Lymphocyte cell counts in middle age are positively associated with subsequent all-cause and cardiovascular mortality

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Abstract

**Background:** There is an association between higher white blood cell counts and all-cause and cardiovascular disease mortality. However, little is known about the prognostic significance of circulating lymphocyte and lymphocyte subset numbers.

**Aims:** The present study examined the association between T, CD4, CD8, and B cell numbers, and the CD4:CD8 ratio, and all-cause and cardiovascular disease mortality.

**Methods:** Lymphocyte and lymphocyte subset numbers were measured by flow cytometry in a cohort of 4256 male middle-aged Vietnam-era US veterans. Mortality was tracked for 15 years and cause of death was determined from death certificates.

**Results:** In fully-adjusted survival analyses, high circulating T cells numbers were associated with increased risk of both all-cause (HR = 1.75, 95%CI 1.15 – 2.66) and cardiovascular (HR = 3.57, 95%CI 1.53 – 8.33) mortality. The former association appeared to reflect an effect for high CD8 cells numbers, the latter an effect for high CD4 cell numbers. For all-cause mortality, a high CD4:CD8 ratio was protective (HR = 0.58, 95%CI 0.41 – 0.81) Cardiovascular mortality was also predicted by high B cells numbers (HR = 1.87, 95%CI 1.10 – 3.17).

**Conclusion:** Circulating lymphocyte and lymphocyte subset numbers may have substantial prognostic significance for both all-cause and cardiovascular disease mortality.

Keywords: all-cause mortality; B cells; cardiovascular mortality; lymphocytes, T cells

Acronyms: BMI = Body Mass Index; CVD = cardiovascular disease; ICD = International Classification of Diseases; IQ = Intelligence Quotient; SBP = systolic blood pressure;
Introduction

Prospective studies attest to an association between higher white blood cell count and all-cause and cardiovascular disease (1-8), confirmed in a meta-analysis of individual-participant data (9). However, much less is known about relationship between lymphocyte and lymphocyte subset cell counts and all-cause and, in particular, cardiovascular disease mortality. Two studies have reported that low total lymphocyte numbers were related to risk of death (10,11), whereas others have found no association (12,13). A low CD4:CD8 cell ratio has also been associated with increased mortality risk (14-16), although again null findings exist (12,13). However, previous studies on lymphocyte cell counts and survival have been conducted on the elderly; virtually nothing is known about lymphocyte numbers and mortality in middle-aged adult populations. Ageing can be characterised by a change in T cell numbers (14,15), increased circulating memory T cells, and a reduction in circulating naïve T cell numbers (16-18); so the prognostic significance of lymphocyte and lymphocyte sub-population counts may vary across the life course.

Previous studies have also largely confined their analyses to total lymphocyte numbers. Few have explored the prognostic significance of lymphocyte subsets, although there is a recent report that older adults’ CD8 cell numbers were positively associated with all-cause mortality across a three-year follow-up (13). Given that previous findings are scant and inconsistent, relate to older populations, and have for the most part restricted their analyses to total lymphocyte numbers and all-cause mortality, the present study examined the association between total T cell, CD4, CD8, and B cell numbers, and the CD4:CD8 ratio, and all-cause and cardiovascular mortality in a large sample of middle-aged male Vietnam-era veterans.
Methods

Study Design and Sample

This is a prospective cohort study. Participants were 4256 Vietnam-era military veterans. Ethical approval for the study was given by the US Centers for Disease Control. Sampling at each stage of data collection is described elsewhere (19,20). Inclusion criteria were: entered military service between January 1, 1965 and December 31, 1971; served only one term of enlistment; served at least 16 weeks of active duty; earned a military specialty other than “trainee”/“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 military archives. From a telephone survey in 1985, socio-economic position was measured using household income in midlife and educational grade achieved. Alcohol units consumed per week, smoking habits, and marital status were also ascertained. Participants were also asked whether or not they had a range of somatic physician-diagnosed health problems including hypertension, cancer, diabetes and coronary heart disease.

In 1986, participants underwent a medical examination. Mean age at medical examination was 38.3 yr. (range: 31.1 to 49.0). Participants fasted from 7pm on the evening before the examination until blood was drawn the following morning. Peripheral blood mononuclear cells were stained with fluorescent tagged monoclonal antibodies. Antibodies used were: OKT3 for T lymphocytes; OKT4A for CD4 lymphocytes; OKT8 for CD8 lymphocytes; CCB1 for B lymphocytes. The percentages of 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. Total cholesterol level was ascertained
using a Kodak Ektachem 700 autoanalyzer. The laboratory assays were assured by using bench
and blind repeat controls. 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. Bench controls yielded coefficients of variation that were all < 10%.

With the participant in a sitting position, a registered nurse, using a standard mercury
sphygmomanometer measured blood pressure twice consecutively, from both arms. For analysis,
an average of the two right arm systolic and diastolic blood pressure values was computed.
Measurements from the left arm were used to verify individual results. At the medical
examination, participants also completed the Army General Technical Test, from which a
cognitive ability score, hereafter referred to as IQ, was derived.

Vital status post-medical examination was tracked until 31st December 2000 using a variety of
databases supplied by the US army, the Veterans Administration (Beneficiary Identification and
Records Locator Subsystem), the Social Security Administration, the Internal Revenue Service,
and the National Center for Health Statistics (National Death Index). Mortality due to major
cardiovascular disease (CVD) was classified using the International Classification of Diseases
rheumatic fever; chronic rheumatic heart diseases; hypertensive diseases; ischaemic heart
diseases; pulmonary heart disease and diseases of pulmonary circulation; other forms of heart
disease; cerebrovascular diseases; diseases of arteries, arterioles and capillaries. The CVD
mortality variable thus encompasses death from a variety of disorders; the bulk of the deaths,
however, were from ischaemic heart diseases.
Statistical Analysis

As the lymphocyte sub-population absolute counts had a skewed distribution, they were subjected to natural logarithmic transformation. Simple comparisons of the social, clinical, and behavioural characteristics of those who had died during follow-up and those who had not, as well as those who had and had not died, were undertaken using \( \chi^2 \) and ANOVA. The main analyses tested various Cox proportional hazard models, with cell counts of the various lymphocyte sub-populations as the independent variables. Models that adjusted only for age were tested first, followed by models which additionally adjusted for service, demographic, traditional risk factor, health behaviour, cognitive ability, and chronic illness variables. These covariates have all been implicated in all-cause and/or cardiovascular mortality. Finally, sensitivity analyses were run excluding those who died in the first seven years following assessment in order to remove the likelihood of reverse causality and confounding. That is, if participants had undiagnosed disease at study entry, this would affect their lymphocyte values and their mortality risk. Thus, it may simply be occult disease at baseline that is causing elevated mortality risk. Removal of deaths in the first few years of follow-up means that men with existing undiagnosed illness are likely to have been excluded. This analysis, then, focuses on the long-term prognostic significance of lymphocyte counts.

Results

The baseline characteristics of the veterans who had died (\( N = 236 \)) or survived during the 15 years of follow-up are presented in Table 1. Those who had died had a higher systolic blood pressure, a lower IQ score, drank more alcohol per week, were more likely to be suffering from a chronic disease, be non-white, not married, a current smoker, have left school earlier, and have a
lower household income in midlife. The means (SDs) of the lymphocyte cell counts by mortality status are also presented in Table 1. Age-adjusted hazard models revealed that higher numbers of circulating T cells, CD4 cells, and CD8 cells were associated with all-cause mortality. Circulating B cell numbers were not related to all-cause mortality. A high CD4:CD8 ratio appeared protective. In models which adjusted for all the covariates in Table 1, there were still positive, albeit somewhat attenuated, associations between the overall number of T cells, and the number of CD8 cells, and all-cause mortality. A high CD4:CD8 cell ratio was still predictive of lower likelihood of death from all causes. The hazard ratios (HR) for these associations are shown in Table 2.

[Insert Tables 1 and 2 about here]

Participants who had died of CVD (N = 63) were slightly older, had a higher systolic blood pressure, higher cholesterol, a lower IQ, were more likely to be suffering from a chronic disease, be non-white, have left school earlier, and have a lower household income in midlife. Current smokers also tended to be more likely to have died of CVD. Age-adjusted analyses indicated that higher numbers of circulating T cells, CD4 cells, CD8 cells, and B cells were all associated with increased risk of CVD mortality. The association between the CD4:CD8 ratio and CVD mortality was not statistically significant. In the fully-adjusted models, the associations with CVD mortality were attenuated but remained statistically significant for circulating T cell, CD4 cell, and B cell numbers. The other predictors of CVD mortality in these models were age, cholesterol, and pre-existing chronic illness. The main associations between lymphocytes and CVD mortality are presented in Table 2.
Sensitivity analysis

The fully-adjusted hazard models were re-run excluding participants who had died in the first seven years of follow up. With few exceptions, much the same associations emerged as those reported above from the full analyses. High overall T cell, CD4 cell, and CD8 cell numbers were associated with an increased risk of death from all causes (N = 153). CVD mortality (N = 45) was predicted by higher T cell, CD4 cell, CD8 cell, and B cell numbers.

Discussion

In both age- and fully-adjusted survival analyses, higher circulating T cells numbers were positively associated with both all-cause and cardiovascular disease mortality. The relationship were stronger for cardiovascular mortality. In addition, whereas the association with all-cause mortality appeared to be largely driven by circulating CD8 cell numbers, the cardiovascular mortality association reflected a relationship with CD4 cell numbers. Cardiovascular mortality was also predicted by high B cells numbers. In sensitivity analyses that excluded deaths in the first seven years of follow-up, the hazard ratios in these truncated analyses were larger than those seen in the main analyses. There was also a significant negative association between the CD4:CD8 ratio and all-cause mortality, suggesting that higher ratios are protective. However, Figure 1 intimates a quadratic relationship, such the lowest death rates are found among those with CD4:CD8 ratios in the middle tertile. However, it should be appreciated that the figures are for illustrative purposes only as they are derived from non-adjusted analyses. Further, as the relationship was not evident in the sensitivity analyses, it is possible that the consequences of the CD4:CD8 ratio for all-cause mortality is short lived.
We know of no other studies that have examined the consequences of circulating lymphocyte numbers for all-cause and cardiovascular mortality in a middle-aged sample, with one exception where, similar to our findings, it was shown that total white blood cell count predicted increased risk of death or myocardial infarction (MI) (22). However, in contrast, total T-cell count was negatively associated with risk of death or MI (22), although this finding may reflect the strong predictive power of neutrophilia together with relative lymphopenia in these patients with coronary artery disease. It is also possible that in patients with existing coronary artery disease, the associations between cell counts and mortality differ in direction from those in a relatively healthy population such as in the present study. Previous studies of older adults have also yielded mixed results (10-13); few have examined lymphocyte subsets. There is one recent report that older adults’ CD8 cell numbers were positively associated with all-cause mortality across a three-year follow-up (13). However, the sample size was small and the association held for women but not men. High overall white blood cells counts are now recognised to be a risk factor for cardiovascular morbidity and mortality (9). Lymphocyte sub-populations have been rarely studied in this context, although high numbers of circulating T and B cells have been associated with increased intima-media thickness of the carotid artery in a cross-sectional study (23). This resonates with the current results showing that high T and B cell numbers were associated with a long-term increased risk of death from cardiovascular disease. Given that immunological and inflammatory processes are recognised as mechanisms involved in the aetiology and progression of atherosclerotic disease, further attention should be paid to prospective role of high levels of circulating lymphocytes, and their sub-populations. It is, however, difficult to disentangle cause and effect; increased circulating lymphocytes, by enhancing their migration into the arterial vessel wall, may contribute to the development and/or progression of atherosclerosis or, alternatively, increased numbers of circulating lymphocytes may be a function of nascent
atherosclerotic disease (23). Although we did adjust for diagnosed chronic disease, including cardiovascular disease, we cannot fully dismiss the possibility that early stage, but still occult, cardiovascular disease was driving the observed relationships between lymphocyte counts and subsequent cardiovascular disease mortality.

This study has other limitations. First, the sample was exclusively male and so the results may not be generalisable to women. Second, 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 position such as years in education and household income in midlife, strongly predicted all-cause in the expected manner. Third, in observational studies, the possibility of residual confounding can never be completely discounted. However, we did adjust for many potential confounders.

In conclusion, simple cell counts of circulating lymphocytes measured in middle age would appear to hold prognostic significance. High T cell numbers predicted all-cause mortality, an effect largely driven by high circulating CD4 cell numbers. High T, specifically high CD8 cell numbers, and B cell numbers were associated with death from cardiovascular mortality. As such, lymphocyte and lymphocyte subset counts could provide rich early warning information about the risk of premature death in general and from cardiovascular disease in particular.

Conflict of Interest
The authors have no conflicts of interest

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References


