Predictive value of multiple cytokines and chemokines for mortality in an admixed population: 15-year follow-up of the Bambui-Epigen (Brazil) cohort study of ageing

Short title: Inflammatory markers and mortality

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Abstract

Inflammation, particularly elevated IL-6 serum levels, has been associated with increased mortality risk, mostly in Caucasians. The influence of genetic ethno-racial background on this association is unknown. We examined associations between baseline serum levels of Interleukin-6 (IL-6) and other cytokines (IL1-2, TNF, IL-10, and IL1β) and chemokines (CCL2, CCL5, CXCL8, CXCL9 and CXCL10) with 15-year mortality in 1,191 admixed Brazilians aged 60 years and over. Elevated IL6 level (but not other biomarkers) was associated with increased risk of deaths with fully adjusted hazard ratios of 1.51 (95% CI = 1.15, 1.97), 1.54 (95% CI = 1.20, 1.96) and 1.79 (95% CI = 1.40, 2.29) for the 2nd, 3rd and the highest quartiles, respectively. Genomic African and Native American proportions did not modify the association ($p>0.05$). The discriminatory ability to predict death of a model based on IL-6 alone was similar as that of a comprehensive morbidity score (C statistics = 0.59 and 0.60, respectively). The abilities of IL-6 and the morbidity score models to predict death remained stable for very long term after the baseline measurement. Our results indicate that genome-based African and Native American ancestries have no impact on the prognostic value of IL-6 for mortality.

Key words: Inflammatory markers, Interleukin-6, cytokines, chemokines, mortality, genomic ancestry, admixed population

Introduction

The aging process results from molecular and cellular damage over time that leads to a gradual decrease in physical and mental functioning, an increased risk of disease, and ultimately, death [1]. Epidemiologic studies conducted in the United States and in Europe have reported that elevated baseline levels of Interleukin-6 (IL-6), which is a pro-inflammatory cytokine [2], is associated with subsequent mortality risk in middle-aged [3] and older adults [4-11]. The prognostic value of other chemokines and chemokines for mortality (e.g. TNF, CXCL8, IL-10 and others) have been examined in more recent studies with inconsistent results [5,9, 12-15].

Inflammation, particularly elevated IL-6 serum levels, has been associated with a number of important age-related conditions, such as cardiovascular diseases, diabetes, physical functioning and cognitive decline, which in turn increase the risk of death [12]. Greater waist circumference, smoking and depression are positively associated with elevated IL-6 levels, while continuous physical exercise appears to have a protective effect [12]. Levels of several cytokines, but specially IL-6 and TNF, also appear to increase with age, even in apparently healthy individuals [12]. Therefore, there is a debate on whether "inflammation" is an independent risk factor for mortality or an expression of the burden of chronic conditions in later life [12]. Furthermore, it has been postulated that inflammatory markers may be better predictors of mortality, in relation to co-morbidity indexes, because measures of inflammation may capture sub-clinical conditions [5,16]. To the best of our knowledge, however, there is a lack of large epidemiological studies quantifying the ability of inflammatory markers to predict mortality in comparison with those of co-morbidity indexes. This issue may be of particular relevance in developing countries where the burden of diseases is greater [17].

Previous studies examining the association between inflammation and subsequent mortality were conducted predominantly in Caucasians [3-15]. To our knowledge, no previous study has specifically examined the influence of genetic ethno-racial background on those associations. Brazil, the largest Latin American country, offers a valuable opportunity to explore this issue. The Brazilian population originates from African, European and Native American ancestral roots [18]. The absence of legal segregation and other factors contributed to an emergence of a highly admixed population [19].

We used 15-year follow-up data from the Bambui-Epigen study, the longest community-based cohort study of aging in Brazil [20], with three main objectives: (1) to examine the association between multiple cytokines and chemokines, particularly IL-6, with very long term mortality; (2) to examine whether genomic African and Native American ancestry levels affect the ability of those biomarkers in predicting mortality; and (3) to compare the ability of IL-6 to predict mortality as that of a comprehensive morbidity score. A well-defined community-based sample, the ethno-racially admixed nature of our study population, and the very long follow-up period provide a rare opportunity to address these questions.

Materials and Methods

Study design and population

The Bambui Cohort Study of Aging is ongoing in Bambuí, a city of approximately 15,000 inhabitants in the State of Minas Gerais in Southeast Brazil. From an ethno racial perspective, the cohort population consists of an admixture of African (≅ 10%), Native American (≅ 5%), and European (≅ 85%) genomic ancestries, in similar proportions to that estimated for the Brazilian population, excluding the Amazon region (19). All three

groups are substantially admixed with considerable overlap. Detailed information on this cohort can be found elsewhere [20]. Briefly, the population eligible for the cohort consisted of all residents aged 60 years and over on 1 January 1997 (92% of the 1,742 inhabitants in this age group participated). Annually, from 1997 to 2011, cohort members underwent subsequent annual follow-up by face-to-face interview. Blood collection and other procedures for the current analysis where performed at the baseline survey.

Mortality data source

Deaths occurring from study enrolment to 31st December 2011 were included in this analysis. Deaths were reported by next of kin during the annual follow-up interview and were ascertained through the Brazilian mortality information system (in Portuguese, Sistema de Informações sobre Mortalidade) with the consent of the Brazilian Ministry of Health. Death certificates were obtained for 96.0% of all deceased participants. Deaths assigned to any cause were considered in this analysis.

Inflammatory markers (cytokines and chemokines)

Blood samples for measurement of cytokines and chemokines were collected at the baseline survey in early morning and stored at -80º C until use. The Cytometric bread array assay (CBA immunoassay kit; Becton Dickinson Biosciences Pharmingen, USA) was used for the quantitative determination of the serum cytokines (Human Inflammatory kit) and chemokines (Human Chemokines kit). The Inflammatory CBA kit comprises micro beads coupled to monoclonal antibody (MoAb) against the following cytokines IL-6, IL-12, TNF, IL-10, and IL-1β, and, the chemokine CBA kit detect CXCL8, CCL2, CXCL9, CCL5 and CXCL10. A second fluorescently labelled phycoerythrin (PE)-anti-cytokine antibody was used and the concentration of the individual cytokines was

indicated by their fluorescent intensity. Data was acquired using a FACSVerse flow cytometer (Becton Dickinson, USA). BD FCAP Array 3.0 software (Becton Dickinson, USA) was used for sample analysis. The coefficients of variation intra and inter-assays were 5-10% and 7-12%, respectively. Based on their distributions, IL-6, CXCL8, CCL2, CXCL9, CCL5 and CXCL10 were categorized into tentiles for exploratory analyses. IL-12, TNF and IL-1β showed very low detectable levels and were considered as dichotomous variables (non-detectable vs. ≥ 0.01 pg/mL). IL-10 showed a highly asymmetric distribution and was also considered as a dichotomous variable (non-detectable vs. ≥ 0.14 pg/mL).

Genetic and ancestry analyses

Cohort participants were genotyped with the Omni 2.5M array (Illumina, USA) [18]. Ancestry inference was performed by using the model-based method [21] implemented in the Admixture software. We used 370,539 SNPs to estimate each individual African, European and Native American tri-hybrid ancestry proportions, based on public datasets parental populations. We used the matrix of kinship coefficients and a network-based approach [22] to identify families, and identified them as categorical variables for the association tests described below. Pairs of individuals were considered as related if they had a kinship coefficient > 0.1 (first and second-degree relatives). Further details on how kinship and ancestry analyses of the Bambui cohort population were performed are described in a previous publication [18].

Morbidity

Health conditions considered in our analysis were: hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or treatment); diabetes

(fasting blood glucose ≥ 126 mg/dL and/or treatment); intermittent claudication (assessed by the Rose's questionnaire); arthritis (any joint condition), myocardial infarction and stroke, all as defined by a previous medical diagnosis for the condition; high fasting non HDL (high density lipoprotein cholesterol) (≥ 130 mg/dL); high plasma B-type natriuretic peptide level (> 100 pg/mL) [23]; low serum albumin level (< 3.8 g/dL); high serum creatinine level (≥ 1.3 mg/dL for men or ≥ 1.1 mg/dL for women); anaemia (haemoglobin < 13g/dL for men and < 12g/dL for women); high waist circumference (≥ 102 cm for men and ≥ 88 cm for women) [24]; depressive symptoms, activity of daily living disability and cognitive impairment (see below). Depressive symptoms were assessed using the 12-item version of the General Health Questionnaire. A score ≥ 5 was considered the cut-off for defining the exposure status, as recommended for the study population [25]. Activity of daily living disability was defined by a great level of difficulty or inability to carry out any of six basic activities of daily living, including showering, toileting, dressing, eating, getting in or out of a bed and walking across a room on the same floor. Possible cognitive impairment was defined by a Mini-Mental State Examination score below the 25th percentile (score < 22) or by the need for a proxy to respond the interview (87 participants). Fasting total and HDL cholesterol, glucose, albumin and creatinine were determined by using standard enzymatic methods (Merck, Germany). Plasma B-Type Natriuretic Peptide was measured using a micro particle-based immunoassay (AxSYM MEIA; Abbott, USA). Haemoglobin level was measured using an electronic counter (Coulter Counter T 890; Coulter Electronics, USA). We used principal component analysis [26] to create a morbidity score that included the above mentioned health conditions (all as dichotomous variables). Scores may range from - ∞ to + ∞. Higher scores indicated worse health.

Covariates

Other variables included socio-demographic characteristics (age, gender and household income), lifestyle (current smoking, physical activity and alcohol consumption). Monthly household income per capita was divided into tertiles (< USD 90.00 was the lowest tertile). Current smokers were those who had smoked at least 100 cigarettes during their lifetimes and who were still smokers. Physical activity was estimated based on the metabolic equivalent task (MET) for 25 physical activities in previous 3 months, as described elsewhere [27]. Insufficient physical activity was defined by energy expenditure less than 450 MET, which corresponds to at least 150 minutes per week of moderately to vigorously physical activity [28]. Alcohol consumption was defined by consumption of 14 doses per week in previous 12 months.

Because Bambui is a former endemic area for the protozoan *Trypanosoma cruzi*, we considered infection status for secondary analysis (see below). *T. cruzi* infection was assessed by seropositivity in three different assays performed concurrently: a hemagglutination assay (Biolab Merieux, Brazil) and two enzyme-linked immunosorbent assays (Abbott, USA and Wiener, Argentina). Further details are described elsewhere [29].

Statistical analysis

Our multivariable analyses were based on hazard ratios (HR) and 95% confidence intervals for mortality estimated by Cox proportional hazards model, after confirming that the assumption of proportionality of hazards was met (p value >0.05 - Schoenfeld residuals test). All models using genomic ancestry variables include a clustered robust variance term to avoid potential bias resulting from analysis of genomic ancestry of individuals who were related (764 cohort participants were first- and second-degree

relatives). We also examined the possibility of hazard ratios to differ by excluding early deaths (prior to 2 years) in all analysis and by excluding events prior to 5 years in our main analysis.

Our multivariate analyses were based on two models. First, we estimated the association between the biomarker and mortality in models adjusted for socio-demographic, lifestyle and health variables (morbidity score in quartiles). Then, we further adjusted for genome ancestry variables (fully adjusted model). Additionally, we examined the significance of the effect of multiplicative interactions between each biomarkers and genomic African and Native American ancestry proportions on mortality by means of cross-product terms in Cox proportional hazards fully adjusted models. We also fit Cox proportional hazards to model IL-6 and morbidity score, both in quartiles, to examine their association with death. In secondary analysis, we examined the influence of *T. cruzi* infection status on the above mentioned associations.

We conducted additional analyses to quantify and compare the discriminatory ability of IL-6 and morbidity score, both in quartiles, to predict death by using the area under the receiver operating characteristic curve (C-statistic). C-statistics were estimated for IL-6 and the morbidity score alone, as well as for those two measures plus age continuum. Further, we estimated the time-dependent discriminatory ability of those measures to predict mortality over time and plotted the results [30, 31].

Because we did not have statistical power to stratify the analysis, males and females were pooled and gender was considered as a covariate in our analyses. Statistical analyses were conducted using Stata, version 13.0 (StataCorp LP, USA), except for determination of the C-statistic and differences between predictors, which were estimated using the survC1 in R package [32]. Time-dependent discriminatory ability of predictors for mortality was estimated by using the risksetROC package in R [33] (R Foundation for

Statistical Computing, Austria). All P values were 2-tailed ($p<0.05$).

Results

Of 1,606 baseline cohort participants, 1,495 had their blood samples analysed and were eligible for this analysis. Among these, 1,191 had complete information on all other study variables and were included in the current analysis. During the study period, 601 participants died (death rate = 46.0 per 1,000 person-years at risk [pyrs]), and 101 were lost, yielding 13,060 pyrs of observation. The median follow-up period per participant was 12.8 years. The leading causes of deaths were cardiovascular diseases (33.7%), malignant neoplasms (14.2%), respiratory diseases (11.0%), and infectious diseases (10.7%); only 19 (2.2%) deaths were due to external causes.

Baseline socio-demographic characteristics, lifestyle and health conditions and their age-gender adjusted association with mortality are shown in Table 1. The age of study participants ranged from 60 to 93 years (mean = 68.8 and median = 67.0), and 58.6% were women. Besides age and sex, low household income, current smoking, insufficient physical activity, hypertension, diabetes, myocardial infarction, intermittent claudication, stroke, high B-type natriuretic peptide, high serum creatinine, anaemia, depressive symptoms, activity of daily living disability, possible cognitive impairment, *T. cruzi* infection and genomic African and Native American ancestries were all associated with increased risk of death.

Table 2 shows baseline serum levels of inflammatory markers and their association with mortality. Hazard ratios for death were statistically significant for IL6 tentiles in the model adjusted for socio-demographic, lifestyle and health variables (HR = 1.08; 95% CI: 1.05, 1.11). Further adjustments for genomic ancestry had no impact on this association (HR = 1.07; 95% CI: 1.04, 1.11). In the fully adjusted models, the confidence

interval slightly overlapped the null death hazard ratio for CXCL8, CXCL9 and CCL5. Other biomarkers showed no statistically significant association with mortality. Additionally, we found no evidence of statistically significant interactions affecting the ability of any biomarker to predict mortality neither for African nor for Native American ancestry (p for interaction >0.05 for all)

The association between IL-6 levels and mortality was also found in supplementary univariate analysis based on quartile levels of this biomarker, as illustrated in Figure 1. (p<0,001; long-rank test).

Figure 1. Kaplan-Meier survival estimates over 15-yeas, by quartile of their baseline Interleukin-6 level, The Bambui-Epigen Cohort Study of Aging, 1997–2011.

Hazard ratios for mortality by baseline level of IL-6 and the morbidity score in quartiles are shown in Table 3. Both measures were associated with increased mortality risk, independently of each other and an array of relevant variables. Regarding IL-6, the fully HR were 1.51 (95% CI: 1.15, 1.97), 1.54 (95% CI: 1.20, 1.96) and 1.79 (95% CI: 1.40, 2.29) for those in the 2^{nd}, 3^{rd} and the highest quartiles, respectively. The corresponding hazard ratios for the health score were 1.34 (95% CI: 1.05, 1.71), 1.65 (95% CI: 1.30, 2.10) and 2.16 (95% CI: 1.68, 2.77), respectively. Importantly, the exclusion of deaths occurred prior to 2 years had little impact on the above mentioned estimates. Furthermore, the exclusion of events prior to 5 years attenuated the associations, but both IL-6 and the morbidity score remained significantly associated with the risk of mortality

The baseline prevalence of *T. cruzi* infection was 35.1% in the study population. In

secondary analysis, we found no evidence for an influence of *T cruzi* infection on the associations between both IL-6 and the morbidity score with mortality, as compared with results shown in Table 3, as follows. HR for IL-6 in the model adjusted for socio-demographic, lifestyle, morbidity score, and genomic ancestry variables plus *T. cruzi* infection were 1.52 (95% CI: 1.15, 2.00), 1.53 (95% CI: 1.20, 1.06) and 1.77 (95% CI: 1.38, 2.26) for those in the 2nd, 3rd and highest quartiles, respectively. The corresponding hazard ratios for the morbidity score were 1.34 (95% CI: 1.05, 1.72), 1.72 (95% CI: 1.34, 2.20) and 2.13 (95% CI: 1.66, 2.75), respectively.

The discriminatory ability to predict death (C statistic) of a Cox proportional hazard model based on quartiles of IL-6 alone was 0.59 (95% CI: 0.57, 0.62). C Statistics for the model based on quartiles of the morbidity score alone (C = 0.60; 95% CI: 0.58, 0.62) was similar as that of the above mentioned model for IL-6 (difference = 0.00 95% CI: -0.03, 0.03; p>0.05). The addition of age to the previous models increased the C statistics values to 0.67 (95% CI: 0.65, 0.70) for IL6 and to 0.68 (95% CI: 0.65, 0.70) for the morbidity score. Again, the difference between the predictive value of those two measures was not statistically significant (0.00 95%CI: -0.02, 0.01; p>0.05). As Figure 2 shows, the discriminatory ability of a model based on IL6 and that of the morbidity score alone, both as continuous variables, remained unchangeable over 15-year of follow-up. The corresponding models plus age continuum decreased slightly over time, particularly after 5 years since the baseline.

Fig 2. Time-dependent ability of models to predict 15-year mortality, The Bambui-Epigen Cohort Study of Aging, 1997-2011.

Models were based on baseline interleukin-6 level or baseline health score in quartiles.

Subsequent models included age continuum.

Discussion

The main findings of this population-based study of older adults were that: (1) baseline IL-6 elevated serum levels were associated with very-long term subsequent mortality, independently of an array of important covariates, and the association appears not to be explained by reverse causation; (2) other biomarkers were not associated or showed weak associations with mortality; (3) genomic ethno-racial background did not modify the associations between any inflammatory marker with mortality; (4) the discriminatory ability of IL-6 to predict deaths was similar as that of a morbidity score based on an array of age-related chronic conditions and diseases.

To the best of our knowledge, our results show for the first time that the predictive value of IL-6 level for mortality is not affected by African or Native American genomic ancestry. This is also the first study to quantify the discriminatory ability of IL-6 to predict mortality in terms of C-statistics. Our analysis showed that the discriminatory ability to predict 15-year death of a model based on IL-6 alone was similar as that of a morbidity score based on an array of health conditions (C statistics = 0.59 and 0.60, respectively). However, both C statistics values did not reach 0.70, which is the classical cut-off point to define a good predictive model [34]. The addition of age to the previous model increased C statistics to 0.67 and 0.68, respectively. Interestingly, the discriminatory ability of IL-6 to predict mortality remained stable over a very long period, in a similar fashion as that of the morbidity score.

There are only few studies examining the association between other cytokines and chemokines with mortality. An earlier report, involving 525 participants of the Framingham Heart Study showed an association between baseline TNFα with 4-year

mortality [7]. Other study, involving 385 participants of the Memo Study in German, showed that both IL-8 (CXCL8) and IL-10 were associated with 9 year increased risk of death [9]. IL-8 was also found to be associated with increased risk of 8 year deaths in women (n = 504), but not in men (n = 504) in the PIVUS Study in Sweden [15]. A more recent study, involving 415 participants of the Belfrail Study in Belgium, examined the association between a battery of cytokines and chemokines with 3-year mortality, and found weak associations for IL-10 and IL-1β [5]. Our results suggest an association between CXCL8, CXCL9 and CCCL5 with 15-year mortality, but the hazard ratios were at the borderline of statistical significance. Given the inconsistency of results across studies, we believe that those associations need further verification.

Strengths of the present study include its large community-based sample followed for a very long period, standardized and systematic measurement of parameters at baseline, continuous surveillance of mortality according to standardized criteria and minimal loss of participants over the follow-up. Our study also incorporated a robust set of measures that could confound the association between the inflammatory markers and mortality. Another strength was the use of genome-wide measures of ancestry instead of ethno racial self-classification, which is prone to misclassification, particularly in admixed populations [19]. A limitation in our study is that we did not adjust for multiple testing in our initial analysis, which could potentially provide false positive results. However, the association between log-transformed IL-6 values with mortality found in our initial analysis has been confirmed in a subsequent analysis based on the quartiles distribution of the biomarker. Therefore, it is unlikely this is a false positive result. Because the circulating level of inflammatory markers were assessed using a single measurement (as was the practice in most previous studies) [3-15], our results may be subject to the effects of regression to the mean, which tend to underestimate the strength of the associations

found. However, it is unlikely that this biased our estimates, because the risk discrimination by IL-6 and the morbidity score decreased only slightly over the long term. The exact mechanism linking increased IL6 level to mortality is unclear. Because IL6 is associated with increased risk of chronic diseases that, in turn, predispose deaths, there is a debate about whether IL6 is a direct cause of death or simply summarize the burden of illness in older adults [12]. Our results indicate that IL6 predicts all-cause mortality, independently of several health and health-related measures, but we were not able to capture changes over time. Therefore, the key issue is to disentangle the complex pathway linking IL6 to mortality, which was not possible in our analysis.

Summarizing, our results showed that a single measure of IL-6 has prognostic value for short and long term mortality in a highly admixed population. Genome-based African and Native American ancestries had no impact on those prognostic values.

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Author Contributions

Conceived and designed the experiments: MFL-C JVdMM KCLT. Performed the experiments: MFL-C JVdMM KCLT SVP ET-S AT-C OAM-F. Analyzed the data: MFL-C JVdMM. Wrote the paper: MFL-C JVdMM KCLT SVP CO ET-S AT-C and OAM-F.

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Table 1. Baseline characteristics of study participants and their associations with 15-year mortality, The Bambui Cohort Study of Aging (1997-2011)

Variables	% baseline[a] (n = 1,191)	Age-sex adjusted HR	
		HR	95% CI
Age, mean (SD)	68.8 (7.0)	1.89	1.08, 1.10
Gender (women)	58.6	0.67	0.57, 0.79
Monthly Family income per capita (lowest tertile)	29.2	1.41	1.09, 1.68
Current smoking	17.6	1.86	1.52, 2.27
Insufficient physical activity (< 150 minutes physical activity per week)	31.2	1.68	1.41, 2.00
Alcohol consumptions in previous 12 months (> 14 doses per week)	3.0	0.82	0.50, 1.34
Hypertension (SBP ≥ 140 e/or DBP ≥ 90mm Hg and/or treatment)	68.8	1.19	1.01, 1.40
Diabetes (fasting blood glucose > 126 mg/dL and/or treatment)	15.2	1.70	1.39, 2.09
Arthritis[b]	24.9	1.04	0.86, 1.25
Myocardial infarction[b]	5.0	1.67	1.21, 2.30
Intermittent claudication[c]	2.5	1.96	1.28, 3.02
Stroke[b]	3.3	1.65	1.09, 2.49
Non-HDL cholesterol ≥ 130 mg/dL	88.3	0.96	0.75, 1.22
B-type natriuretic peptide > 100 pg/mL	11.1	1.57	1.34, 1.85
Albumin < 3.8 g/dL	5.9	1.16	0.85, 1.58
Creatinine (≥ 1.3 mg/dL for men and ≥ 1.1 mg/dL for women)	4.6	1.51	1.09, 2.09
Anemia (hemoglobin < 13g/dL for men and < 12g/dL for women)	4.2	2.35	1.61, 2.48
Waist circumference ≥ 102 cm for men and ≥ 88 cm for women)	43.5	1.01	0.83, 1.23
Depressive symptoms (General Health Questionnaire score ≥ 5)	37.8	1.35	1.14, 1.60
Activity of daily living disability[d]	12.4	2.01	1.62, 2.48
Possible cognitive impairment[e]	24.9	1.64	1.38, 1.95
African, median (p25, p75)[f]	9.6 (4.8, 17.6)	1.86	1.16, 3.00
Native American (p25, p75)[f]	5.3 (2.8, 8.4)	7.35	1.10, 49.1
European (p25, p75)[f]	84.0 (73.9, 91.4)	0.53	0.34, 0.82

HR = hazard ratio; 95% CI = 95% confidence interval; SD = standard deviation; p25, p75 = 25th and 75th percentiles; SBP = systolic blood pressure; DBP = diastolic blood pressure.

[a] Data are presented as percentage, except when specified.

[b] Previous medical diagnosis.[c] Rose's questionnaire.[d] Great level of difficulty or inability to carry out any of six basic activities of daily living, including showering, toileting, dressing, eating, getting in or out of a bed and walking across a room on the same floor.[e] Mini Mental State Examination score < 75th percentile or proxy respondent.[f] Genomic African, European and Native America ancestry were continuous variables and all models included a robust variance term corresponding to cluster in family.

Table 2. Hazard ratios of 15-year mortality according with baseline multi-inflammatory serum levels, The Bambui Cohort Study of Aging (1997-2011)

Biomarkers	HR adjusted for socio-demographic [a], lifestyle [b] and health variables[c]		HR adjusted for socio-demographic [a], lifestyle [b], health [c] and genomic ancestry variables[d]			
			All		Excluding deaths prior to 2 years	
	HR	95% CI	HR	95% CI	HR	95% CI
IL-6[e]	1.08	1.05, 1.11	1.07	1.04, 1.11	1.07	1.04, 1.11
CXCL8[e]	1.02	0.99, 1.05	1.02	0.99, 1.05	1.02	0.98, 1.05
CCL2[e]	0.99	0.96, 1.02	0.99	0.96, 1.02	0.99	0.96, 1.02
CXCL9[e]	1.03	1.00, 1.06	1.03	1.00, 1.07	1.04	1.00, 1.08
CCL5[e]	1.02	0.99, 1.05	1.02	0.99, 1.05	1.02	0.98, 1.05
CXCL10[e]	1.01	0.98, 1.04	1.01	0.97, 1.04	1.01	0.98, 1.04
IL-10[f]	1.07	0.89, 1.29	1.06	0.88, 1.28	0.99	0.81, 1.21
IL-12[g]	1.12	0.84, 1.51	1.12	0.86, 1.45	1.09	0.82, 1.45
TNF[g]	0.94	0.75, 1.16	0.93	0.74, 1.17	0.93	0.73, 1.19
IL-1β[g]	1.22	0.98, 1.52	1.22	0.97, 1.53	1.15	0.90, 1.47

HR = hazard ratio; 95% CI = 95% confidence interval.

[a] Age (continuous), sex, monthly household income per capita

[b] Current smoking, insufficient physical activity, alcohol consumptions, high waist circumference.

[c] A latent variable based on the following health conditions: hypertension, diabetes, intermittent claudication, arthritis, myocardial infarction, stroke, non-HDL cholesterol, B-type natriuretic peptide, serum albumin, serum creatinine, anemia, waist circumference, depressive symptoms, activity of daily living disability and cognitive impairment, as specified in Table 1.

[d] Previous model plus genomic African and Native American ancestry proportions (as continuous variables) and cluster in family.

[e] Divided in tentiles.

[f] Dichotomous variables, having the lowest detectable value (≥ 0.14 pg/mL) as exposure category.

[g] Dichotomous variables, having a detectable value (≥ 0.01 pg/mL) as exposure category.

Table 3. Hazard ratios of 15-year mortality according with baseline serum levels of interleukin-6 and the morbidity score, and stratified by time to death, The Bambui-Epigen Cohort Study of Aging (1997-2011)

Measure in quartiles	Fully adjusted hazard ratio (95% confidence interval)[a]					
	All		Excluding deaths within 2 years		Excluding deaths within 5 years	
	HR	95% CI	HR	95% CI	HR	95% CI
Interleukin-6 (IL-6)[b]						
Lowest	1.0		1.0		1.0	
2nd	1.51	1.15, 1.97	1.47	1.11, 1.94	1.41	1.04, 1.91
3rd	1.54	1.20, 1.96	1.46	1.13, 1.87	1.44	1.09, 1.90
Highest	1.79	1.40, 2.29	1.74	1.34, 2.24	1.56	1.17, 2.09
Morbidity score[c]						
Lowest	1.0		1.0		1.0	
2nd	1.34	1.05, 1.71	1.38	1.07, 1.79	1.29	0.97, 1.70
3rd	1.65	1.30, 2.10	1.62	1.26, 2.10	1.50	1.13, 2.01
Highest	2.16	1.68, 2.77	2.24	1.71, 2.92	2.11	1.55, 2.86

HR = hazard ratio; 95% CI = 95% confidence interval.

[a] Estimated by Cox proportional regression. Hazard ratios are mutually adjusted for the variables listed in the table and adjusted for age (continuous), sex, monthly household income per capita, current smoking, insufficient physical activity, alcohol consumption, waist circumference (as specified in Table 1), genomic African and Native American ancestry proportion (as continuous variables) and cluster in family.

[b] Interleukin-6 levels by quartiles - lowest: ≤ 0.43; 2nd: 0.44-1.03; 3rd: 1.04-2.10; highest: > 2.10 pg/mL.

[c] A latent variable based on the following health conditions: hypertension, diabetes, intermittent claudication, arthritis, myocardial infarction, stroke, non-HDL cholesterol, B-type natriuretic peptide, serum albumin, serum creatinine, anemia, waist circumference, depressive symptoms, activity of daily living disability and cognitive impairment, as specified in Table 1.