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# CMV infection, CD19<sup>+</sup> B cell depletion, and Lymphopenia as predictors for unexpected admission in the institutionalized elderly

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## Abstract

**Background:** Chronic infections played a detrimental role on health outcomes in the aged population, and had complex associations with lymphocyte subsets distribution. Our study aimed to explore the predictive roles of chronic infections, lymphopenia, and lymphocyte subsets on unexpected admission and mortality in the institutionalized oldest-old during 3 year follow-up period.

**Results:** There were 163 participants enrolled prospectively with median age of 87.3 years (IQR: 83.1–90.2), male of 88.3%, and being followed for 156.4 weeks (IQR: 136.9–156.4 weeks). The unexpected admission and mortality rates were 55.2 and 24.5% respectively. The Cox proportional hazards models demonstrated the 3rd quartile of cytomegalovirus IgG (OR: 3.26, 95% CI: 1.55–6.84), lymphopenia (OR: 2.85, 95% CI: 1.2–6.74), and 1st quartile of CD19<sup>+</sup> B cell count (OR: 2.84, 95% CI: 1.29–6.25) predicted elevated risks of unexpected admission after adjusting for potential confounders; while the 3rd quartile of CD3<sup>+</sup> T cell indicated a reduced risk of mortality (OR: 0.19, 95% CI: 0.05–0.71). Negative association between CMV IgG and CD19<sup>+</sup> B cell count suggested that CMV infection might lead to B cell depletion via decreasing memory B cells repertoire.

**Conclusions:** CMV infection, lymphopenia, and CD19<sup>+</sup> B cell depletion might predict greater risk of unexpected admission, while more CD3<sup>+</sup> T cell would suggest a reduced risk of mortality among the oldest-old population. A non-linear or U-shaped relationship was supposed between health outcomes and CMV infection, CD3<sup>+</sup> T cell, or CD19<sup>+</sup> B cell counts. Further prospective studies with more participants included would be needed to elucidate above findings.

**Keywords:** CD3<sup>+</sup>, CD19<sup>+</sup>, Chronic infection, Cytomegalovirus, The elderly, Immunosenescence, Lymphopenia

## Background

In contrast to acute infections that trigger pro-inflammatory cytokine storms, chronic infections indicate a unique status of dynamic equilibrium between pathogens replication and host immune response [1].

The global prevalence of chronic infections estimated > 90% for Varicella zoster virus (VZV), 83% for cytomegalovirus (CMV), 5% for hepatitis B virus (HBV), and 2.5% for hepatitis C virus (HCV), and the prevalence is expected to be higher for HBV and HCV in Asia-Pacific region [1–3]. The majority of chronic infections are believed to be harmless that cause merely subclinical illness in immunocompetent hosts, but opportunistic clinical diseases among immunocompromised persons, congenital abnormalities in neonates, or tumor growth

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in specific target organs due to intrinsic oncogenicity [1, 2].

Accumulating evidence suggests a detrimental role of chronic infection on physical function, cognition, and other health outcomes in immunocompetent populations, especially in the older population. Chronic infection also had a positive association with geriatric syndromes among the elderly. The Northern Manhattan Study reported that chronic infection burden had a negative impact on cognitive performance, predicted accelerated cognitive decline, dementia onset, and stroke incidence [4]. Chronic herpesviral and *Chlamydia pneumoniae* (*C. pneumoniae*) infections were also reported as risk factors of Alzheimer's disease and cardiovascular mortality [5, 6]. Moreover, CMV infection was proven a key indicator for cognitive impairment, frailty, also fatality in the Women' Health and Aging studies I and II (WHAS I & II), the third National Health and Nutrition Examination Survey (NHANES III), and other prospective cohorts in the aged population [7–9]. Other than seropositivity, elevated CMV immunoglobulin (IgG) was considered as a biomarker for increased physical vulnerability and dysregulatory immunomodulation that driven by repetitive antigen exposure from reactivation of latent infection [10].

Adaptive immunity and lymphocyte subset distribution have complex interaction with physiological aging and chronic infection [11]. Absolute depletion in total lymphocyte count, or lymphopenia, predicted acute infection and mortality among the elderly and was taken an indicator for impaired adaptive immunity in aging process [12, 13]. While reduced CD19<sup>+</sup> B cell count was reported as one parameter of “immune risk profile” along with poor T cell mitogenicity, accumulated CD8<sup>+</sup> T cell count, inverted CD4<sup>+</sup>/CD8<sup>+</sup>, and CMV seropositivity, that predicted mortality and adverse health outcomes in the Swedish OCTO/NONA immune longitudinal studies in older persons [10]. However, the influence on lymphocyte subset distribution by chronic infection showed a great variation between different pathogens. Depletion in CD8<sup>+</sup> and sometimes in CD4<sup>+</sup> T cells by apoptotic pathway, called “T cell exhaustion”, were reported in HBV, HCV, and human immunodeficiency virus infection [11, 14]. In contrast, clonal expansion of antigen-specific CD8<sup>+</sup> T cell by repetitive antigen stimulation, called “T cell senescence”, was disclosed in CMV infection [11, 15]. Reduced CD19<sup>+</sup> B cell had been reported among middle-aged males with positive CMV infection, but the association still remained inconclusive [16].

Although chronic infection was frequently associated with adverse health outcomes in the elderly, the results based on accumulating evidences still remained controversial. The possible influence on geriatric syndromes,

and complex interactions with lymphocyte subset distribution by chronic infection was not fully explored. Moreover, being more prevalent in Asia-Pacific region, the potential influence of HBV and HCV infections in the older generation was not yet investigated. The Veteran Affairs - Comprehensive Geriatric Assessment (VA-CGA) study in Taiwan was initiated since 2012, enrolled residents receiving elder care and assist-living care in the retirement communities for the veterans, and provided periodic assessment of geriatric syndromes by well-trained research staff every 6 months [17]. Residents were basically physically independent, cognitively intact, had less social engagement, and experienced common environmental exposures after allocation. Extended research by serological tests on chronic infection including CMV, VZV, HBV, HCV, and *C. pneumoniae* was started since 2016 among residents receiving elder care.

Thus, our study aimed to answer the possible impacts of chronic infections, lymphopenia, and lymphocyte subsets on unexpected admission and mortality in 3-year follow-up period among the older population at the retirement communities for veterans, as well as the association between chronic infection and lymphocyte subset distribution.

## Results

There were 163 participants enrolled with a median age of 87.3 years (IQR: 83.1–90.2 years), male of 88.3%, and being followed for a median of 156.4 weeks (IQR: 136.9–156.4 weeks). The physical function and cognitive status were relatively intact among participants, with median Barthel index score of 95 (IQR: 90–100) and Mini-Mental State Examination (MMSE) score of 26 (IQR: 23–29). Seropositivity was 99.4% of CMV (median IgG 236.8 IU/mL, IQR: 174.1–722.1 IU/mL), 92% of VZV (median IgG 3.46 S/CO, IQR: 2.51–4.7 S/CO), 57.1% of HBV surface antigen (HBsAg) (median IgG 17 mIU/mL, IQR: 3.8–50.8 mIU/mL), 7.4% of HCV (median IgG 0.07 S/CO, IQR: 0.05–0.1 S/CO), and 84.7% of *C. pneumoniae* (median IgG 1.49 S/CO, IQR: 1.28–1.63 S/CO) respectively. Five participants (3.1%) moved out the retirement communities without registration of final health outcome. The unexpected admission rate was 55.2% (57.7% by infectious disease, 12.2% by cardiovascular disease, and 30% by others including traumatic injury, acute delirium, acute kidney injury, etc.), and the 3-year mortality rate was 24.5% (37.5% by infectious diseases, 17.5% by cardiovascular disease, and 45% by others including malignancy, brainstem hemorrhage, acute gastrointestinal bleeding, etc.). Demographic characteristics, chronic illness, geriatric syndromes, laboratory parameters in details were listed in Table 1.

In the Cox proportional hazards model, lymphopenia was shown an important predictor for both unexpected

**Table 1** Baseline demographic characteristics of participants

Demographic and clinical characteristics	Participants N = 163 (100%)
<b>Age, years</b>	87.3 (83.1–90.2)
<b>Male gender (%)</b>	144 (88.3%)
<b>Follow-up duration, weeks</b>	156.4 (136.9–156.4)
<b>Move out from retirement communities</b>	5 (3.1%)
<b>Unexpected admission rate (%)</b>	90 (55.2%)
Infectious diseases-related (%)	52 (57.7%)
Cardiovascular diseases-related (%)	11 (12.2%)
Others (%)	27 (30%)
<b>Mortality rate (%)</b>	40 (24.5%)
Infectious diseases-related (%)	15 (37.5%)
Cardiovascular diseases-related (%)	7 (17.5%)
Others	18 (45%)
<b>Body mass index, kgs/m<sup>2</sup></b>	24.2 (21.1–26)
<b>Cigarette Smoking</b>	
Ex-smoker (%)	17 (10.4%)
Active smoker (%)	20 (12.3%)
<b>Alcohol consumption</b>	
Ex-drinker (%)	10 (6.1%)
Active drinker (%)	28 (17.2%)
<b>Education</b>	
< 6 years (%)	53 (32.5%)
6–9 years (%)	51 (31.3%)
> 9 years (%)	48 (29.4%)
<b>Flu vaccine uptake (%)</b>	109 (66.9%)
<b>Underlying diseases</b>	
Cerebrovascular disease (%)	9 (5.5%)
Chronic liver disease (%)	2 (1.2%)
Chronic kidney disease (%)	4 (2.5%)
Chronic lung disease (%)	24 (14.7%)
Congestive heart failure (%)	7 (4.3%)
Dementia (%)	7 (4.3%)
Depression (%)	8 (4.9%)
Diabetes mellitus (%)	45 (27.6%)
Hypertension (%)	118 (72.4%)
Peptic ulcer disease (%)	26 (16%)
Peripheral arterial disease (%)	32 (19.6%)
Malignancy (%)	11 (6.7%)
<b>Charlson comorbidity index</b>	1 (0–2)
<b>Geriatric syndromes</b>	
Physical function by BI score	95 (90–100)
Risk of falls by JHFRAT score	12 (10–16)
Polypharmacy (%)	83 (50.9%)
Types of medicine	5 (3–6)
Cognition by MMSE score	26 (23–29)
Depression by GDS-5 score	0 (0–1)
Malnutrition by MUST score	0 (0–0)
Visual impairment (%)	52 (31.9%)

**Table 1** Baseline demographic characteristics of participants (*Continued*)

Demographic and clinical characteristics	Participants N = 163 (100%)
Hearing impairment (%)	27 (16.6%)
Sleep disorder (%)	46 (28.2%)
Use of hypnotic agents (%)	36 (22.1%)
Constipation (%)	44 (27%)
Stool incontinence (%)	3 (1.8%)
Urinary incontinence (%)	5 (3.1%)
<b>Laboratory tests</b>	
White blood cell count, cells/cumm	5900 (4900- 6700)
Hemoglobin, gm/dL	12.7 (11.6–13.6)
Platelet count, ×1,000 cells/cumm	180.5 (148.7–221)
Neutrophil count, cells/cumm	3157 (2472- 3914)
Lymphocyte count, cells/cumm	1800 (1500- 2300)
CD3 <sup>+</sup> T cell count, cells/cumm	1136 (903–1526)
CD4 <sup>+</sup> T cell count, cells/cumm	718 (557–926)
CD8 <sup>+</sup> T cell count, cells/cumm	390 (262–576)
CD19 <sup>+</sup> B cell count, cells/cumm	135 (81–205)
CD4 <sup>+</sup> /CD8 <sup>+</sup>	1.81 (1.25–2.54)
Albumin, gm/dL	4 (3.8–4.2)
Cholesterol, mg/dL	163 (141–188)
Triglyceride, mg/dL	87 (62–122)
HDL-C, mg/dL	48 (36–57)
LDL-C, mg/dL	97 (77–117)
BUN, mg/dL	19.5 (16–25)
Serum creatinine, mg/dL	0.96 (0.79–1.24)
Aspartate aminotransferase, U/L	21 (17–25)
hs-CRP, mg/dL	0.14 (0.08–0.32)
Lymphopenia (%)	12 (7.4%)
CD4 <sup>+</sup> /CD8 <sup>+</sup> < 1	18 (11%)
<b>Chronic infection</b>	
CMV seropositivity (%)	162 (99.4%)
VZV seropositivity (%)	150 (92%)
HBsAg seropositivity (%)	93 (57.1%)
HCV seropositivity (%)	12 (7.4%)
<i>Chlamydia pneumoniae</i> seropositivity (%)	138 (84.7%)
CMV IgG, IU/mL	236.8 (174.1–722.1)
VZV IgG, S/CO	3.46 (2.51–4.7)
HBsAg IgG, mIU/mL	17 (3.8–50.8)
HCV IgG, S/CO	0.07 (0.05–0.1)
<i>Chlamydia pneumoniae</i> IgG, S/CO	1.49 (1.28–1.63)

Abbreviations: *BI* Barthel index, *CD* cluster of differentiation, *CMV* Cytomegalovirus, *GDS-5* Geriatric Depression Scale-5 items, *HBsAg* hepatitis B virus surface antigen, *HCV* hepatitis C virus, *HDL-C* high density lipoprotein-cholesterol, *hs-CRP* highly sensitive C reactive protein, *IgG* Immunoglobulin G, *JHFRAT* John Hopkins Fall Risk Assessment Tool, *LDL-C* Low density lipoprotein-cholesterol, *MMSE* Mini-Mental Status Examination, *MUST* Malnutrition Universal Screening Test, *VZV* Varicella zoster virus

admission (OR: 2.97, 95% CI: 1.51–5.82) and mortality (OR: 2.32, 95% CI: 1–5.33) in model 1, remained significant for unexpected admission (OR: 2.85, 95% CI: 1.2–6.74) but less significant for mortality (OR: 1.9, 95% CI: 0.66–5.4) after adjustment for competing risk factors of

mortality in model 2. The 3rd quartile of CMV IgG (OR: 3.26, 95% CI: 1.55–5.2) and the 1st quartile of CD19<sup>+</sup> B cell count (OR: 2.84, 95% CI: 1.29–6.25) indicated greater risk of unexpected admission even after adjustment of competing risk factors of mortality in model 2,

while the 3rd quartile of CD3<sup>+</sup> T cell count had a reduced risk of mortality (OR: 0.19, 95% CI: 0.05–0.71) (Table 2). Due to uneven distribution in gender among participants, we performed another Cox regression surviving analysis including only males as model 2. The 3rd (OR: 3.76, 95% CI: 1.75–8.0), 4th (OR: 2.26, 95% CI: 1.07–4.76) quartile of CMV IgG, lymphopenia (OR: 2.8, 95% CI: 1.18–6.61), the 1st quartile of CD19<sup>+</sup> B cell count (OR: 2.8, 95% CI: 1.24–6.29) still suggested greater risk of unexpected admission, and the 3rd quartile of CD3<sup>+</sup> T cell count (OR: 0.19, 95% CI: 0.05–0.71) had a protective effect against mortality.

The CMV IgG positively correlated with age, flu vaccine uptake, risks of fall, usage or hypnotic agents, *C. pneumoniae* IgG, while negatively associated with education, cognition, and CD19<sup>+</sup> B cell count (Table 3). For

all lymphocyte, CD3<sup>+</sup> T cell, and CD19<sup>+</sup> B cell counts, there were positive associations with female gender, body mass index (BMI), cognitive performance, hemoglobin, albumin, cholesterol, low density lipoprotein-cholesterol, while negative correlations with age and flu vaccine uptake. Other results of Spearman correlation analysis were listed in Table 3.

Additional adjustment for associating factors was performed in final model and still reported an elevated risk of unexpected admission for the 3rd quartile of CMV IgG (OR: 2.66, 95% CI: 1.14–6.2). With adjustment for correlating factors after removing white blood cell count, lymphocyte and subset counts, lymphopenia still indicated an increased risk of unexpected admission (OR: 3.59, 95% CI: 1.34–9.59), and

**Table 2** The Cox proportional hazards model for mortality and unexpected admission

Variables	Unexpected admission: OR (95% CI)			Mortality: OR (95% CI)		
	Unadjusted	Model 1	Model 2	Unadjusted	Model 1	Model 2
<b>CMV IgG, IU/mL</b>						
< 174.1	1	1	1	1	1	1
174.1–236.8	1.23 (0.63, 2.39)	1.24 (0.62, 2.47)	2.05 (0.93, 4.51)	1.31 (0.52, 3.34)	1.37 (0.53, 3.5)	1.63 (0.57, 4.67)
236.8–722.1	2.56 (1.41, 4.64)**	2.84 (1.56, 5.2)**	3.26 (1.55, 6.84)**	1.36 (0.54, 3.38)	1.15 (0.45, 2.89)	0.92 (0.31, 2.66)
> 722.1	1.65 (0.88, 3.12)	1.7 (0.89, 3.24)	2.02 (0.97, 4.18)	1.49 (0.6, 3.71)	1.17 (0.46, 2.95)	0.97 (0.35, 2.67)
<b>HCV IgG, S/CO</b>						
< 0.0575	1	1	1	1	1	1
0.0575–0.07	0.85 (0.45, 1.58)	0.96 (0.51, 1.83)	1.1 (0.5, 2.42)	0.41 (0.17–0.97)*	0.51 (0.21, 1.26)	0.53 (0.18, 1.53)
0.07–0.1	1.03 (0.55–1.91)	1.39 (0.72, 2.67)	1.62 (0.77, 3.39)	0.58 (0.25, 1.32)	0.85 (0.35, 2.06)	0.95 (0.34, 2.58)
> 0.1	1.63 (0.91, 2.91)	1.71 (0.92, 3.18)	1.84 (0.91, 3.72)	0.53 (0.22, 1.26)	0.65 (0.26, 1.59)	0.65 (0.23, 1.81)
<b>Lymphopenia</b>	3.43 (1.8, 6.5)***	2.97 (1.51, 5.82)**	2.85 (1.2, 6.74)**	2.91 (1.29, 6.6)*	2.32 (1, 5.33)*	1.9 (0.66, 5.4)
<b>CD3<sup>+</sup> T cell count, cells/cumm</b>						
> 1526	1	1	1	1	1	1
1136–1526	0.97 (0.5, 1.88)	0.77 (0.38, 1.53)	0.43 (0.18, 1)	0.43 (0.13, 1.4)	0.21 (0.06, 0.73)*	0.19 (0.05, 0.71)*
903–1136	1.42 (0.77, 2.65)	0.98 (0.49, 1.92)	0.72 (0.34, 1.54)	1.12 (0.45, 2.76)	0.5 (0.19, 1.33)	0.59 (0.2, 1.73)
< 903	2.22 (1.23, 4.02)**	1.54 (0.78, 3.03)	1.04 (0.47, 2.28)	2.07 (0.91, 4.7)	1 (0.4, 2.47)	0.94 (0.33, 2.7)
<b>CD4<sup>+</sup> T cell count, cells/cumm</b>						
> 926	1	1	1	1	1	1
718–926	1.34 (0.66, 2.69)	1.06 (0.51, 2.22)	0.63 (0.27, 1.46)	1.45 (0.46, 4.58)	1 (0.31, 3.18)	0.95 (0.27, 3.38)
557–718	2.15 (1.13, 4.11)*	1.55 (0.77, 3.12)	1.11 (0.52, 2.37)	2.23 (0.77, 6.42)	1.24 (0.42, 3.68)	1.31 (0.41, 4.13)
< 557	2.78 (1.46, 5.3)**	1.75 (0.84, 3.67)	1.57 (0.71, 3.46)	3.88 (1.42, 10.61)**	2.15 (0.75, 6.14)	2.55 (0.8, 8.06)
<b>CD19<sup>+</sup> B cell count, cells/cumm</b>						
> 205	1	1	1	1	1	1
135–205	2.07 (1.06, 4.04)*	1.76 (0.86, 3.6)	1.59 (0.73, 3.46)	1.46 (0.46, 4.62)	0.97 (0.3, 3.12)	0.99 (0.28, 3.5)
81–135	1.54 (0.77, 3.08)	1.24 (0.57, 2.71)	1.05 (0.45, 2.48)	2.42 (0.84, 6.98)	1.35 (0.44, 4.12)	1.11 (0.34, 3.64)
< 81	3.1 (1.64, 5.84)***	2.24 (1.09, 4.63)*	2.84 (1.29, 6.25)**	3.67 (1.34, 10.03)*	1.99 (0.68, 5.81)	2.41 (0.79, 7.36)

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

Model 1: Adjusted by age, gender, body mass index, highly-sensitive C-reactive protein, and multimorbidity by Charlson comorbidity index

Model 2: Adjusted by education status, cigarette smoking, alcohol consumption, albumin, low density lipoprotein cholesterol, medical history of acute myocardial ischemia, congestive heart failure, cerebrovascular accident, and diabetes mellitus, in addition to age, gender, body mass index, and multimorbidity

the 3rd quartile of CD3<sup>+</sup> T cell remained a reduced risk of mortality (OR: 0.38, 95% CI: 0.15–0.95).

## Discussion

Comparing with other chronic infections, CMV infection was a potential predictor for unexpected admission but not mortality, and the 3rd quartile of CMV IgG suggested an elevated risk of unexpected admission among the oldest-old persons even after adjustment for competing risk factors of mortality. For the CMV seroprevalence of 99.4% in our participants and 91.7% of blood donors in Taiwan, the CMV seropositivity would not be a good biomarkers on outcomes prediction [18]. Increased frailty incidence had been disclosed in the elderly with the highest CMV IgG in WHAS I & II, as well as poor survival possibility and rising incidence of cardiovascular disease in the Sacramento Area Latino Study on Aging and the population-based European Prospective Investigation of Cancer- Norfolk cohort study [8, 19, 20]. On the other hand, the BELFRAIL and another cohort studies among the oldest-old population revealed a neutral role of CMV infection on frailty and mortality [21, 22]. A non-linear or U-shaped relationship was supposed between CMV infection and adverse health outcomes in the oldest-old population, which would lead to diverse findings between studies. To the best of our knowledge, this study would be the first one to adopt unexpected admission for outcome assessment, and to present a non-linear pattern of CMV infection on outcome prediction. High CMV seroprevalence in Asian population since their childhood might account for the difference from previous studies including mostly Caucasian population [23].

The bottom quartile of CD19<sup>+</sup> B cell count showed a positive association with unexpected admission in 3-year follow-up duration, and the effect weaned when adjusted for CMV IgG in final model. When we created interaction term between CMV IgG and CD19<sup>+</sup> B cell count, there was significant interaction found in Cox proportional hazard model. Even CMV infection was shown a better predictor for risk of unexpected admission, reduced CD19<sup>+</sup> B cell count was believed to be the mediator for adverse health outcome. The paradoxically negative correlation was disclosed between the CMV IgG and CD19<sup>+</sup> B cell count, which was supposed the CMV-driven memory B cell exhaustion as previous study in middle-aged males [16]. Depletion in CD19<sup>+</sup> B cell had been recognized an important indicator of the “immune risk profile”, showed positive association with male gender, advanced age, and CMV infection [16, 24]. Lower CD19<sup>+</sup> B cell count was reported an independent predictor of mortality in patients under hemodialysis, and was associated with frailty and poor physical

function in the elder population [25–27]. However, due to limited publications available at present, more studies would be needed for approaching the underlying pathogenic mechanism of CMV infection on CD19<sup>+</sup> B cell count and long-term health outcomes.

Lymphopenia was shown an important risk factor of unexpected admission but not of mortality in our participants. The NHANES study retrospectively recognized a positive association between lymphopenia and mortality of either cardiovascular or non-cardiovascular causes in the general population [12]. The Copenhagen General Population Study demonstrated lymphopenia as a risk factor of admission and mortality due to infectious disease prospectively [13]. There were also prolonged length of stay and elevated in-hospital mortality reported among the older inpatients with lymphopenia [28]. Even lymphopenia predicts infection incidence, it failed to indicate an increased risk of mortality in the institutionalized elderly in our previous study [29]. Variation in lymphocyte subset distributions might be the possible explanation for this equivocal findings on predicting mortality by lymphopenia [11].

Lowest risk of mortality in the elderly was reported in the 3rd quartile group of CD3<sup>+</sup> T cell count in our participants even after adjustment for all correlating factors. There had been a prospective study revealing a higher CD3<sup>+</sup>, CD4<sup>+</sup> T cell counts and CD4<sup>+</sup>/CD8<sup>+</sup> among survivors in a 2-year follow-up in the healthy Chinese elderly [30]. But our study failed to discover the ability by CD4<sup>+</sup>, CD8<sup>+</sup> T cell counts or CD4<sup>+</sup>/CD8<sup>+</sup> on outcomes prediction in the 3-year period.

Several limitations were noticed in our study. First, the non-linear or U-shaped relationship between risk factors and health outcomes might relate to the baseline characteristics of study participants at enrollment. We had tried adjusting all possibly confounding factors of all-cause mortality as possible to increase the strength of study, but more studies with larger sample size were still needed to prove our findings. Even limited participants were enrolled, with minimal drop-out rate of 3.1% and a follow-up period of 3 years, we believed above findings were convincing enough for male elderly through adequate statistical methods. Second, the proinflammatory cytokines were not included at initial study design except highly sensitive-C reactive protein, thus the complex interactions between chronic infection, lymphocyte subsets, inflammation, and health outcomes were not fully evaluated. Final, the current categories by flow cytometry could not distinguish the naïve or well-differentiated cells from the same lymphocyte subsets. Further studies to explore the possible influence of naïve or well-differentiated cell distributions would be needed for answering more questions.

**Table 3** Results of Spearman correlation analysis between baseline characteristics

Indicators	Variables	Correlation coefficient	p value
CMV IgG	Age	0.19	0.017*
	Education	-0.17	0.036*
	Flu vaccine uptake	0.278	0.001**
	JHFRAT score	0.219	0.006**
	MMSE score	-0.197	0.013*
	Usage of hypnotic agents	0.169	0.043*
	CD19 <sup>+</sup> B cell count	-0.179	0.026*
	<i>Chlamydia pneumoniae</i> IgG	0.194	0.015*
Lymphocyte count	Age	-0.291	< 0.001***
	Female Gender	0.226	0.004**
	Body mass index	0.296	< 0.001***
	Flu vaccine uptake	-0.192	0.017*
	Smoking history	-0.194	0.014*
	Diabetes mellitus	0.18	0.024*
	JHFRAT	-0.225	0.004**
	MMSE score	0.227	0.004**
	MUST score	-0.259	0.001**
	White blood cell count	0.5	< 0.001***
	Hemoglobin	0.261	0.001**
	Platelet count	0.185	0.018*
	CD3 <sup>+</sup> T cell count	0.862	< 0.001***
	CD4 <sup>+</sup> T cell count	0.757	< 0.001***
	CD8 <sup>+</sup> T cell count	0.632	< 0.001***
	CD19 <sup>+</sup> B cell count	0.449	< 0.001***
	Albumin	0.297	< 0.001***
	Cholesterol	0.319	< 0.001***
	Triglyceride	0.177	0.024*
	LDL-C	0.234	0.003**
hs-CRP	-0.186	0.018*	
CD3 <sup>+</sup> T cell count	Age	-0.337	< 0.001***
	Female Gender	0.304	< 0.001***
	Body mass index	0.328	< 0.001***
	Flu vaccine uptake	-0.209	0.01*
	Diabetes mellitus	0.195	0.015*
	JHFRAT score	-0.168	0.035*
	MMSE score	0.193	0.015*
	MUST score	-0.224	0.005
	White blood cell count	0.401	< 0.001***
	Hemoglobin	0.263	0.001**
	Platelet count	0.17	0.032*
	Lymphocyte count	0.862	< 0.001***
	CD4 <sup>+</sup> T cell count	0.835	< 0.001***
	CD8 <sup>+</sup> T cell count	0.792	< 0.001***
	CD19 <sup>+</sup> B cell count	0.423	< 0.001***

**Table 3** Results of Spearman correlation analysis between baseline characteristics (*Continued*)

Indicators	Variables	Correlation coefficient	p value
CD19 <sup>+</sup> B cell count	Albumin	0.239	0.003**
	Cholesterol	0.364	< 0.001***
	LDL-C	0.281	< 0.001***
	Triglyceride	0.206	0.01*
	<i>Chlamydia pneumoniae</i> IgG	-0.17	0.033*
	Age	-0.471	< 0.001***
	Female gender	0.337	< 0.001***
	Body mass index	0.265	0.001**
	Flu vaccine uptake	-0.278	0.001**
	Alcohol consumption	0.215	0.007**
	Chronic liver disease	0.184	0.02*
	Barthel index score	0.23	0.004**
	MMSE score	0.24	0.002**
	White blood cell count	0.202	0.011*
	Hemoglobin	0.232	0.003**
	Lymphocyte count	0.449	< 0.001***
	CD3 <sup>+</sup> T cell count	0.423	< 0.001***
	CD4 <sup>+</sup> T cell count	0.587	< 0.001***
	CD4 <sup>+</sup> /CD8 <sup>+</sup>	0.343	< 0.001***
	Albumin	0.192	0.016*
	Cholesterol	0.299	< 0.001***
	LDL-C	0.258	0.001**
	Creatinine	-0.168	0.035*
CMV IgG	-0.179	0.026*	

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

Abbreviations: CD cluster of differentiation, CMV cytomegalovirus, HDL-C high density lipoprotein-cholesterol, hs-CRP highly sensitive C reactive protein, IgG Immunoglobulin G, JHFRAT John Hopkins Fall Risk Assessment Tool, LDL-C Low density lipoprotein-cholesterol, MMSE Mini-Mental Status Examination, MUST Malnutrition Universal Screening Test, VZV Varicella zoster virus

## Conclusions

CMV infection, lymphopenia, and CD19<sup>+</sup> B cell depletion predicted greater risk of unexpected admission, while more CD3<sup>+</sup> T cell would suggest a reduced risk of mortality among the oldest-old population at institutes. A non-linear or U-shaped relationship was supposed between health outcome and CMV infection, CD3<sup>+</sup> T cell, or CD19<sup>+</sup> B cell counts. Further prospective studies with more participants included would be needed to elucidate above findings.

## Methods

### Participants

This prospective study enrolled residents receiving elder care with relatively intact physical and cognitive function at three retirement communities for veterans in Taiwan from July 1st, 2016 to January 31st, 2017. Potential candidates were invited for participation if they fulfilled the following criteria, (a) age  $\geq 65$  years, (b) stayed at the retirement communities for more than 3 months, (c) free

of acute illness 2 weeks prior to study enrollment, and (d) with life expectancy for  $\geq 1$  year. Participants were excluded for those who (a) could not complete the baseline assessment and laboratory tests, (b) would not like to be followed in study period, or (c) would request for study withdrawal. All participants received baseline anthropometric measurements, comprehensive geriatric assessment, and venous blood sampling at enrollment by well-trained staffs. Demographic characteristics, underlying diseases, and laboratory parameters were collected, while a composite score of Charlson Comorbidity Index (CCI) was calculated for adjustment [31].

### Measurements

#### Geriatric syndromes

Physical performance was approached by the Barthel index for activities of daily living. The higher Barthel index score suggests a better physical function [32]. Risk of falls was evaluated by Johns Hopkins Fall Risk Assessment Tool (JHFRAT) for home health care [33]. Scores



of JHFRAT were calculated from established items, including (1) age, (2) fall history  $\leq 6$  months, (3) bowel and urine elimination, (4) usage of high risk medications, (5) patient care equipment, (6) mobility, and (7) cognition. The more JHFRAT score indicated elevated risks of fall. Polypharmacy was identified if residents concurrently took  $\geq 5$  types of medications [34]. Types of medications were counted and registered for analysis.

Cognition was assessed by the MMSE, Chinese version. The lower MMSE score indicates a poor cognition [35]. Depressive symptom was approached by the geriatric depression scale-5 item version (GDS-5). Participants with higher GDS-5 had more possibility for depression [36]. Risk of undernutrition was defined by Malnutrition Universal Screening Tool (MUST). Participants with higher MUST score faced more risks of undernutrition [37]. Visual impairment, hearing difficulty, sleep disorder, as well as urinary and stool incontinence were also recorded.

#### Laboratory parameters

Peripheral venous blood samples were sampled via the antecubital vein in the morning after an overnight fasting. Complete blood counts and differential counts were analyzed by the automated cellular analysis system Beckman Coulter DxH 800 hematology analyzer (Beckman Coulter, Miami, FL). Biochemical parameters of albumin, lipid profile, high sensitive-C reactive protein (hs-CRP), fasting glucose, aspartate transaminase, blood urea nitrogen, and serum creatinine were measured by the ARCHITECT i2000SR immunoassay analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Lymphopenia was defined by absolute lymphocyte count  $< 1000/\text{cumm}$  [29].

Lymphocyte subsets were determined flow-cytometric analysis system Beckman Coulter FC500 flow cytometer (Beckman Coulter, Miami, FL) in freshly drawn peripheral blood after adequate processing as the manufacturer's instruction. Serum CMV IgG, VZV IgG, HBsAg IgG, HCV IgG, and *C. pneumoniae* IgG were measured by a chemiluminescent immunoassay on the ARCHITECT i2000SR immunoassay analyzer (Abbott Diagnostics, Abbott Park, IL, USA).

#### Outcome measurement

Unexpected admission and mortality were recorded of each individual in 3-year follow-up period, and the main etiology of each event was also registered. For those who lost to follow-up in study period would be treated as censored cases, and time for follow-up were documented until the last available information.

#### Statistical analysis

Results for data analysis are expressed as median with interquartile range (IQR) for continuous variables, and

numbers (%) for categorical variables. Quartile grouping was performed on CMV IgG, VZV IgG, HBsAg IgG, HCV IgG, *C. pneumoniae* IgG, CD3<sup>+</sup> T cell count, CD4<sup>+</sup> T cell count, CD8<sup>+</sup> T cell count, CD19<sup>+</sup> B cell count, and CD4<sup>+</sup>/CD8<sup>+</sup>. Cox proportional hazard model with bootstrapping for 1000 was used to assess the possible impacts on unexpected admission and mortality of lymphopenia and each quartile groups. Adjustment for age, gender, nutritional status by BMI, inflammatory status by hs-CRP, and multimorbidity by CCI was performed in model 1, while further adjustments for competing risk factors of all-cause mortality including education status, cigarette smoking, alcohol consumption, serum albumin, low density lipoprotein-cholesterol, congestive heart failure, cerebrovascular disease, and diabetes mellitus were performed in model 2. Non-parametric method of Spearman correlation analysis was used for comparison in case of possibly non-Gaussian distribution of numerical data. Another Cox proportional hazard model with additional adjustment for associated factors was performed finally. All data analyses were carried out with the Statistical Package for the Social Sciences for Windows version 20.0 (SPSS, Chicago, IL, USA), and variables were considered as statically significant if  $P < 0.05$ .

#### Abbreviations

CD: Cluster of differentiation; CMV: Cytomegalovirus; *C. pneumoniae*: *Chlamydia pneumoniae*; GDS-5: Geriatric Depression Scale-5 items; HBsAg: Hepatitis B virus surface antigen; HBV: Hepatitis B virus; HCV: Hepatitis C virus; IgG: Immunoglobulin G; hs-CRP: Highly sensitive-C reactive protein; JHFRAT: John Hopkins Fall Risk Assessment Tool; MMSE: Mini-Mental Status Examination; MUST: Malnutrition Universal Screening Test; NHANES: The National Health and Nutrition Examination Survey; VZV: Varicella zoster virus; WHAS: The Women' Health and Aging studies

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#### Authors' contributions

LYC, ACH, and CYH designed the study protocol, collected the data, performed statistical analyses, and drafted the manuscript. LKC, FDW, and YJC coordinated laboratory tests, modified study protocols, rechecked statistical analyses, and provided critical suggestions before submission. All authors had read and approved the final manuscript.

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#### Availability of data and materials

All collected data and analyses during the current study are available from the corresponding author on reasonable request at email address: [yjchan@vghtpe.gov.tw](mailto:yjchan@vghtpe.gov.tw)

#### Declarations

##### Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Taipei Veterans General Hospital, which was conformed to the provisions of the World Medical Association's Declaration of Helsinki (IRB-TPEVGH No.: 2016-06-009A). The written informed consent for each participant was obtained before study enrollment.

##### Competing interests

The authors declare no conflicts of interests.

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