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Association between inflammatory markers and hospitalizations: evidence from the Bambuí cohort of aging baseline

Associação entre marcadores inflamatórios e ocorrência de hospitalizações: evidências da linha de base da coorte de idosos de Bambuí

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ABSTRACT: ***Introduction:*** Inflammation plays an important role in the aging process. ***Objective:*** This cross-sectional study aims to examine the association between inflammatory markers and hospitalizations among older adults, considering as potential confounding factors the predisposing and enabling factors for the use of health services and health conditions. ***Methods:*** We used data from 1,393 participants (≥ 60 years) in the baseline cohort from Bambuí. The markers assessed were ten cytokines and chemokines [interleukin (IL)-1, IL-6, IL-10, IL-12, tumor necrosis factor (TNF), CCL2, CCL5, CXCL8, CXCL9, and CXCL10]. The outcome variable was one or more hospitalizations in the preceding 12 months. ***Results:*** Elevated serum levels of IL-6 were significantly associated with hospitalizations [prevalence ratio (PR) = 1.38; confidence interval of 95% (95%CI) 1.02 – 1.87 and PR = 1.38; 95%CI 1.01 – 1.88 for the intermediate and highest tertiles, respectively]. High levels of CXCL9 were also independently associated with the outcome (PR = 1.38; 95%CI 1.01 – 1.89 and PR = 1.46; 95%CI 1.07 – 2.00, respectively). Other markers showed no statistically significant association with hospitalizations. ***Conclusion:*** Among the ten markers analyzed, only IL-6 and CXCL9 were associated with hospitalizations.

Keywords: Inflammation. Chemokines. Cytokines. Hospitalization. Aging.

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RESUMO: ***Introdução:*** A inflamação exerce um importante papel no processo de envelhecimento. ***Objetivo:*** Este estudo transversal objetiva examinar a associação entre marcadores inflamatórios e a ocorrência de hospitalizações entre idosos, considerando fatores predisponentes e facilitadores do uso de serviços de saúde e condições de saúde como potenciais fatores de confusão. ***Métodos:*** Foram utilizados dados de 1.393 participantes (≥ 60 anos) da linha de base da coorte de Bambuí. Os marcadores considerados foram dez citocinas e quimiocinas (interleucina (IL)-1, IL-6, IL-10, IL-12, fator de necrose tumoral (TNF), CCL2, CCL5, CXCL8, CXCL9 e CXCL10). A variável de desfecho foi a ocorrência de uma ou mais hospitalizações nos 12 meses precedentes. ***Resultados:*** Níveis séricos elevados da IL-6 apresentaram associações significantes com a ocorrência de hospitalizações (razão de prevalência — RP = 1,38; intervalo de confiança — IC95% 1,01 – 1,87; e RP = 1,38; IC95% 1,02 – 1,88, para os tercis intermediário e superior, respectivamente). Níveis elevados da CXCL9 também apresentaram associações independentes com o desfecho (RP = 1,38; IC95% 1,01 – 1,89 e RP = 1,46; IC95% 1,07 – 2,00, respectivamente). Os demais marcadores não apresentaram associações estatisticamente significantes com a ocorrência de hospitalizações. ***Conclusão:*** Entre os 10 marcadores examinados, IL-6 e CXCL9 apresentaram associação com a ocorrência de hospitalizações.

Palavras-chave: Inflamação. Quimiocinas. Citocinas. Hospitalização. Envelhecimento.

INTRODUCTION

Inflammation plays an important role in the aging process[1]. Previous researches show that the increase in serum levels of inflammatory markers, particularly interleukin-6 (IL-6) and/or tumor necrosis factor-alpha (TNF-alpha), is associated with the rise in mortality risk, as well as the higher incidence of cardiovascular diseases, diabetes, physical and cognitive decline, among other adverse events[2]. This evidence suggests that inflammatory markers can be useful in stratifying the risk of adverse events among older adults, in order to identify vulnerable groups for early intervention and rehabilitation[2-4].

The prevalence and incidence of chronic diseases and functional limitation increase with old age, leading to a higher need for health care. According to the classic model by Andersen & Newman[5], in addition to health needs, factors associated with the use of health services include predisposing (such as age and gender) and enabling (such as schooling and income) characteristics.

Two recent longitudinal studies suggest that the increase in serum levels of some inflammatory markers is associated with the rise in hospitalization risk among older adults. The first of these studies, conducted in Alabama, comprised 370 adults aged 65 years or older. The results showed that an inflammation score based on four markers, among them IL-6 and C-reactive protein, was associated with future hospitalizations, despite some predisposing and enabling factors[3]. A more recent study, held in Belgium, assessed the prognostic value of a battery of cytokines and chemokines in predicting hospitalization among 415 adults aged 80 years or older. The results indicated that only IL-6 had prognostic value for hospitalization in the study population[4].

The exact mechanisms involved in these association are still not clear. Inflammation can lead to a higher risk of hospitalization by demonstrating the individual's disease burden as it acts directly in the organism (for instance, the catabolic effect on muscle mass, predisposing the person to functional limitation) or due to a combination of these factors[2-4]. To the best of our knowledge, no study analyzed this association in Brazil and the literature on the topic is restricted to the two studies aforementioned, indicating the need for further investigations to confirm or deny these associations.

The present cross-sectional study used data from the baseline cohort of older adults from Bambuí with two objectives:

1. examine the existing associations between multiple inflammatory markers (cytokines and chemokines) and hospitalizations;
2. verify if these associations are independent of health conditions and predisposing and enabling factors for the use of health services.

METHODS

STUDY POPULATION

The Bambuí cohort was established in the city of the same name (with approximately 15,000 inhabitants), located in the state of Minas Gerais. Previous publications[6,7] described the recruitment of participants and the procedures used in the baseline cohort. Briefly, the eligible study population consisted of all city residents aged 60 years or older on January 1, 1997 (n = 1,742). Out of them, 1,660 participated in the baseline survey. The participants signed the Informed Consent Form. The Research Ethics Committee of Fundação Oswaldo Cruz, Rio de Janeiro, approved the baseline survey.

The Ethics Committee of Fundação Oswaldo Cruz, Rio de Janeiro, approved this cohort in 1996.

STUDY VARIABLES

The dependent variable of this study was the incidence of hospitalizations in the 12 months prior to the interview date of the baseline survey. Hospitalization was defined as at least one night of hospital stay and discharge.

The inflammatory markers included in this analysis were five cytokines (IL-1, IL-6, IL-10, IL-12, and TNF) and five chemokines (CCL2, CCL5, CXCL8, CXCL9, and CXCL10). The Cytometric Bead Array Assay (CBA immunoassay kit; Becton Dickinson Biosciences Pharmingen, San Diego, USA) performed the quantitative analysis of these markers. Data were collected using the flow cytometer FACSVerse (Becton Dickinson, USA) and the samples were analyzed with the aid of the software BD FCAP Array 3.0 (Becton Dickinson,

USA). Standard curves determined marker concentrations and results were expressed in picograms per milliliter (pg/mL). Doses of those markers were stored in aliquots at -80°C, in 2014. The blood sample for these tests was collected during the baseline cohort survey.

The selection of potential confounding variables for the study followed the theoretical framework proposed by Andersen & Newman[5], mentioned above. The predisposing and enabling factors for the use of health services considered in this study were age, gender, and schooling (categorized as < 4 and 4 years). Health needs were established by systolic blood pressure and total cholesterol levels, and presence of coronary heart disease (myocardial infarction or angina pectoris), stroke, heart failure, diabetes, and functional limitation. Systolic blood pressure was stipulated by the mean of two out of three blood pressure measurements, dismissing the first one. Coronary heart disease was determined using the Rose Questionnaire[8]. Medical history established the presence of myocardial infarction and stroke. Heart failure was associated to individuals with serum levels of B-type natriuretic peptide greater than 100 pg/mL, as recommended by the manufacturer (AxSYM MEIA; Abbott Laboratories, Inc., Abbott Park, Illinois). Diabetes mellitus was defined by fasting blood glucose > 126 mg/dL and/or use of oral hypoglycemic agents or insulin. Functional limitation was related to the report of major difficulty in performing the following basic activities of daily living: dressing, bathing/showering, toileting, feeding, ambulating – walking from one room to another on the same floor –, and transferring – moving from bed to chair. The blood sample for biochemical tests was collected early in the morning, with a recommendation of 12 hours of fasting. Cholesterol and glucose levels were measured based on traditional enzymatic methods (Merck, Darmstadt, Germany). More details can be found in a previous publication[6].

STATISTICAL ANALYSIS

The unadjusted analysis used the χ^2, Student's t, and Kruskal-Wallis tests to assess the statistical significance of the differences between frequencies, means, and medians, respectively. These analyses considered the serum levels of IL-10, IL-12, TNF, and IL-1 as dichotomous variables (detected versus undetected), since their detection levels were very low. The other markers were evaluated as continuous variables, estimating the median and quartiles.

Markers that showed statistically significant associations ($p < 0.05$) with the outcome in the unadjusted analysis were selected for multivariate analyses. These analyses were based on prevalence ratios estimated by Poisson regression with robust variance[9], considering the distribution of each marker into tertiles. Initially, we estimated the prevalence ratios adjusted for age and gender. Next, the models included the other variables (enabling and need factors). Lastly, we used a binary logistic regression to estimate the predicted probability of hospitalization in different ages, according to serum levels (in tertiles) of the two markers (IL-6 and CXCL9) that remained associated with the outcome in previous analyses, and plotted these results. All analyses used the statistical package Stata[a] (version 14.1).

RESULTS

This analysis included 1,393 participants in the Bambuí cohort who had complete information for all study variables. Table 1 presents the sociodemographic and health characteristics of these individuals, according to the incidence of hospitalizations. The mean age of the study participants was 69.9 years [standard deviation (SD) = 7.0)], 60.9% were women, and 64.9% had less than 4 years of schooling. The following variables showed positive and statistically significant associations ($p < 0.05$) with hospitalizations: age, gender, schooling, stroke, coronary heart disease, heart failure, and functional limitation.

Table 2 indicates the distribution of serum levels of inflammatory markers, according to the incidence of hospitalizations. Compared to non-hospitalized older adults, those who were hospitalized in the preceding 12 months had higher serum levels of IL-6 (median = 1.31 pg/mL among hospitalized and 0.98 pg/mL among non-hospitalized; $p = 0.003$),

Table 1. Sociodemographic characteristics and health conditions of participants in the baseline cohort of older adults from Bambuí, according to the incidence of hospitalizations in the preceding 12 months.

Variables	Total (n = 1,393)	Hospitalizations		P-value
		Yes (n = 289)	No (n = 1,104)	
Predisposing factors				
Age, mean (SD)	69.9 (7.0)	69.9 (7.4)	68.7 (6.9)	0.007
Women	60.9	68.2	59.0	0.004
Enabling factors				
Less than 4 years of schooling	64.9	69.6	63.0	0.040
Health needs				
Systolic blood pressure in mmHg, mean (SD)	137.2 (22.7)	137.8 (24.6)	137.1 (22.2)	0.623
Total cholesterol in mg/dL, mean (SD)	233.7 (49.3)	235.1 (51.5)	233.3 (48.8)	0.586
Stroke[a]	3.6	6.2	2.9	0.007
Coronary heart disease (myocardial infarction[1] or angina pectoris[b])	12.4	19.2	10.6	< 0.001
Heart failure[c]	40.6	49.3	38.3	0.001
Diabetes (fasting blood glucose > 126 mg/dL and/or treatment)	14.6	17.7	13.9	0.105
Functional limitation[d]	5.9	10.0	4.8	0.001

All values are expressed as percentage, except when specified; SD: standard deviation; p-value: Pearson's χ^2 test and Student's t test for differences between frequencies and means; [a]medical history of the disease; [b]Rose questionnaire (World Health Organization); [c]B-type natriuretic peptide > 100 pg/mL; [d]major difficulty or impairment in performing basic activities of daily living (dressing, bathing/showering, toileting, feeding, ambulating – walking from one room to another on the same floor –, and transferring – moving from bed to chair).

CXCL9 (median = 2,973 pg/mL and 2,135 pg/mL, respectively; $p < 0.001$), and CXCL10 (median = 3,336 pg/mL and 2,956 pg/mL, respectively; $p = 0.033$). Other cytokines and chemokines showed no statistically significant association with the outcome ($p > 0.05$ for all).

As seen in Table 3, elevated serum levels of IL-6 were significantly associated with hospitalizations in the analysis adjusted for gender and age [prevalence ratio (PR) = 1.48; confidence interval of 95% (95%CI) 1.09 – 1.99 and PR = 1.52; 95%CI 1.12 – 2.05 for the highest tertile, compared to the lowest one], and the analysis subsequently adjusted for schooling and health conditions (PR = 1.38; 95%CI 1.02– 1.87 and PR = 1.38; 1.01 – 1.88, respectively). High levels of CXCL9 were also independently associated with the outcome, both in the analysis adjusted for gender and age (PR = 1.38; 95%CI 1.02 – 1.83 and PR = 1.55; 95%CI 1.14 – 2.10 for the intermediate and highest tertiles, respectively) and the analysis subsequently adjusted for other covariates (PR = 1.38; 95%CI 1.01 – 1.89 and PR = 1.46; 95%CI 1.07 – 2.00, respectively). With respect to CXCL10, the association previously identified in the unadjusted analysis lost statistical significance after adjustments for age and gender, and the lack of association persisted in the model adjusted for other covariates.

Figure 1 illustrates the predicted probability of hospitalization, according to age and serum levels of IL-6 and CXCL9 in tertiles. In all ages, individuals with serum levels of IL-6 in the intermediate and highest tertiles had significantly higher chances of hospitalization, compared to their peers with values in the lowest tertile. CXCL9 presented a graded

Table 2. Distribution of serum levels of inflammatory markers among participants in the baseline cohort of older adults from Bambuí, according to the incidence of hospitalizations in the preceding 12 months.

Marker	Total (n = 1,393)	Hospitalizations		p-value
		Yes (n = 289)	No (n = 1,104)	
IL-6	1.04 (0.45 – 2.11)	1.31 (0.66 – 2.24)	0.98 (0.41 – 2.04)	0.003
CXCL8	3.05 (1.60 – 5.63)	3.35 (1.71 – 6.24)	2.93 (1.58 – 5.44)	0.086
CCL2	38.4 (25.3 – 57.6)	39.0 (23.8 – 59.5)	38.4 (25.4 – 57.3)	0.800
CXCL9	2,305 (1,225 – 4,091)	2,973 (1,178 – 3,895)	2,135 (1,178 – 3,805)	< 0.001
CCL5	870 (548 – 1,638)	844 (509 – 1,662)	888 (562 – 1,635)	0.226
CXCL10	3,039 (2,007 – 4,789)	3,336 (2,088 – 5,134)	2,956 (1,980 – 4,667)	0.033
Marker	Percentage above detectable value	Percentage above detectable value	Percentage above detectable value	p-value
IL-10	42.5	45.7	41.7	0.220
IL-12	7.5	5.5	8.0	0.160
TNF[b]	17.3	18.0	17.1	0.727
IL-1β[c]	22.3	24.9	21.6	0.222

P25–P75: 25 and 75 percentiles. All values are presented as median in pg / ml (25-75th percentile), unless otherwise specified.

association, that is, the probability of hospitalization in all ages gradually increased from the lowest to the intermediate and highest tertiles, respectively.

DISCUSSION

The results of this study, conducted in a large population of older adults, revealed that, out of 10 inflammatory markers, only IL-6 and CXCL9 showed statistically significant associations with hospitalizations in the preceding 12 months. The associations found did not depend on predisposing and enabling factors for the use of health services, as well as indicators of health conditions and functional capacity.

IL-6 is a glycoprotein produced mainly by leukocytes, adipocytes, and endothelial cells in response to various inflammatory stimuli, such as toxins, infections, and other cytokines[10]. Several studies have demonstrated that the serum concentration of this marker increases with age and that it is involved in the development of the chronic inflammatory condition characteristic of aging[10]. High levels of IL-6 are also associated with cognitive decline, changes in respiratory function, increase in physical frailty, and a higher risk of cardiovascular, metabolic, and neoplastic diseases[10-12].

Table 3. Multivariate analysis results of the association between selected inflammatory markers and hospitalizations in the preceding 12 months among participants in the baseline cohort of older adults from Bambuí.

Markers in tertiles (pg/mL)	Percentage of hospitalizations	PR (95%CI) adjusted for gender and age	PR (95%CI) adjusted for gender, age, schooling, and health conditions[a]
IL-6			
Lowest (< 0.63)	15.2	1.0	1.0
Intermediate (0.63 – 1.62)	23.3	1.48 (1.09 – 1.99)	1.38 (1.02 – 1.87)
Highest (³ 1.63)	23.9	1.52 (1.12 – 2.05)	1.38 (1.01 – 1.88)
CXCL9			
Lowest (< 1,536)	15.3	1.0	1.0
Intermediate (1,536 – 3,349)	21.3	1.38 (1.02 – 1.83)	1.38 (1.01 – 1.89)
Highest (³ 3,350)	25.6	1.55 (1.14 – 2.10)	1.46 (1.07 – 2.00)
CXCL10			
Lowest (< 2,346)	18.5	1.0	1.0
Intermediate (2,347 – 3,961)	18.5	0.96 (0.71 – 1.30)	0.95 (0.70 – 1.29)
Highest (³ 3,962)	25.2	1.27 (0.96 – 1.69)	1.21 (0.91 – 1.62)

PR: prevalence ratio; 95%CI: confidence interval of 95% estimated by Poisson regression; [a]systolic blood pressure, total cholesterol, stroke, coronary heart disease, heart failure, diabetes, and functional limitation as specified in Table 1.

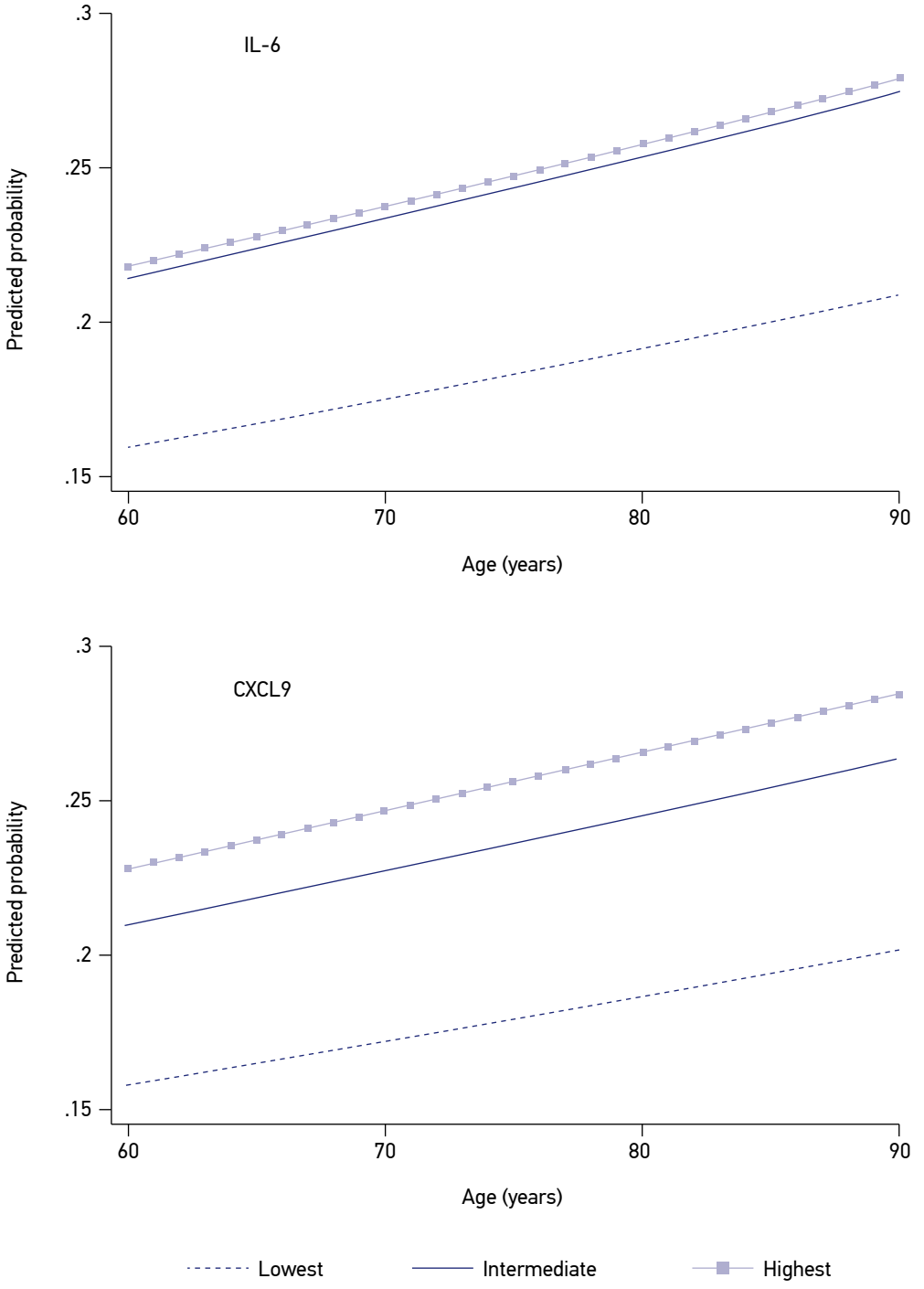

Figure 1. Predicted probability of hospitalizations in the preceding 12 months among participants in the baseline cohort of older adults from Bambuí, according to age and serum levels of inflammatory markers in tertiles.

As previously mentioned, two longitudinal studies showed that IL-6 has prognostic value for hospitalizations among older adults[3,4]. Therefore, its association with a higher incidence of hospitalizations in the study population is not unexpected. Our results add to previous studies by showing that adjustments for important indicators of health conditions reduce the strength of this association, but it remains statistically significant. Also, we underline that relatively low levels of IL-6 (> 0.63 pg/mL) were associated with the outcome.

To the best of our knowledge, no previous studies examined the association between serum levels of CXCL9 and hospitalizations among older adults. In the present analysis, this marker revealed a strong graded association with the outcome. CXCL9, CXCL10, and CXCL11 are part of a group of chemokines induced by interferon gamma (IFN-gamma) and play an important role in leukocyte recruitment to inflammation and infection sites and inhibition of angiogenesis[13]. Studies have shown that high levels of these chemokines are present in some neurological and rheumatic diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. This finding suggests that these chemokines could contribute to inducing or exacerbating a chronic inflammatory response associated with autoimmune diseases[13]. Thus, the association between elevated serum levels of CXCL9 and hospitalizations found in this study is biologically plausible.

This study has advantages and limitations. The main advantages are the large population base and the use of robust indicators of health conditions that could confound the associations examined. Another benefit is the analysis of various cytokines and chemokines, which only became possible more recently, with the availability of reliable kits to measure these dosages. The main limitation of this study is its cross-sectional nature, which prevents us from establishing temporal relationships between inflammatory markers and the outcome. In other words, it is not possible to know if the inflammation preceded or was a consequence of hospitalization, a situation that characterizes reverse causality[14]. Lastly, both hospitalizations and dosage of inflammatory markers occurred at baseline cohort, that is, about 20 years ago. Although the prevalence or incidence of these measures could have changed over time, it is unlikely that these changes happened differentially, affecting the magnitude of the associations found.

CONCLUSION

Our results show that older adults hospitalized in the preceding 12 months had high levels of two inflammatory markers (IL-6 and CXCL9) which, in turn, were associated with an increased risk of developing several diseases, chronic conditions, and other adverse events, as demonstrated by the literature on the topic[2-4,10-13]. Our results are limited to allow inferences about the prognostic value of these markers for hospitalizations. Longitudinal analyses are underway for a better understanding of these relationships, and to determine if the increase in serum levels of these markers precedes or is a consequence of hospitalization.

REFERENCES

1. Franceschi C, Valensin S, Lecai F, Olivieri F, Licastro F, Grimaldi LM, et al. Neuroinflammation and the genetics of Alzheimer's disease: the search for a pro-inflammatory phenotype. Aging (Milano) 2001; 13(3): 163-70.
2. Singh T, Newman AB. Inflammatory markers in population studies of aging. Ageing Res Rev 2011; 10(3): 319-29.
3. Salanitro AH, Ritchie CS, Hovater M, Roth DL, Sawyer P, Locher JL, et al. Inflammatory biomarkers as predictors of hospitalization and death in community-dwelling older adults. Arch Gerontol Geriatr 2012; 54(3): e387-91.
4. Adriaensen W, Matheï C, Vaes B, van Pottelbergh G, Wallemacq P, Degryse JM. Interleukin-6 as a first-rated serum inflammatory marker to predict mortality and hospitalization in the oldest old: A regression and CART approach in the BELFRAIL study. Exp Gerontol 2015; 69: 53-61.
5. Andersen R, Newman JF. Societal and individual determinants of medical care utilization in the United States. Milbank Mem Fund Q Health Soc 1973; 51(1): 95-124.
6. Lima-Costa MF, Firmo JO, Uchoa E. Cohort profile: the Bambui (Brazil) cohort study of ageing. Int J Epidemiol 2011; 40(4): 862-7.
7. Lima-Costa MF, Firmo JOA, Uchoa E. The Bambui Cohort Study of Aging: methodology and health profile of participants at baseline. Cad Saúde Pública 2011; 27(Suppl. 3): S327-35.
8. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 1962; 27(6): 645-58.
9. Long S, Freese J. Regression models for categorical dependent variables using Stata. 2nd ed. College Station, TX: StataCorp LP; 2006.
10. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in Aging and Chronic Disease: A Magnificent Pathway. J Gerontol A Biol Sci Med Sci. 2006; 61(6): 575-84.
11. Chang SS, Vaz Fragoso CA, Van Ness PH, Fried LP, Tinetti ME. Association between combined interleukin-6 and c-reactive protein levels and pulmonary function in older women: results from the Women's Health and Aging Studies I and II. J Am Geriatr Soc 2011; 59(1): 113-9.
12. Volpato S, Guralnik JM, Ferrucci L, Balfour J, Chaves P, Fried LP, Harris TB. Cardiovascular disease, interleukin-6, and risk of mortality in older women the women's health and aging stud. Circulation 2001; 103(7): 947-53.
13. Lacotte S, Brun S, Muller S, Dumortier H. CXCR3, inflammation, and autoimmune Diseases. Send to Ann N Y Acad Sci 2009; 11731(1): 310-7.
14. Szklo M, Javier Nieto E. Epidemiology: Beyond the Basics. 2nd ed. Massachusetts : Jones & Bartlett Publishers; 2007.

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