, household income, and education grade. The association between lymphocyte counts and the individual MetS components was examined in further fully adjusted models. Linear regression, with full adjustment, was used to test the relationship between circulating lymphocytes cell counts and the number of MetS components participants possessed.

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Results

Five hundred and eighty-four (14%) of the men were identified as having MetS. Aside from differing on all the components of MetS, participants with MetS were slightly older, tended to have a briefer education, were less likely to be divorced, widowed or separated and more likely to come from ethnic groups other than white or black (Table 1). The unadjusted lymphocyte sub-population cell counts for those with and without MetS are also presented.

In age-adjusted logistic regression analyses, men with higher T lymphocyte cell counts were more likely to exhibit MetS, OR = 2.50, 95%CI 1.90 – 3.28, $p < .001$. The same was true for T4, OR = 2.20, 95%CI 1.70 – 2.86, $p < .001$, and T8, OR = 1.80, 95%CI 1.45 – 2.24, $p < .001$, sub-populations. Higher circulating B cell counts were also associated with a greater likelihood of MetS, OR = 1.80, 95%CI 1.51 – 2.15, $p < .001$. The associations were undiminished in the fully adjusted analyses, as shown in Table 2. As might be anticipated from Table 1, the other predictors of MetS in these models were ethnicity, years in education, and marital status. In addition, those with the lowest household income in mid-life were more likely to have MetS.

One thousand and eighty-four of the veterans met the criteria for hypertension. Higher T cell, T4 cell, T8 cell and B cell counts were associated with an increased risk of hypertension in fully adjusted models (see Table 2). There were 550 participants categorized as obese and the same held true for obesity in fully adjusted analyses (see Table 2). Eight hundred and nine of the veterans had high triglyceride values. Again, as shown in Table 2, lymphocyte cell numbers positively predicted triglyceride status for T,

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T4, T8, and B cells. Low HDL cholesterol values were evident in 1656 of the participants and were also more common among those with high lymphocyte subset counts, as shown in Table 2. Finally, 207 participants had fasting glucose ≥ 6.1 mmol/l (110 mg/dl) or taking diabetes medication. There were no associations between T cells or T cell sub-populations and high fasting glucose/diabetes. However, there was a small positive relationship with circulating B cell numbers, OR = 1.21, 95%CI 1.02 – 1.43, p = .03.

The average numbers of MetS components in this sample was 1.13 (SD = 1.19). In fully adjusted hierarchical linear regression analyses, higher circulating lymphocyte numbers were associated with a greater number of MetS components: for T cells, $\beta = .13$, $t = 8.70$, $p < .001$, $\Delta R^2 = .017$; for T4 cells, $\beta = .12$, $t = 8.13$, $p < .001$, $\Delta R^2 = .015$; for T8 cells, $\beta = .09$, $t = 5.92$, $p < .001$, $\Delta R^2 = .008$; for B cells, $\beta = .14$, $t = 8.68$, $p < .001$, $\Delta R^2 = .017$.

The main analyses were re-visited excluding participants who reported a physician diagnosis of coronary heart disease at the telephone interview in 1985. This reduced the sample to 4199 and the number with MetS to 567 (again 14%). Higher circulating T cells, OR = 2.63, 95%CI 1.95 – 3.54, $p < .001$, T4 cells, OR = 2.26, 95%CI 1.70 – 3.02, $p < .001$, T8 cells, OR = 1.80, 95%CI 1.44 – 2.27, $p < .001$, and B cells, OR = 1.80, 95%CI 1.49 – 2.17, $p < .001$, were still strongly associated with an increased likelihood of having MetS in fully adjusted models. Analyses of the individual MetS components produced the same outcomes as those reported above. Finally, the same positive associations between lymphocyte sub-populations and the number of MetS components

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that were seen in the full sample emerged from fully adjusted linear regression analyses of this truncated sample: T cells, $\beta = .14$, $t = 8.62$, $p < .001$, $\Delta R^2 = .017$, T4 cells, $\beta = .13$, $t = 7.88$, $p < .001$, $\Delta R^2 = .014$, T8 cells, $\beta = .09$, $t = 6.02$, $p < .001$, $\Delta R^2 = .008$, B cells, $\beta = .13$, $t = 8.35$, $p < .001$, $\Delta R^2 = .016$.

Discussion

Higher circulating lymphocyte sub-population counts were associated with an increased likelihood of having MetS. This held true for all the sub-populations studied: overall T cells, T4 cells, T8 cells, and B cells. As such the present results extend the findings of earlier studies showing a positive association between overall white blood cell numbers and MetS (10, 24). High lymphocyte sub-population counts were also associated with an increased likelihood of possessing virtually all the component symptoms of MetS. The exception was high fasting glucose/diabetes, where only high B cell numbers were indicative. This again extends previous research showing that total white blood cells numbers are associated with the individual component symptoms of MetS (12-15). Only one study that we know of has examined the relationship between lymphocyte counts and MetS component symptoms (14); overall lymphocyte numbers were positively related to postprandial, but not fasting, glucose and to triglycerides. The present much larger scale study indicates that high numbers of the main lymphocyte sub-populations are also implicated in the aetiology of individual MetS components.

The focus of the few studies examining associations between lymphocytes and MetS has been on the relationship between overall lymphocyte counts and the number of MetS

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components exhibited by participants. A reasonable consensus is emerging that as circulating lymphocyte numbers increase so too does the number of MetS components (12, 14, 15). There is also one report of a positive association between T4 cell numbers and the number of MetS components (15). The present analyses indicate that increased numbers of all of the major sub-populations of lymphocytes are related to increased numbers of MetS components.

With cross-sectional analyses it is impossible to determine the direction of the association. It is possible that the associations observed in this study reflect the effects of inflammatory cardiovascular disease on lymphocyte traffic. There is evidence that T4 and T8 cells are among the first immune cells to infiltrate the arterial intima during the initial stages of atherosclerosis (20, 25). Further, both T4 and T8 cells have been linked to carotid intima-media thickness in a cross-sectional study (26). Although the same relationships emerged from analyses that excluded participants with diagnosed coronary heart disease, we cannot rule out the possibility that occult inflammatory disease underlies the associations observed in this study. Thus, probably the most parsimonious explanation for the present findings is that MetS can be regarded as a consequence of systemic inflammation, and high lymphocyte subset numbers, like high leukocyte numbers, are also markers of inflammation. Consequently, the definition of MetS might usefully be extended to the immune system and encompass high lymphocyte numbers as part of an expanded definition. Nevertheless, our previous findings in this cohort that high circulating T and B cells predicted death from cardiovascular disease during a 15-year follow-up (27) suggest that this direction of effect may not be the full story. It is,

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accordingly, also possible that high circulating lymphocyte numbers are not only implicated in the initiation and progression of inflammatory cardiovascular disease (20) but that, as they also increase the risk of developing MetS, there is a feasible pathway from increased lymphocyte numbers to MetS to cardiovascular disease. However, such inference must remain speculation as only prospective and experimental studies can resolve issues of causality. Finally, it is always possible that some unmeasured or poorly measured factor is driving the observed associations (28). However, we did adjust for more potential confounders than most previous studies.

The present study may have other limitations. First, the sample was exclusively male and this raises the issue of generalisation; these outcomes may not hold for women. In line with this, a previous study reported that higher circulating lymphocyte numbers characterised men, but not women, with MetS (14). However, this study tested just 162 women, only 18 of whom were diagnosed with MetS; thus, it would not have had sufficient power to detect, as statistically significant, effects of the magnitude observed in the present study. Our sample was also relatively young and the prevalence of MetS was low compared to studies of older populations (29). Nevertheless, this is more likely to have attenuated than exaggerated the magnitude of the present associations. The relative youth of the sample also increases the plausibility of a pathway from lymphocyte numbers to MetS since relatively few participants would have had diagnosed or undiagnosed inflammatory cardiovascular disease at this stage of life. Finally, in terms of the socio-demographic characteristics, the present sample was rather homogeneous. Nevertheless, even given range restriction in the sample, measures of socio-economic

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position, such as years in education and household income in mid-life, were associated in fully adjusted models with MetS in the expected direction. The present analyses, however, are not without their strengths. These include its large size, the very well characterised study population, and the rigour of the assays.

In conclusion, higher circulating lymphocyte sub-population numbers were associated with an increased likelihood of MetS, with virtually all of the components of MetS, and with the number of MetS components participants possessed. These novel findings in a large epidemiological study extend previous research which has largely been confined to total white blood cell numbers or to overall lymphocyte counts. They also suggest that the definition of MetS might be expanded to encompass immune system inflammatory markers such as lymphocyte subsets. Finally, if these associations were to emerge from prospective research, the further possibility is raised that simple lymphocyte cell counts could provide a prognostic indicator of risk for MetS.

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Table 1. Characteristics of participants with and without metabolic syndrome

	Metabolic syndrome (N = 584)		No metabolic syndrome (N = 3671)		*p*
	Mean	SD	Mean	SD	
Metabolic Syndrome Markers:					
BMI (kg/m^2)	30.73	4.15	25.17	3.04	<.001
Triglycerides (mg/dL)	228.16	216.14	96.98	65.03	<.001
HDL cholesterol (mg/dL)	34.64	7.83	46.26	12.28	<.001
SBP (mmHg)	133.33	12.50	121.36	11.11	<.001
DBP (mmHg)	91.85	9.29	82.88	8.84	<.001
Blood glucose (mg/dL)	106.75	30.79	92.33	12.58	<.001
Predictor Variables:					
T cells 10^3mm^3	1.68	0.55	1.53	0.51	<.001
T4 cells 10^3mm^3	1.15	0.38	1.05	0.36	<.001
T8 cells 10^3mm^3	0.66	0.32	0.60	0.26	<.001
B cells 10^3mm^3	0.31	0.17	0.26	0.14	<.001
Covariates:					
Age at medical examination (years)	38.74	2.52	38.26	2.51	<.001
Units of alcohol per week	7.28	16.78	7.05	14.01	.72
	N (%)		N (%)		*p*
Metabolic Syndrome Markers:					
Obese	347 (59)		203 (6)		<.001
Hypertension diagnosis	169 (29)		271 (7)		<.001

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Diabetes diagnosis		24 (4)	25 (1)	<.001
Covariates:				
Place of service	Ever in Vietnam	339 (58)	2009 (55)	.32
	Other overseas	142 (24)	953 (26)	
	US only	103 (18)	709 (19)	
Ethnicity	White	473 (81)	3017 (82)	.03
	Black	60 (10)	435 (12)	
	Other	51 (9)	219 (6)	
Household income in midlife	<$20,000	183 (31)	1018 (28)	.11
	-$40,000	289 (50)	1840 (50)	
	>$40,000	112 (19)	813 (22)	
Years in education	<11	93 (16)	419 (11)	.004
	-12	218 (37)	1346 (37)	
	>12	273 (47)	1906 (52)	
Smoking status	Non smoker	146 (25)	938 (26)	.96
	Ex smoker	166 (28)	1043 (28)	
	Current smoker	272 (47)	1690 (46)	
Marital status	Married	459 (79)	2672 (73)	.006
	Divorced/separated/widowed	79 (13)	688 (19)	
	Never married	46 (8)	314 (8)	

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Table 2: Fully-adjusted associations between lymphocyte subset counts and MetS.

Lymphocyte subsets	OR	95%CI	*p*
Metabolic Syndrome			
T cells	2.68	1.99 – 3.61	$p < .001$
T4 cells	2.37	1.78 – 3.15	$p < .001$
T8 cells	1.79	1.43 – 2.24	$p < .001$
B cells	1.82	1.51 – 2.19	$p < .001$
Hypertension			
T cells	1.46	1.16 – 1.83	$p = .001$
T4 cells	1.47	1.18 – 1.84	$p = .001$
T8 cells	1.20	1.00 – 1.43	$p = .05$
B cells	1.20	1.04 – 1.38	$p = .01$
Obesity			
T cells	2.36	1.73 – 3.20	$p < .001$
T4 cells	2.50	1.86 – 3.36	$p < .001$
T8 cells	1.41	1.10 – 1.78	$p = .004$
B cells	1.96	1.61 – 2.38	$p < .001$

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High triglycerides			
T cells	3.50	2.67 – 4.57	$p < .001$
T4 cells	3.02	2.33 – 3.90	$p < .001$
T8 cells	1.90	1.55 – 2.33	$p < .001$
B cells	1.64	1.40 – 1.93	$p < .001$
Low HDL			
T cells	2.09	1.69 – 2.57	$p < .001$
T4 cells	1.62	1.32 – 1.98	$p < .001$
T8 cells	1.58	1.34 – 1.86	$p < .001$
B cells	1.64	1.44 – 1.87	$p < .001$

NB The fully adjusted model adjusts for place of service, ethnicity, marital status, alcohol consumption, smoking, household income, and education grade.