Original Article

Nutritional indicators, peripheral blood lymphocyte subsets and survival in an institutionalised elderly population

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The objectives of this study were to determine the percentage and absolute counts of the peripheral blood lymphocyte subsets, and to examine the relationship between lymphocyte subsets and nutritional status, and total mortality in an institutionalised elderly population. *Design The study had a c*ross-sectional and observational design. The sample of 115 permanent elderly residents was drawn from large geriatric institution in Melbourne, Australia. The main outcome measures were as follows*:* (i) percentages and absolute counts of lymphocyte subsets, (ii) association between biochemical indices of nutritional status (ferritin, iron and zinc) and peripheral blood lymphocyte subsets, (iii) total mortality during a 22-month period in relation to baseline lymphocyte subset counts. Women had higher absolute counts of various lymphocyte subsets than men. Positive correlations of serum ferritin with the number of CD8 (T-suppressor cell) and of serum iron with CD56 (natural killer, NK cells) were observed in men. In women, serum zinc was positively correlated with the absolute counts of CD3 (total T-cells), CD4 (T-helper cell) and CD19 (total B-cell). The analysis of survival data after 22 months showed that the mean number of CD4 cells of non-survivors ($524 \pm 292 \times 10^6$ cells/L) was significantly lower than that of survivors ($759 \pm 292 \times 10^6$ cells/L). The biochemical indicators of iron and zinc status partly account for variations in lymphocyte subset counts, consistent with known effects of iron overload and of zinc deficiency on immunocompetence. The number of CD4 T-cells may be useful in the prediction of total mortality in an institutionalised elderly population.

Key Words: lymphocytes, T-cells, B-cells, natural killer cells, iron status, zinc status, survival, total mortality

Introduction

Many studies have demonstrated that undernutrition contributes to immunodeficiency in the elderly and that nutritional support improves immune responses.[1,2] The elderly represent a large expanding group with significant numbers of individuals possessing poor nutritional status. Comparative studies report that the institutionalised elderly are more nutritionally vulnerable than their free-living counterparts.[3-5] Restricted meal times may limit the capacity to 'graze' and where inadequate staffing may not allow sufficient time to assist those who cannot feed themselves. Nutritional inadequacy in the elderly could account for the impairment of several aspects of the immune system,[2,6] which could contribute to the development of nosocomial infection in the institutionalised elderly.[7-10] There is now good evidence that immunodeficiency is in part reversible by nutritional means, to the extent that it has been nutritionally caused.[1, 11]

The human host defends itself against toxins, foreign organisms or malignant cells in two ways, innate immu-nity, which provides barriers to infection and a rapid first line of defense with broad specificity, and adaptive immunity, which provides a very high degree of targeted specificity and 'memorisation' to enhance the speed of the response during any future re-infection.[12,13] Macrophages are particularly involved in the innate immunity, while lymphocytes in the adaptive immunity. Lymphocytes may be T (so named because they develop in the thymus gland), B (in reference to development in the bursal equivalent) and natural killer (NK) cells. B cell function is mediated by the types of antibodies produced. The main types of antibody molecules (immunoglobulin, Ig) are IgM, IgA and IgG. T cells are divided into two main subclasses: CD4 and CD8 cells. CD4 cells operate by secreting immunomodulatory cytokines that can promote T cell growth, facilitate antibody production by B cells, or activate macrophage functions. These CD4 T cells are frequently referred to as T helper

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cells. CD8 cells serve as cytotoxic cells that are able to recognise and kill other cells infected with intracellular pathogens, such as viruses.

Immunocompetence is likely to be a key factor predicting ultimate outcome in terms of morbidity and mortality,[14] especially in the elderly who are at risk for malnutrition. Failure to recognise or to anticipate the development of malnutrition can allow the needless presence of nutrition-related immunodeficiency and proneness to infection, with increased morbidity and mortality. A decline in immune function with age could contribute to the social problems, economic burden and need for medical services. A cross-sectional study of nutritional determinants of lymphocyte subsets and their possible contribution to survival was conducted in the nutritionally vulnerable institutionalised elderly.

Subjects and methods

Study population

Subjects were recruited from the Kingston Centre, Cheltenham, Victoria, Australia, during February 1993 to June 1994. A total of 115 elderly subjects (37 men and 78 women), aged 67 to 100 years agreed to have anthropometric measurements and blood tests. There were no exclusion criteria. Morbidity and mortality data were obtained from medical records over a period up to nearly two years and used for survival analyses. All subjects gave informed consent and the study protocol was approved by the Monash University Standing Committee on Ethics in Research on Humans, and the Ethics and Research Committees at Kingston Centre.

Anthropometric measurements

Body weight, height, skinfold thicknesses and circumferences were measured at various body sites. Body mass index (BMI, body weight in kilograms divided by height in metres squared), and % body fat estimated from the sum of the skinfold thicknesses in millimetres, measured at the triceps, biceps, subscapular and supra-iliac sites, were used as indices of body fatness,[15] even though such reference data are not available for the over 72 year olds. Waist-to-hip ratio (measured at the umbilical and maximal gluteal protrusion levels) was used as an index of abdominal body fatness.

Biochemical and haematological measurements

Fasting blood samples were collected for routine biochemical measurements including total protein, albumin, iron and ferritin. Serum zinc was also measured. Full blood tests included haemoglobin, hematocrit, and numbers of erythrocytes, leukocytes and lymphocytes. All of these measurements were done in the Department of Clinical Pathology, Monash Medical Centre, Southern Healthcare Network, Melbourne, Australia.

Lymphocyte subset analysis

The analysis of lymphocyte subsets, namely CD3 (total T-cells), CD4 (T-helper cells), CD8 (T-suppressor cells), CD19 (total B-cells), and CD56 (natural killer (NK) cells) was performed in peripheral venous whole blood, with the method described elsewhere.[16] Enumeration of lymphocyte subsets was done using an EPICS 752 Flowcytometer (Coulter, USA) after the addition of monoclonal fluorescent antibodies, at Monash Centre for Inflammatory Diseases, Monash University Department of Medicine at Monash Medical Centre. Total lymphocyte count was used to obtain the absolute counts of lymphocyte subsets.

Statistical analyses

Data analyses were performed using a Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, USA). Wilcoxon rank-sum tests were performed to determine the differences in measures of central tendency between groups with different characteristics. Spearman's rank correlation coefficient (r_s) was used to determine the degree and direction of the association between two variables of interest. The Kaplan-Meier product limit method was used in the survival analyses.[17] The significance level was set at 5%.

Table 1. Characteristics of the study population

Parameter	Men		Women	
	N	Mean ± SD	*N*	Mean ± SD
Age (years)	37	78.8 ± 6.6	78	83.0 ± 7.2 **
Selected body composition parameters				
Weight (kg)	33	74.8 ± 15.0	74	58.9 ± 11.2 ***
Stature (cm)	33	168 ± 7	72	153 ± 6 ***
Body mass index (kg/m^2)	33	26.6 ± 5.0	73	25.1 ± 4.2
Body fat mass (kg)[a]	32	20.7 ± 7.8	71	20.1 ± 6.7
Percent body fat (%)[a]	32	27.0 ± 5.6	71	33.5 ± 5.0 ***
Waist-to-hip ratio[b]	26	0.998 ± 0.051	64	0.968 ± 0.066 *
Selected biochemical parameters				
Serum total protein (g/L)	36	69.2 ± 0.9	76	70.0 ± 0.7
Serum albumin	36	36.3 ± 0.6	77	36.4 ± 0.3
Serum iron	36	13.3 ± 1.1	77	13.0 ± 0.6
Serum ferritin	35	177.1 ± 33.9	74	97.6 ± 14.7 *
Serum zinc	35	12.0 ± 0.3	76	11.8 ± 0.2

Significantly different from men (Wilcoxon rank-sum test): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

[a]Body fat mass and percent body fat were estimated using Durnin and Womersley.[15]

[b]Waist-to-hip ratio was obtained from the circumferences measured at the umbilical and maximal gluteal levels.

Table 2. Lymphocyte subsets of the study population

Lymphocyte subsets	Men		Women	
	N	Mean ± SD	*N*	Mean ± SD
CD3 (total T-cell)				
Percentage	37	62.6 ± 14.6	78	65.9 ± 10.3
Count (x10^6/L)	36	969 ± 350	75	1261 ± 679 **
CD4 (T-helper cell)				
Percentage	37	41.8 ± 12.9	78	41.8 ± 10.9
Count (x10^6/L)	36	639 ± 260	75	773 ± 314 *
CD8 (T-suppressor cell)				
Percentage	36	18.7 ± 8.5	71	22.5 ± 11.8
Count (x10^6/L)	35	295 ± 160	68	463 ± 574 *
CD19 (total B-cell)				
Percentage	36	7.4 ± 3.5	77	8.7 ± 4.2
Count (x10^6/L)	35	121 ± 74	74	158 ± 99 *
CD56 (natural killer cell)				
Percentage	31	12.7 ± 7.1	59	11.5 ± 5.4
Count (x10^6/L)	30	202 ± 162	57	193 ± 103

Significantly different from men (Wilcoxon rank-sum test): * $P < 0.05$; ** $P < 0.01$.

Table 3. Spearman's partial correlation coefficients of the relationships between selected biochemical measurements and peripheral blood lymphocyte subset counts, after controlling for albumin and total protein, in 36 men and 77 women

	Absolute count (x 10^6 cells/L)				
	CD3	CD4	CD8	CD19	CD56
Total					
Serum iron (μmol/L)	0.15	0.08	0.09	0.33 **	0.32 **
Serum ferritin (μg/L)	0.03	−0.01	0.09	−0.10	0.16
Serum zinc (μmol/L)	0.20	0.23 *	0.09	0.33 **	0.03
Men					
Serum iron (μmol/L)	−0.04	−0.08	0.16	0.30	0.47 **
Serum ferritin (μg/L)	0.26	0.05	0.40 *	0.06	0.16
Serum zinc (μmol/L)	−0.03	0.21	−0.08	0.12	0.06
Women					
Serum iron (μmol/L)	0.21	0.15	0.06	0.35 **	0.21
Serum ferritin (μg/L)	−0.02	−0.02	0.00	−0.15	0.17
Serum zinc (μmol/L)	0.32 **	0.28 *	0.21	0.50 ***	−0.11

Significantly different from zero: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Results

As expected, men were heavier and taller than women ($P < 0.0001$), but there were no differences in BMI. Men had a lower % body fat mass but a higher waist-to-hip ratio compared to women (Table 1). Results of lymphocyte subset analysis are shown in Table 2. Although no gender difference was observed in the percentage of lymphocyte subsets, women had significantly higher absolute counts of CD3, CD4, CD8 and CD19 cells, than men did.

Relationships of iron and zinc status to immuno-competence

Table 3 summarises the associations between serum iron, ferritin or zinc, and the absolute counts of peripheral blood lymphocyte subsets after plasma total protein and serum albumin concentrations were adjusted for. Positive relationships between serum iron and the number of CD56 cells ($r_s = 0.47$, $P < 0.01$), and between ferritin and the number of CD8+ cells ($r_s = 0.40$, $P < 0.05$) were found in men. Women with a high serum zinc concentration tended to have high numbers of CD3, CD4 and CD19 cells. A positive relationship was also observed between serum iron and CD19 cells ($r_s = 0.35$, $P < 0.01$) in women.

Lymphocyte subset and total mortality

Of 115 subjects, five men and 10 women died from various causes during an observation period of 22 months (Table 4). In Figure 1, it can be seen that the mean number of CD4 cells of the survivors (759±292 x10^6 cells /L) was significantly higher than that of elderly subjects who died during the 22-month observation period (542 ± 290 x10^6 cells/L). A survival analysis was performed in two groups of subjects with different CD4 cell counts, using a cut-off point of 559 x10^6 cells/L, which was the 25th percentile of a representative population of free-living, apparently healthy elderly (unpublished data). The survival curve for subjects with a CD4 cell count ≥ 559 x10^6 cells/L was consistently above the curve for those with a CD4 cell count < 559 x10^6 cells/L (Figure 2). However, the difference between two curves was not significant based on the Kaplan-Meier product limit method.[17]

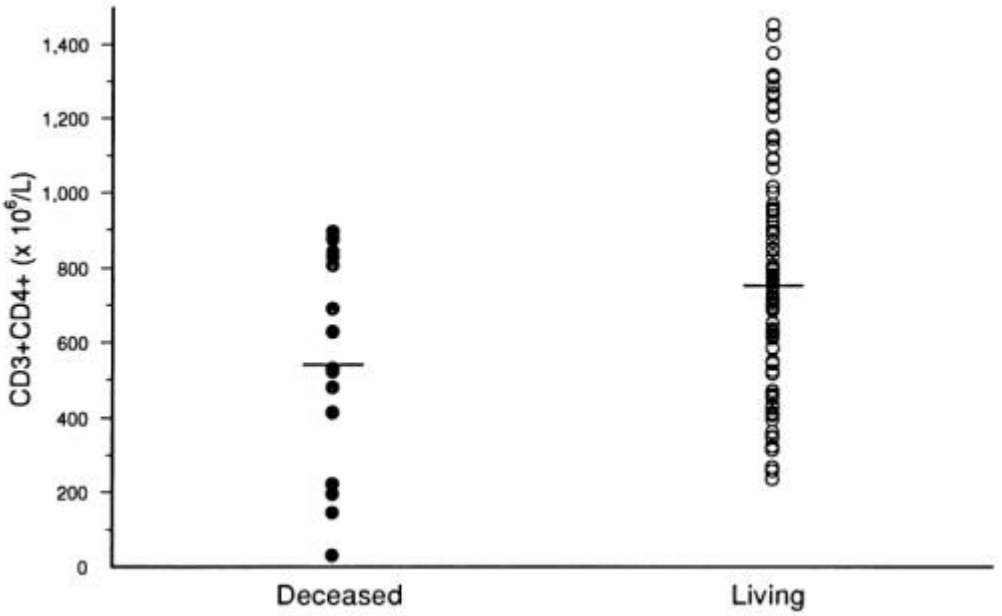

Figure 1. Mean and distribution of CD4 T-cells in deceased (N = 15) and living (N = 98) subjects.

Table 4. Elderly subjects who died during the observation period with CD4 T-cell counts and antecedent morbid event

Subject	Gender	Age	CD4 ($x10^6$ cells/L)	Antecedent morbid event[a]
1	F	86	630	septicaemia
2	F	85	692	perforated ulcer
3	M	79	876	oesophageal carcinoma
4	F	87	898	heart failure and IHD
5	F	78	146	IHD
6	F	86	415	cerebrovascular accident
7	F	77	831	heart failure and IHD
8	F	91	196	colorectal carcinoma and pneumonia
9	M	72	483	cholangiocarcinoma
10	F	83	809	heart failure and IHD
11	M	86	31	heart failure and IHD
12	M	69	223	prostate carcinoma
13	F	91	845	acute myocardial infarction
14	F	74	532	cardiac arrest
15	M	67	523	lung carcinoma

[a] IHD: ischaemic heart disease; M: male; F: female

Discussion

The elderly represent a unique subset of the population with limited nutritional and physiological reserve capacities. Studies on the health status of the institu-tionalised elderly population have been the subject of much interest and investigation, especially in an effort to improve the geriatric health care system. Flint and colleagues have reported that the nutritional status of institutionalised elderly is less favourable than their free-living counterparts.[3,18] The institutionalised elderly have significantly lower intake of energy, protein and zinc. Aged individuals, however, have minimal risk of developing iron deficiency. Iron stores increase throughout adult life in men and after menopause in women.[19] The present study examined the following two major hypotheses: firstly, whether iron and zinc status were associated with immunocompetence measured by peripheral blood lymphocyte subsets; and secondly, whether immunocompetence predicted total mortality among the institutionalised elderly. The present study indicates that there is a gender difference in immuno-competence in the study population (Table 2). It was found that both male and female subjects had similar iron and zinc status, but female subjects had

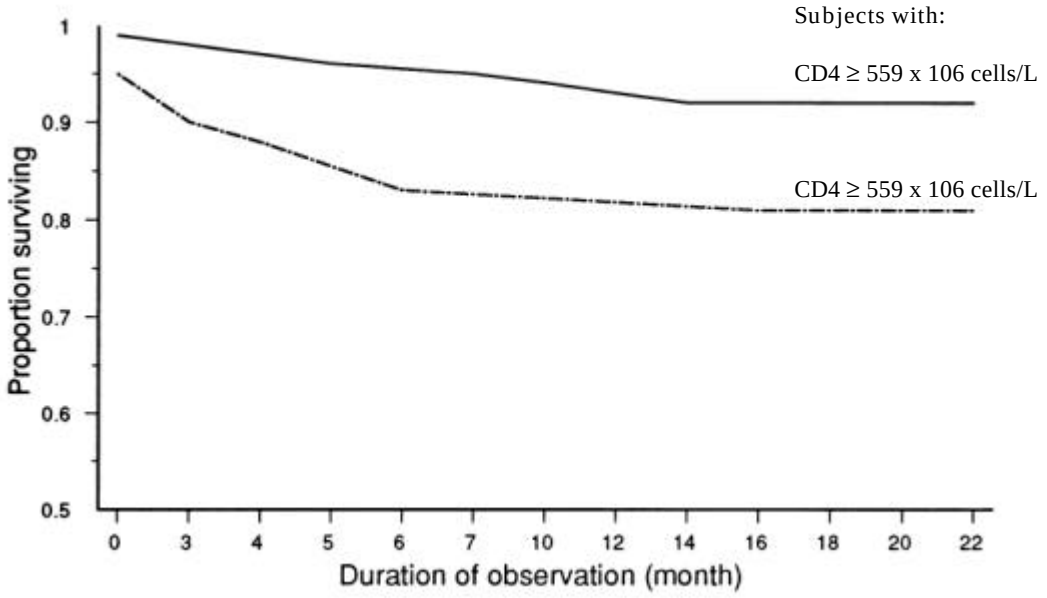

Figure 2. Survival probabilities for 113 institutionalised elderly individuals according to immunological status, assessed by the absolute counts of CD4 T-cells

higher counts of CD3 and CD19 cells. Given the fact that all female subjects were postmenopausal, this raises the possibility that being female may confer immuno-advantage, which may partly be attributable to the residual effect of sex
hormones. Oestrogen at a physiological concentration has been reported to have a favourable effect on lymphocyte subsets.[20] It has also been shown that oestrogen and progesterone, at a low dose, enhance immune response,[21] whereas these hormones at high doses can be immuno-suppressive.[22]

Relationships of iron and zinc status to immuno-competence

Both iron deficiency and iron overload have been reported to contribute to a variety of immunological abnormalities.[2,23-24] The observation of a positive association between serum ferritin and the number of CD8 cells in men is in agreement with the finding that absolute numbers of CD8 lymphocytes are elevated in untreated hereditary haemochromatosis patients.[25]

Grady and colleagues showed that the percentage of CD8 T-suppressor cells increased linearly with the amount of blood transfused in patients with thalassemia.[26] Despite the finding in the present study that serum iron correlates positively with NK-cells, a study on iron overload has suggested that iron overload contributes to the suppression of NK-cell activity.[27] This may represent a biphasic relationship between iron and NK-cell status, since the iron storage of the subjects in the present study would have not been excessive as observed in the thalassemia major.

Serum zinc concentration has been reported to decline with age.[28] Several clinical symptoms in the aged, such as decreased taste acuity[29] and delayed wound healing,[30] may be partly associated with zinc deficiency. Zinc is also known to have an immunomodulatory function.[24] Results of the present study showing the positive association between serum zinc and T-cells, especially CD4 (T-helper) cells, in women are consistent with other previous studies.[31-32] In zinc deficiency, T lymphocytes, and therefore cell-mediated immunity, decrease along with other components of the immune system.[31] Certain subpopu-lations of T-cells may be affected differentially.[24,32]

T-helper (CD4) cell counts and total mortality

Impaired cell-mediated immunity was associated with increased mortality in a study of 273 initially healthy individuals, aged 60 and over.[33] The vigour of the delayed-type hypersensitivity (DTH) skin test is known to depend on T-helper or CD4 cells.[34] It has also been suggested that a low CD4 T-cell count is a predictor of mortality in HIV-positive patients.[35] This could be the case for institutionalised elderly populations, as it was observed in the present study that, over the study period of 22 months, the survivors had a higher absolute count of CD4 cells compared to the non-survivors. However, when the Kaplan-Meier survival analyses were performed, subjects with CD4 cell counts ≥ 559 x10^6/L, or 'immuno-competent' subjects had a higher, but not significant, survival probability, compared to those with CD4 cell counts < 559 x10^6/L. The lack of significance may be due to the small sample size, the limited duration of the observation period or the existing co-morbidities.[36] Combining information on CD4 T-cells with other indicators of health status, such as disability, would provide a useful measure of the risk for adverse outcome.[37]

In conclusion, biochemical indicators of iron (serum iron and ferritin) and zinc status partly account for the variations in different circulating lymphocyte subsets, consistent with known data on the adverse effects of iron overload and of zinc deficiency on immunocompetence. The observation made in this study suggests that the number of CD4 cells may be used to predict total mortality. Future studies should examine the hypothesis whether improving immunocompetence by nutritional

interventions leads to better quality of life and survival of institutionalised aged individuals.

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