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Elevated levels of CRP and IL-8 are related to reduced survival time:

1-year follow-up measurements of different analytes in frail elderly nursing home residents

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Introduction: There are only few studies with specific focus on predictors of survival in nursing home residents. The aim was to study whether 1-year changes in complete blood count (including haemoglobin, red blood cells, erythrocyte volume fraction, mean corpuscular volume, mean corpuscular haemoglobin concentration, white blood cells count, and platelet count), C-reactive protein and interleukin-1β (IL-1β), IL-1Ra, IL-6, IL-8 and IL-10, are associated with 8-year survival in elderly nursing home residents, aged ≥80 years.

Methods: Complete blood count, C-reactive protein and interleukins were measured at baseline, after 6 and 12 months from 167 nursing home residents aged 80-101 years, mean age 88±4.5 years, 75% of whom were women. Dates of death were collected from the National Death Register 8 years after baseline.

Results: Levels of haemoglobin, red blood cells and mean corpuscular haemoglobin concentration were lower after 1-year, but higher for mean corpuscular volume and IL-1β, compared to baseline or 6 month follow-up. In the Cox regression model with a time-dependent covariate, raised levels of C-reactive protein and IL-8 were associated with reduced survival time.

Conclusions: Elevated levels of C-reactive protein and IL-8 during 1-year follow-up were related to reduced lengths of survival in elderly nursing home residents.

Key words: aging, frailty, blood cell count, c-reactive protein, interleukins, survival

Introduction

Although nursing home residents (NHRs) are often frail, with multi-morbidity and reduced functional abilities [1, 2] limiting their survival, the survival may be of significant length for some NHRs. For example, in a study of 5-year survival in two cohorts of NHRs, the mean time for survival was 57.4+36 months and 44.5+31 months, respectively, and the 5-year survival rate was 23% and 28%, respectively [3]. It may, even in this NHR population, be of clinical value to determine which factors are the most important predictors of survival.

There are only few studies with specific focus on predictors of survival in NHRs [3, 4, 5]. One study in this type of setting reports that dependence in activities of daily living (ADLs) reduces 5-year survival by about 25–30% in both men and women [3]. Moreover, conflicting results have been reported about the effect of body mass index (BMI) on survival in NHRs [5], but in a 1-year follow-up study, those who died during the year had lost weight compared with those who survived [4]. In one study in 1,700 older men living in the community, the following prognostic factors for survival were identified [6]: no history of myocardial infection, stroke, cancer or dementia; a white blood cells (WBC) $<10x10^9$/L; and haemoglobin (Hb) >130 g/L.

Other analytes in blood may also be potential predictors of survival. Previous studies have shown differences in levels of analytes with increasing age [7, 8, 9], for example decreased levels of lymphocytes, red blood cells (RBCs), haematocrit and albumin, and increased levels of WBCs, C-reactive protein (CRP), interleukin (IL) 6 and creatinine [7, 10]. However, the participants in the above mentioned studies seem to have been classified as ‘healthy’ and may not represent the entire population that includes a substantial proportion of less healthy individuals, as in the present NHR population.

In a longitudinal study conducted by Starr & Deary [11], 486 community-dwelling people without identified blood disorder, with a mean age of 79.1 (77.8–80.6) years were examined. At the follow-up 8 years later, the remaining 187 participants, aged 86.7 (85.7–87.4) years, showed decreased levels of RBCs, lymphocytes and eosinophils and increased levels of neutrophils and platelet count (PLT). The authors attributed the decrease in RBCs to impaired renal function and the increase in neutrophils and PLTs to inflammatory processes. In that cohort, none of the blood cell counts at age 79 significantly predicted survival at 87 years [11]. Baune et. al made a 9-year follow-up with the aim to investigate inflammatory biomarkers with all-cause mortality in a cohort of 370 elderly (age 65-83), living in community dwelling [12]. Among the participants, 110 deaths occurred after 9 years

and IL-6, IL-8 and IL-10 were associated with all-cause mortality [12]. Within the EU project, Genetics of Healthy Ageing (GEHA), 1160 Italian sibling aged 90+, were investigated with the aim to inter alia identify survival predictors [13]. Most of them lived in own houses at the inclusion. Blood samples for common clinical haematological tests were performed. After six years, 718 out of 1160 died, and survival probability increased with high levels of Hb and low levels of WBC, neutrophil granulocytes and PLT [13].

Hence, indicators of survival time may be of clinical interest in a nursing home population. Despite previous research showing differences in analytes depending on age, there is a knowledge gap regarding analytes that may indicate survival in less healthy elderly populations.

The aim of the present investigation was to study whether 1-year changes in complete blood count (CBC) (including Hb, RBC, erythrocyte volume fraction (EVF), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), WBCs, and PLT), CRP and ILs (including IL-1β, IL-1Ra, IL-6, IL-8 and IL-10) are associated with 8-year survival in elderly nursing home residents, aged ≥80 years.

Method

Study population

In the present study, blood samples from 167 NHRs aged 80–101 years, mean age 88±4.5 years, 75% of whom were women, were collected over the period 2007–2009 in two municipalities in the south of Sweden [3]. During the year of investigation, the NHRs were carefully monitored for suspected infection by nursing staff and at any sign of medical, physical or cognitive change, which could be an expression of infection, as described elsewhere [3]. Also, blood sampling was done at baseline, after 6 and 12 months, i.e. when the NHRs were stable in their disease status and habitual condition. These occasions were separated with at least two weeks from occasions with suspected infection mentioned above. Physical status was assessed through interviews with the resident or the nursing assistants, using Katz personal (P) and instrumental (I) ADL index [14, 15]. The residents were graded from 0 to 10, where 0 = independency in all variables and 10 = dependency in all variables. All residents needed daily care and support, of whom 6% managed personal ADL with minor assistance and they all lived in group housing for the elderly. Dementia was diagnosed in 62%, diabetes mellitus type 2 in 19%, heart disease in 59%, malignancy in 25% and stroke in 35%. Data on chronic diseases were collected from medical records.

In the morning, non-fasting venous blood samples for analyses of CBC (described in Table 1), CRP and IL, including IL-1β, IL-1Ra, IL-6, IL-8 and IL-10, were collected in vacutainer tubes with ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. Complete blood count and CRP were measured with accredited routine methods at laboratories in Eksjö and Boxholm. Methodological principles in Eksjö were optical light scatter and colorimetric determination for CBC with CELL-DYN 3200 analyser (Abbott Diagnostics, St Clara, CA, USA) and for CRP immunoassay with ADVIA 1200 was used (Siemens Medical Solutions Diagnostics Inc., NY USA). In Boxholm Swelab alfa (Boule Diagnostics AB, Spånga, Sweden) was used for CBC and CRP, using impedance and photometric as methodological principle. Blood samples (EDTA tubes) for cytokines were centrifuged and plasma was transferred into tubes kept at -80°C until analysis. The cytokines were later analysed at Ryhov Hospital, Jönköping, using Luminex (Bio-Rad Laboratories, Hercules, CA, USA). Inter-assay coefficient of variation (CV) of multiplexed bead-based assays for cytokine detection has been shown to be 10-14% and inter-assay precision of duplicate wells averages <10% CV [16].

Analysis of CBC and CRP are commonly used in care for the elderly and cytokines are interesting because they react early in immunological responses. Blood samples were collected at baseline and 6 and 12 months. Depending on mortality and missed sampling, the number of individuals with available blood samples had decreased by the 6 and 12-month follow-up (Table 1). Dates of death were collected from the National Death Register 8 years after baseline. This time point was chosen because it (or a similar length of time) is often used, and since most individuals in a cohort of this setting will die during this period. At the end of the follow-up period, ten individuals were alive.

Table 1 Number of blood samples collected at baseline and at the 6- and 12-month follow-up, and reason for unavailable samples (death or missed sampling).

Analyte	Baseline	Death	Missed sampling	6 months	Death	Missed sampling	12 months	Baseline and 6 months	Baseline and 12 months	Baseline, 6 and 12 months
	n	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*	*n*
CBC	165	29	11	125	14	6	105	124	104	102
CRP	165	29	11	125	14	6	105	125	105	103
ILs	152	29	8	115	14	11	90	104	81	69

CBC = complete blood count, which includes haemoglobin (Hb), red blood cells (RBCs), erythrocyte volume fraction (EVF), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), white blood cells (WBCs) and platelet count (PLT). CRP = C-reactive protein; ILs = interleukins, including IL-1β, IL-1Ra, IL-6, IL-8 and IL-10.

Statistics

For comparisons of mean levels of analytes at baseline and at 6 and 12 months' follow-up, Student's *t*-test was used. With Bonferroni correction, an adjusted p value of 0.0167 was required for statistical significance when comparing the three time points. To explore predictors of survival, the Cox regression model with segmented time-dependent covariates was used. Survival in days from the inclusion date, up to a maximum of 8 years, was the dependent variable, with levels of analytes, age and gender as independent variables. Cox regression with a segmented time-dependent covariate was used to correct for the samples collected at baseline, and at 6 and 12 months. Age intervals of 5 year were used; 80–84 years, 85–89 years, 90–94 years and 95–101 years. The group of individuals aged 80–84 years was considered as a reference for the other age groups. The level of significance was set to $p<0.05$. For statistical calculations, PASW Statistics 25 (IBM SPSS Statistics, Chicago, IL, USA) was used.

Ethics

All participants, or their next of kin, gave written informed consent. It was made clear that participation was voluntary and could be withdrawn at any time. The study complied with the Declaration of Helsinki [17] and was approved by the Regional Ethical Board in Linköping, Sweden (Dnr: M-8206). The health service directors of community care gave their permission to conduct the study.

Results

No intraindividual differences in the absolute values of EVF, WBC, PLT, CRP, IL-1Ra, IL-6, IL-8 and IL-10 were found between baseline and 6 months, or between the 6 and 12 months' follow-up. For Hb, the mean value was lower at 12 months compared with 6 months ($p<0.01$). Regarding RBCs, mean value at 12 months were lower than at baseline ($p<0.01$). MCHC ($p<0.001$) was lower at 12 months compared with the 6 months' follow-up, and MCV was higher at 12 months compared with baseline ($p<0.01$). Of the analysed cytokines, only IL-1β showed differences with higher levels at 12 months ($p<0.001$) compared with baseline and 6 months (Table 2).

Table 2 Comparison of significant differences in mean values for analytes at baseline (0 months), and at 6 and 12 months' follow-up using Student's paired *t*-test.

Analyte	Months	N	Mean	SD	*p*-value
Hb	0/6	124	127.8/129.6	15.4/15.2	0.044
(g/L)	6/12	103	130.0/127.0	12.9/12.9	**0.002***
	0/12	104	128.3/126.8	14.6/12.7	0.160

RBCs	0/6	124	4.21/4.20	0.52/0.48	0.832
(x 10^{12})	6/12	103	4.22/4.15	0.45/0.47	0.036
	0/12	104	4.24/4.15	0.50/0.47	**0.008***
MCV	0/6	124	91.7/92.2	4.9/5.5	0.120
(fL)	6/12	103	92.3/92.7	4.8/5.1	0.169
	0/12	104	91.4/92.7	4.6/5.1	**0.001***
MCHC	0/6	124	332/334	9.5/9.0	0.076
(g/L)	6/12	103	334/331	8.6/9.3	**<0.001***
	0/12	104	332/331	10.1/9.4	0.284
IL-1β	0/6	104	0.71/0.49	1.20/0.35	0.040
(ng/L)	6/12	77	0.57/0.94	0.36/0.15	**<0.001***
	0/12	82	0.69/0.94	0.35/0.16	**<0.001***

* $p<0.0167$ (adjusted p value after Bonferroni correction).
EVF = erythrocyte volume fraction; Hb = haemoglobin; IL-1β = interleukin 1β; MCHC = mean corpuscular Hb concentration; MCV = mean corpuscular volume; RBCs = red blood cells.

To be included in this Cox analyses, the individual had to have survived >6 months and analytes from at least two of the three consecutive measuring occasions were needed. When taking all the results of the Cox regression model with a segmented time-dependent covariate into consideration, 94 participants were included. In this regression, raised levels of CRP ($p<0.001$) and IL-8 ($p<0.05$) were significantly associated with reduced survival time (Table 3). In total 102 participants had CBC, 103 had CRP and 69 participants had cytokines from all three measuring occasions.

Table 3 Days of survival, related to analytes, age and gender, at baseline and at 6 and 12 months, using the Cox regression model with segmented time-dependent covariates (n=94).

	n	**Exp[B]**	**95% CI**	***p*-value**
Hb		1.077	0.804–1.442	0.620
RBC		0.062	0.003–1.323	0.075
EVF		1.035	0.379–2.827	0.946
MCV		0.916	0.812–1.034	0.158
MCHC		0.978	0.879–1.088	0.677
WBC		0.964	0.851–1.092	0.567
PLT		1.001	0.997–1.004	0.684
CRP		1.059	1.030–1.088	<0.001***
IL-1β		0.499	0.204–1.222	0.128
IL-1Ra		1.003	1.000–1.007	0.080
IL-6		1.014	0.974–1.056	0.503
IL-8		1.024	1.002–1.046	0.029*
IL-10		0.973	0.873–1.085	0.622
Age 80–84 yrs	22			
Age 85–89 yrs	36	2.026	1.076–3.815	0.029*
Age 90–94 yrs	34	2.542	1.340–4.822	0.004**
Age 95–101 yrs	2	0.730	0.155–3.442	0.691
Sex m/f 25/69		1.001	0.596–1.684	0.996

*$p<0.05$; **$p<0.01$; ***$p<0.001$.

Exp[B] = exponentiation of B coefficient, odds ratio; 95% CI = 95% confidence interval; CRP = C-reactive protein; EVF = erythrocyte volume fraction; Hb = haemoglobin; IL = interleukin; MCHC = mean corpuscular Hb concentration; MCV = mean corpuscular volume; RBCs = red blood cells; PLT = platelet count; WBCs = white blood cells.

Discussion

In the present study, levels of analytes were investigated in elderly NHR individuals during a period of 1 year and in relation to days from inclusion up to a maximum of 8 years. The most prominent result was that elevated levels of CRP at baseline were connected to shorter survival as shown by the Cox regression model with segmented time-dependent covariates. Also, elevated IL-8 at baseline was connected to shorter survival. These elevated levels might be related to inflammatory processes that are common in elderly. Even if all participants were ≥80 years old, there was still variation in age, as the oldest participant had reached the age of 101 years. In this statistical analysis, however, neither age nor sex affected the results for CRP and IL-8 and lack of correlation between CRP measurement and increased age are supported in previous studies [18]. In the interpretation of these results it should be considered that the ageing process is associated with chronic low-grade inflammation, which may shorten survival time [12]. Other authors have found an association between higher levels of high-sensitivity CRP (hs-CRP) and increased risk of death [9]. High-sensitivity CRP is a standard method that has been validated to detect lower levels of CRP than detected applying the traditional CRP method we used. As infectious diseases in frail elderly people are challenging to detect because of lack of specific signs and symptoms [19, 20], measuring CRP with a bedside instrument could be an option in the nursing home setting to detect suspected infection early on in this population. When measuring CRP it is crucial to have baseline values for comparison. This was not a focus in the present study, so it needs to be further investigated. Even though it may not be possible to increase survival rates, adequate diagnosis and treatment of symptoms due to inflammation could improve wellbeing at end of life.

Interestingly, we found that IL-8 was significantly associated with shorter survival, which probably is related to inflammatory processes, which also is in line with results of Baune et al. in their study on short and long-term mortality rates [12]. They also investigated IL-1β, IL-6 and IL-10, but did not find any association for IL-1β, which is in agreement with our results. However, Baune et al. report that IL-6 and IL-10 had a prognostic value for mortality [12]. Valiathan et al. found differences in levels of IL-6 in elderly people >70 years old compared with individuals aged <50 years. However, they did not report any differences for CBC, IL-8 or Il-10 [8]. The fact that the results differ between different investigations, may to some extend depend on methodological differences. Regarding IL-8,

most values were within the range of the method but IL-8 occurred in larger quantities than the other cytokines, and this might be a reason why only IL-8 became significant in our statistical analysis.

Because the investigated NHRs have different diseases and could be considered as frail, we have paid a lot of attention to just collect blood samples when they were free from additional stresses, like ongoing infection. The NHRs were monitored carefully from the nursing staff that met them daily and knew them well [3]. Any sign of medical, physical or cognitive change have been paid attention and blood samples to this investigation (i.e. baseline, 6 and 12 months) have been taken at later occasions, at least two weeks afterwards.

Moreover, we also found higher levels of IL-1β after 12 months compared with baseline, which could be due to increased inflammatory activity in the investigated cohort. Compared with baseline, levels for RBC and MCV were lower and higher, respectively, at 12 months. The decrease in levels of RBC between baseline and 12 months is in line with other studies [7, 11] and could be due to impaired renal function [11]. Also, the tendency for a decrease in Hb in our 1-year follow-up is similar to Sebastiani et al., even though they compared Hb between different age groups [7]. During a follow-up period of 8 years, Starr & Deary [11] found increased levels of PLT, and Sebastiani et al. [7] reported increased levels of WBC and IL-6 with increased age. None of these differences were found in our investigation. A possible explanation may be that our 1-year period of blood sampling was too short. In our study, carried out on a NHR population with high burden of co-morbidity and cognitive and physical impairment, a 1-year follow-up must be considered reasonable, as 43 out of 167 participants died during the first year and ten individuals were still alive after 8 years. However, both our and others' results warrant further study of the clinical relevance of these analytes.

When comparing our results regarding cytokines with results reported by others [7, 8, 10], we found both similarities and differences. Explanations of differences could be that different methods were used and it is difficult measure the low levels of circulating cytokines.

A limitation when comparing results from different studies with samples from elderly individuals is that the description of inclusion criteria may be vague [8, 11], since consensus on frailty is lacking. Another problem when studying elderly individuals is that inclusion criteria could mean that very frail elderly people are often excluded, while an elite of the healthiest individuals remains [11]. We noted that only nine (7%) out of 138 NHRs were assessed as 'healthy', in terms of being free from heart disease, autoimmune disease, dementia, stroke, diabetes mellitus type 2, malnutrition and

receiving paracetamol [21]. The health status differs a lot between the included individuals in this investigation. However, all of them are living in nursing homes, and hence their overall health is not that good. The differences among the participants when coming to for example different diseases would of course have affected the results in this study. Further investigations are needed to develop reliable reference intervals when evaluating results from blood tests for this group of people.

Conclusion

In the present study, levels of analytes were investigated in elderly NHR individuals during a period of 1 year and in relation to time of death during a follow-up of 8 years. The most prominent results were that elevated levels of CRP and IL-8 at baseline were connected to shorter survival. These elevated levels might be related to inflammatory processes that are common in elderly.

Abbreviations

NHR: nursing home resident; ADL: activities of daily living; BMI: body mass index; Hb: haemoglobin; RBC: red blood cell; WBC: white blood cell; CRP: C-reactive protein; IL: interleukin; EVF: erythrocyte volume fraction; MCV: mean corpuscular volume; MCHC: mean corpuscular Hb concentration; PLT: platelet count; EDTA: ethylene diamine tetra-acetic acid; hs-CRP: high-sensitivity CRP

Declarations

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author upon request. All data and material will be made available.

Authors´ contributions

MS-L, ME, AM, JE and EG designed the study. MS-L acquired the participants and the data. ME conducted the analyzes and performed the statistical analysis. ME, JE and EG interpreted the data. ME, MS-L and EG drafted the manuscript. ME, MS-L, AM, JE and EG critically revised the manuscript and approved the final manuscript.

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