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Cu to Zn ratio, physical function, disability, and mortality risk in older elderly (ilSIRENTE study)

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Abstract Associations between copper to zinc ratio (CZr) and mortality have suggested CZr as a biomarker of aging. Nevertheless, very limited data exist on the association between serum CZr and physical or functional status of very old people. We examined the relationship between serum CZr and physical performance, muscle strength, functional status, and survival from the ilSIRENTE Study: a longitudinal study of persons aged 80 years or older (n=346). An adjusted linear regression model was subsequently performed to calculate the regression coefficients of the associations between baseline physical and functional measures (dependent variable) with CZr or "Cu and Zn" alone taking also into account the influence of other relevant factors, including hematological (albumin, cholesterol, and urea) and inflammatory biomarkers (IL-6 and CRP) that were significantly different across CZr tertiles. CZr showed significant and stronger relationships than Cu or Zn alone with all baseline physical and functional measures in models that did not include adjustments for inflammatory parameters. CZr was also associated with physical decline, measured as "SPPB% decline" at 2 years of follow-up and mortality at 4 years of follow-up. Subjects in the high CZr tertile had a higher risk of death with an adjusted hazard ratio of 1.92 (95% CI, 1.12–3.29; p=0.02). In conclusion, we have confirmed the role of CZr as a predictor of mortality, whereas the role of CZr as a biomarker or predictor of physical or functional performance seems to be the consequence of its strict relationships with inflammatory parameters. In this context, further investigations need to be carried out.

Keywords Cu to Zn ratio · Physical function · Disability · Mortality · Aging

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Introduction

The growing number and percentage of old and very old people has compelled the scientific community to

increase focus on biomarkers of aging and peculiar consequences of aging itself such as disability, physical function decline, frailty, age-related diseases, and mortality (Gluckman and Hanson 2004; Rockwood et al. 1996). Older elderly (octo- and nonagenarians) are a group characterized by a decreased ability to maintain homeostasis and, consequently, at high risk of serious adverse clinical outcomes, including mortality (Maggio et al. 2005).

Disability is a common condition in older persons and, although a decline in age- and sex-specific rates of physical and functional performances, with subsequent disability, has been recently observed (Manton et al. 2006), the absolute number of disabled older persons will continue to increase in the next decades' growing aging population. A decline in physical performance and function often mark the early stage of the process (Guralnik et al. 1995). Therefore, there is an urgent need to identify biomarkers related to the age-associated decline in physical and functional performance. Powerful biomarkers might be relevant in clinical practice to help clinicians in the early detection of disability, as they can unmask homeostatic failure even in the absence of the most prevalent comorbidity conditions (Walston et al. 2002).

Most studied biomarkers include soluble inflammatory mediators, hormones, free radicals, antioxidants, macro-, and micronutrients (Fried et al. 2009). Within this last class of nutrients, the low serum concentration of some essential elements, such as copper and zinc, seems particularly important to predict the development and course of disability because they are associated with poor nutritional status (Semba et al. 2006).

Although micronutrient deficiencies could potentially increase the risk of disability and mortality through multiple pathways involving oxidative stress, inflammation, muscle and bone metabolism, and immunity (Milaneschi et al. 2010; De Martinis et al. 2006; Fulop et al. 2010), the relationship between micronutrients and the risk of physical decline or disability has not been well characterized. In this context, some trace elements may have an important role in frailty and aging beyond their association with the nutritional status. In fact, the serum concentration of the most important trace elements is strictly regulated by compensatory mechanisms that act to stabilize their concentrations. Hence, the maintenance of homeostasis can be reflected by an appropriate balance among different trace elements (Lukaski and Penland 1996).

The serum or plasma copper to zinc ratio (CZr) is among those parameters that may be associated with reduced ability to maintain or regain homeostasis after a destabilizing event. Increments of this ratio reflect increased inflammatory status or decreased nutritional status with subsequent appearance of some degenerative age-related diseases (Malavolta et al. 2010). Indeed, plasma CZr was found to be higher in hospitalized elderly subjects than in their healthy counterparts (Belbraouet et al. 2007) and has been associated with the risk of CVD death (Leone et al. 2006; Reunanen et al. 1996) and malignancy (Cunzhi et al. 2003; Dìez et al. 1989). Therefore, this parameter may be a useful tool in disability research as well as in altered physical and functional performances, taking also into account that copper and zinc homeostasis may be related to the maintenance of physical performance, muscle strength as well as to the regulation of the inflammatory response (Mocchegiani et al. 2008; Cousins 1985), whose alterations predispose to chronic disease, as well as to the development of disability in the elderly (Cesari et al. 2009). Increased changes of inflammatory parameters or the presence of age-related diseases, such as cardiovascular diseases, have been associated with augmented plasma copper and/or decreased plasma Zn, thus leading to the suggestion that CZr might be a more sensitive biomarker for physical and functional decline, as well as to predict mortality in elderly than the individual values of copper or zinc (Malavolta et al. 2010).

Even though research shows a growing interest in the field of circulating biomarkers associated with disability (Hozawa et al. 2010; McDermott et al. 2004), information on the relationship between CZr and physical performance and function as well as survival in octo–nonagenarians are limited. In the present study, we investigated the association between serum CZr and Cu or Zn with measures of physical performance and functional status as well as their decline and/or all-cause mortality in community-dwelling older persons aged 80 years or older enrolled in the "Aging and longevity in the Sirente geographic area, ilSIRENTE Study."

Subjects and methods

We used data from the ilSIRENTE, a prospective cohort study conducted in the mountain community

living in the Sirente geographic area (L'Aquila, Italy), developed by the teaching nursing home Opera Santa Maria della Pace (Fontecchio, L'Aquila, Italy) in a partnership with local administrators and primary care physicians. The Catholic University of Sacred Heart (Rome, Italy) ethical committee ratified the entire study protocol. All the participants signed an informed consent at baseline visit. The ilSIRENTE study protocol is described in detail elsewhere (Landi et al. 2005).

Study population

A preliminary list of all persons living in this well-defined area was obtained at the end of October 2003 from the registry offices of the 13 municipalities involved in the study. From this preliminary list, potential study participants were identified by selecting all persons born in the Sirente area before 1 January 1924 and actually living in such area (inclusion criteria). No exclusion criteria were identified. General practitioners presented the ilSIRENTE study protocol to their clients, inviting them to participate in the study. Persons who refused to be enrolled were contacted at least two additional times by the study personnel before being considered as refusals. Of the initial 514 subjects screened, 32 men and 53 women died or moved away from the area before the baseline assessment. Among those eligible (n=429), prevalence of refusals was very low (15%), without significant differences across age or gender groups. However, the present analysis was conducted in 346 subjects (115 men and 231 women), after the exclusion of 18 participants because of missing data for copper to zinc ratio.

Data collection

The participants' baseline assessments began in December 2003 and were completed in September 2004. Assessors were trained on how to perform each component of the ilSIRENTE study protocol (Landi et al. 2005). The Minimum Data Set for Home Care (MDS-HC) form was administered to all study participants according to the guidelines published in the MDS-HC manual (Morris et al. 1996). The MDS-HC contains over 350 data elements, including sociodemographics, physical, and cognitive status variables, as well as major clinical diagnoses (Morris et al. 1997). Moreover, the MDS-HC includes information on an extensive array of signs, symptoms, syndromes, and treatments. The MDS items have shown an excellent inter-rater and test–retest reliability when completed by nurses performing usual assessment duties (average weighted Kappa, 0.8) (Landi et al. 2000). Additional information on family history, lifestyle, physical activity, and behavioral factors were collected using specific questionnaires shared with the "Invecchiare in Chianti Study" (Ferrucci et al. 2000).

Physical performance measurement

Physical performance was assessed using the 4-m walking speed and the Short Physical Performance Battery (SPPB) score. The SPPB is composed of three timed tests: the 4-m walking speed test, the balance test, and the chair stand test (Guralnik et al. 1995, 2000). The walking speed was evaluated measuring the participants' usual gait speed (in meters per second) over a 4-m course. To assess the chair stand test, the participants were asked to stand up from a chair with their arms folded across the chest five times in a row as quickly as possible. The time needed to complete the task was recorded. To assess the balance test, the participants were asked to perform three increasingly challenging standing positions: side-by-side position, semi-tandem position, and tandem position. The participants were asked to hold each position for 10 s. Participants were scored 1 if they were able to hold a side-by-side standing position for 10 s, but were unable to hold a semi-tandem position for 10 s; they were scored 2 if they were able to hold a semi-tandem position for 10 s, but were unable to hold a tandem position for more than 2 s; they were scored 3 if they were able to stand in tandem position for 3–9 s; they were scored 4 if they were able to hold the tandem position for 10 s. The participants who were unable to complete the test were scored 0.

Timed results from each test were rescored from 0 (worst performers) to 4 (best performers). The sum of the results from each test (ranging from 0 to 12) was used in the present analysis. Categorization of results from usual gait speed and chair stand test was obtained from the ilSIRENTE population-specific quartiles, as previously described (Landi et al. 2010). The balance test results were categorized

according to previously established cut points provided by Guralnik et al. (1995, 2000). This battery has shown to be a valid and reproducible measure that can discriminate small and clinically meaningful differences in physical function and predict different forms of disability among older adults (Landi et al. 2005; Cesari et al. 2006, 2009).

Muscle strength measure

Muscle strength was assessed by hand grip strength using a handheld dynamometer (North Coast Hydraulic Hand Dynamometer, North Coast Medical Inc., Morgan Hill, CA, USA). One trial for each hand was performed, and the result from the strongest hand was used for the present analyses.

Functional status measures

Basic and instrumental activities of daily living (IADL) were assessed by the assessor using the MDS-HC instrument (Morris et al. 1996, 1997). The activities of daily living (ADL) scale is based on seven levels of self-performance, including dressing, eating, toilet use, bathing, mobility in bed, locomotion, transfer. Similarly, the IADL scale is based on seven levels of self-performance, including meal preparation, house work, managing finance, phone use, shopping, transportation, managing medications.

Blood measurements

Venus blood samples were drawn in the morning after an overnight fast. The samples were immediately centrifuged and stored at −80°C until the final analysis. Standard determinations of serum albumin, cholesterol (HDL and LDL), C-reactive protein (CRP), and urea were performed by commercially available kits (Olympus, Italy).

Serum copper to zinc ratio

The serum Zn and Cu were determined by a Thermo XII Series ICP-MS (Thermo Electron Corporation, Waltham, MA, USA) by adapting methods used for the measurement of trace elements in human plasma with slight modifications (Malavolta et al. 2010). Serum samples were diluted 1:20 with a diluent containing 0.1% Triton X-100 (BDH Chemicals), 0.1% Trace Select Ultra HNO_3 (Sigma-Aldrich, Steinheim, Germany), and 10 ppb Rh (Merck, Darmstadt, Germany) as internal standard. External multielement calibration solutions containing Zn and Cu (blank to 100 ppb) were prepared by serial dilution of a parent multielement solution (Inorganic Ventures, Christiansburg, VA, USA), using the same diluent used for the samples. Data were acquired for ^{64}Zn and ^{63}Cu. The instrument was operated with a MicroMist nebulizer (Glass Expansion, Melbourne, Australia), a Cinnabar Spray chamber with helix (Glass Expansion, Melbourne, Australia) a single piece quartz torch (1.5-mm i.d. injector) together with Xi interface cones and a Cetac ASX 100 autosampler (CETAC Technologies, Omaha, NE, USA). The instrument was operated in collision cell technology with kinetic energy discrimination mode by introducing a mixture of 8% hydrogen in helium into the octapole cell. The gases were introduced into the cell under mass flow control through stainless steel lines. The ICP-MS was operated using 1,250 W of RF power, 0.79 $L min^{-1}$ of nebulizer gas flow, 1.15 $L min^{-1}$ of auxiliary gas flow, 16.0 $L min^{-1}$ of cool gas flow, 20-ms dwell time, 90-s sample uptake, 60-s wash time (three repeats per sample). The instrument was calibrated daily with oxide and doubly charged ions set below 0.03%. The accuracy of the system was routinely checked with quality control samples prepared from Seronorm Trace Element Serum level 1 and level 2 (Sero AS, Billingstad, Norway).

Covariates

Medical diagnoses and drugs were directly collected by general practitioners. Medical diagnoses were defined as conditions that have a relationship with patients' functional, cognitive and behavioral status, medical treatment, and risk of death. The diagnoses were listed on the MDS-HC form in a check-box section containing 27 specific diagnostic categories. A general practitioner collected information on up to 18 different drugs received by each patient in the 7 days preceding the assessment. Drugs were coded using the Anatomical Therapeutic and Chemical codes. Body weight was measured while wearing light clothes using a calibrated bathroom scale. The body height was measured using a standard stadiometer. Body mass index (BMI) was defined as weight (kilograms) divided by the square of height (meters).

Alcohol consumption was assessed asking participants on the number of glasses of wine drunk during a standard day. Alcohol abuse was defined as a consumption of more than half a liter of wine per day. As indicated in the MDS-HC manual, 18 hearing impairment was defined as difficulty to hear during normal conversational speech such as when using the telephone, watching television, responding to doorbells, and engaging in group activities. Vision impairment was defined as difficulty to see regular print in newspaper and/or books.

Follow-up and survival status

The vital status of all the study participants was ascertained by the general practitioners and confirmed using the National Death Registry over at 24th and 48th month period of time after the baseline visit. The participants were censored at the time of their death using the date of death (for participants who died during the follow-up) or at the end of follow-up (for participants who did not die during the study follow-up). SPPB was measured in people who survived after the 24th month to monitor physical decline. Physical performance decline was assessed considering the percent decline in the SPPB score. The SPPB decline or improvement in percentage at 2 years of follow-up was calculated as following:

$$[(\text{SPPB baseline}) - (\text{SPPB (follow} - \text{up)}] \times 100/(\text{SPPB baseline}).$$

Statistical analysis

For analytical purposes, CZr levels were categorized into three groups according to gender-specific tertiles. The characteristics of the study participants were described according to CZr tertiles, and differences were assessed using Fisher's exact test and ANOVA test statistics for categorical and continuous variables, respectively. Adjusted and unadjusted generalized regression models were performed to calculate the regression coefficients (and standard errors, SE) of the associations between physical and functional measures with CZr and Cu or Zn alone.

Variables considered for adjustment were those thought to be clinically significant or showing a statistically significant different distribution across CZr tertiles. Different models were used to test the impact of covariates on the relationship between CZr and physical or functional measures. The final analyses were adjusted for age and gender (model 1); age, gender, BMI, and hypertension (model 2); age, gender, BMI, hypertension, congestive heart failure, albumin, urea, cholesterol (model 3); and age, gender, BMI, hypertension, congestive heart failure, albumin, urea, cholesterol, CRP, and IL-6 (model 4). Adjusted and unadjusted generalized regression models were performed to calculate the regression coefficients (and SE) between physical decline with CZr, Cu, Zn, and inflammatory biomarker levels (independent variables).

Time to death was calculated from the date of baseline assessment to the date of death. We examined all events which occurred during the 2- and 4-year follow-up. Crude and adjusted hazard ratios and 95% confidence intervals (CI) of death by CZr or Cu and Zn were estimated by Cox proportional regression models.

Variables considered for adjustment in Cox regression analysis were age, gender, BMI, hypertension, congestive heart failure, albumin, urea, cholesterol, CRP, and IL-6, 4-m walking speed, short physical performance battery, hand grip strength, ADL score, and IADL score.

All analyses were performed using the SPSS 14.0 package (SPSS Inc., Chicago, IL).

Results

1. Characteristics of the population according to CZr tertiles

   Our population included 115 men (33.2%) and 231 women (66.8%) with a mean age (SD) of 86.08 (4.85) and 85.73 (4.84), respectively. The mean levels of CZr where higher in females than males (2.06±0.04 vs. 1.93±0.04, $p=0.03$). In females, plasma Cu/Zn was positively correlated with age ($r^2=0.037$, $p=0.003$), while no correlation with age was observed in men ($r^2=0.016$, $p=0.174$). A categorization of the study population in gender-specific tertiles of CZr generated the following cutoff thresholds: low tertile (<1.67), medium tertile (1.68–2.07), high tertile (>2.08) in men ($n=115$); low tertile (<1.76), medium tertile (1.77–2.23), high tertile (>2.24) in women ($n=$

231). Characteristics of the study population according to CZr tertiles are summarized in Table 1. Compared with participants in the low CZr tertile, those in the high tertile were older, had lower BMI, albumin and cholesterol, higher levels of inflammatory markers (CRP and IL-6), higher prevalence of congestive heart failure, and hearing impairment (Table 1). Death counts were more frequent in the high CZr tertile compared to the other groups both at 2 and 4 years of follow-up. Moreover, in this unadjusted model, parameters of physical performance and function significantly worsened across CZr tertile (Table 1).

2. Baseline physical performance and function in relation to CZr values

Table 2 shows the results from the adjusted and unadjusted linear regression models performed between physical function measures (dependent variables) and CZr or Cu and Zn as independent variables. The unadjusted model confirms the association between CZr and physical or functional measures shown by the trend across CZr tertiles in Table 1. However, also Cu and Zn were associated with different functional and physical measures thus raising the question about the importance of CZr as a biomarker compared to Cu or Zn alone. However, after adjusting for age and gender (model 1), all the baseline physical performance variables and the functional status measures (ADL and IADL) remained significantly associated with CZr while none remained significantly associated with the sole Cu; only three out of five measures remained significantly associated with Zn (Table 2). These results were also maintained after adding BMI, hypertension, and congestive heart failure to the regression model (Table 2, model 2). The inclusion of the hematological parameters albumin, urea, and cholesterol as independent variables (Table 2, model 3) significantly affected the sole association of CZr with hand grip strength ($p=0.06$). However, all the associations were no longer significant when inflammatory parameters (CRP and IL-6) were added to the regression model (Table 3, model 4). In this model, IL-6 was significantly associated with all physical performance measures and ADL while CRP was associated with IADL.

3. Physical performance decline: 2-year decline of SPPB and baseline CZr

After the 2-year follow-up, data for SPPB were available in $n=221$ subjects with a baseline SPPB score >0. Survivor's mean±SEM of SPPB score changed from 7.08±0.21 at baseline to 6.39±0.24 at follow-up ($p<0.01$ by a paired t test) with a mean decline of 1.41±0.19 (median decline=1). Death events after 2 years significantly affected the distribution of the population across CZr tertiles (see Table 1, unadjusted counts for deaths), with a substantial reduction of people in the high CZr tertile. Therefore, the association of CZr with SPPB decline was studied using CZr as continuous variable by a generalized linear regression model and using SPPB% decline as dependent variable. SPPB% decline offers the advantage to be independent of baseline SPPB levels (data not shown). Mean SPPB% decline is 18.13±2.5% in the survivors. An association of SPPB% decline with the independent variables CZr, Cu, and Zn, IL-6 or CRP by generalized linear regression is shown in Table 3. CZr was the sole variable associated with SPPB% decline in the nonadjusted model, as well as in the adjusted models (Table 3).

4. Cox proportional hazard models for CZr and risk of 4-year mortality

A total of number of 145 deaths (55 men and 90 women) and a total of number of 201 survivors (60 men and 141 women) occurred during the 4-year follow-up. CZr was significantly higher in old subjects who died when compared to those still living, both in men (dead mean±SEM=2.13±0.06 vs. survivors mean±SEM=1.74±0.05; $p<0.01$) and women (dead mean±SEM=2.29±0.06 vs. survivors mean±SEM=1.91±0.04; $p<0.01$).

The hazard ratio (95% CI) for high CZr vs. low CZr tertile from the unadjusted and adjusted Cox proportional hazard models for the 4-year mortality, including various confounders are shown in Table 4. Interestingly, the results observed in model 4 did not change significantly when baseline physical and functional measurement were included in the analysis as additional confounders (Table 4).

Plasma Cu, but not Zn, remained an independent predictor of the 4-year mortality when these variables were substituted by CZr in the survival analysis presented in Table 4. In addition to CZr

Table 1 Characteristics of study participants according to the Cu/Zn tertiles

Tertiles of Cu/Zn	Low (n=114)	Medium (n=119)	High (n=114)
Age	84.5±0.4	86.2±0.4*	86.9±0.5**
Female	76 (67)	79 (66)	76 (67)
Weight loss 12 months	22 (19)	21 (18)	25 (22)
Chewing problems	3 (7)	3 (8)	2 (4)
Marital status			
Married	44 (39)	26 (22)	29 (25)
Widowed	58 (51)	82 (69)	72 (63)
Never married	12 (10)	11 (9)	13 (11)
Living alone	28 (25)	41 (36)	26 (23)
Education years	5.14±0.2	5.13±0.2	5.11±0.1
Specific diseases			
Hypertension	53 (46)	72 (60)	48 (42)**
Congestive heart failure	3 (3)	7 (6)	12 (10)*
Coronary heart disease	15 (13)	13 (11)	12 (10)
Cerebrovascular disease	4 (3)	3 (2)	9 (8)
Cancer	6 (5)	4 (3)	7 (6)
Chronic obstructive pulmonary disease	16 (14)	14 (12)	16 (14)
Diabetes	10 (9)	11 (9)	11 (10)
Osteoarthritis	24 (21)	28 (23)	16 (14)
Parkinson	1 (1)	2 (2)	3 (3)
Alzheimer	6 (5)	4 (3)	11 (10)
Demenzia (non-AD)	2 (2)	4 (3)	7 (6)
Total no. of diseases	2.13±0.12	2.18±0.12	2.18±0.13
Sensory impairment			
Hearing	21 (18)	23 (19)	34 (30)*
Vision	25 (22)	24 (20)	30 (26)
No. of medications	3.13±0.20	3.31±0.21	3.51±3.13
BMI (kg/m^2)	26.30±0.43	25.88±0.38	24.55±0.46*
Alcohol abuse (>1/2 L of wine per day)	16 (14)	16 (13)	12 (10)
Smoker or ex smoker	26 (23)	30 (25)	24 (21)
Hematological parameters			
Albumin (g/dL)	4.22±0.02	4.22±0.03	4.11±0.04*,**
Urea (mg/dL)	44.65±1.53	47.62±1.75	51.65±2.61*
Cholesterol (g/dL)	202.61±4.16	200.06±4.10	184.11±3.96*,**
C-reactive protein (g/dL)	2.57±0.22	3.91±0.28[a]	6.15±0.38*,**
IL-6 (pg/dL)	2.12±0.18	2.33±0.17	4.54±0.30*,**
Unadjusted counts for death			
2-year follow-up	14 (12)	24 (20)	49 (43)*,**
4-year follow-up	26 (23)	46 (39) [a]	73 (64)*,**
Unadjusted physical performance measures			
4-m walking speed (m/s)	0.57±0.03	0.50±0.03	0.38±0.03*,**
SPPB	7.46±0.36	6.86±0.35	5.45±0.37*,**
Unadjusted muscle strength measure			
Hand grip strength (kg)	33.19±1.38	29.58±1.35	27.54±1.40*

Table 1 (continued)

Tertiles of Cu/Zn	Low (n=114)	Medium (n=119)	High (n=114)
Unadjusted functional status measures			
ADL scale score	0.90±0.22	1.25±0.22	2.23±0.22*,**
IADL scale score	2.38±0.23	2.94±0.23	3.94±0.23*,**

Data are given as number (percent) for the following variables: gender, marital status, living alone, specific diseases, sensory impairments, alcohol abuse, smoking habit, urinary incontinency, fecal incontinence. For all the other variables means±SD are reported. Differences in proportions and means of covariates between different Cu/Zn tertiles were assessed using Fisher's exact test and ANOVA test statistics, respectively

*p<0.05 with respect to low tertile; **p<0.05 with respect to medium tertile

and Cu, other independent predictors of the 4-year mortality were CHF, IL-6, cholesterol, IADL, and SPPB.

Discussion

In the present study, we have explored the association between serum CZr and physical performances, functional status, and survival in a population of aged community-dwelling persons. Our study shows that CZr displays significant and stronger relationships than Cu or Zn alone with all baseline physical and functional measures in models not including adjustments for inflammatory parameters. A comparison of unadjusted means shows that all participants (both elderly men and women) in the highest tertile of CZr had significantly lower BMI, cholesterol and albumin, higher incidence of congestive heart failure, higher IL-6, and CRP levels as well as worse in physical and functional performance than those observed in the lower tertiles at baseline. An adjustment for inflammatory parameters (IL-6 and CRP) affects the association of CZr with baseline physical and functional measures, thus limiting the relevance of CZr as biomarker of physical and functional status in comparison with other well-known inflammatory biomarkers (Brinkley et al. 2009; Ferrucci et al. 2002). However, the association between CZr and physical decline shows that CZr levels are significantly associated with the worsening of physical performance in a 2-year follow-up. In this regard, it is interesting to note that Cu and Zn were not correlated with physical decline. CZr was also found to be the sole hematological/inflammatory parameter correlated with physical decline also in models including all potential confounding factors. Other findings show that inflammatory biomarkers, such as IL-6, are strictly related with baseline physical functions (Cesari et al. 2004; Brinkley et al. 2009) and with future physical decline (Schaap et al. 2009) while others confirm only the presence of baseline associations (Taaffe et al. 2000). Our findings confirm the baseline association between IL-6 and physical performances but not its involvement in physical decline where CZr plays the major role. A possible explanation to this dichotomy might arise from the observation that CZr could also be sensitive to other conditions, such as dietary habits and biological aging and subclinical pathological changes (Malavolta et al. 2010) not necessarily associated with a concomitantly marked inflammatory change but related to future physical decline. In addition, people with marked inflammatory profiles in heterogeneous older population, as herein reported, might die before the measure of physical decline at follow-up (Thomas et al. 2001). As such, the association between CZr and physical decline may be limited.

Moreover, the present study confirmed that CZr was directly associated with all-cause mortality regardless of age, gender, and other confounding factors. This study further highlights the crucial role played by CZr on the 4-year mortality risk independent of physical performances (Landi et al. 2010), IL-6 (Walston et al. 2006), cholesterol (Reuben et al. 2002), and congestive heart failure (Volpato et al. 2001) in the aged elderly. In this context, we have already shown that plasma CZr is an important nutritional/inflammatory biomarker and a strong predictor of all-cause mortality (3.5-year follow-up)

Table 2 Adjusted linear regression models predicting physical performance and function measures from CZr or Cu and Zn

	4-m walking speed B (standard error) *p*	SPPB B (standard error) *p*	Hand grip B (standard error) *p*	ADL B (standard error) *p*	IADL B (standard error) *p*
Unadjusted					
Plasma CZr	−0.162 (0.318)<0.01	−1.987 (0.400)<0.01	−6.768 (1.539)< 0.01	1.179 (0.248)<0.01	1.259 (0.261)<0.01
Plasma Cu[a] ($\times 10^{-2}$)	−0.026 (0.008)<0.01	−0.330 (0.099)<0.01	−1.360 (0.379)< 0.01	0.139 (0.062) 0.03	0.126 (0.065) 0.05
Plasma Zn[a] ($\times 10^{-2}$)	0.040 (0.0124)<0.01	0.484 (0.154)<0.01	1.112 (0.592) 0.06	−0.261 (0.097) 0.01	−0.349 (0.102)< 0.01
Model 1					
Plasma CZr	−0.117 (0.308)<0.01	−1.468 (0.390)<0.01	−3.689 (1.135) 0.01	1.832 (0.496)<0.01	0.960 (0.252)<0.01
Plasma Cu[a] ($\times 10^{-2}$)	−0.014 (0.012) 0.07	−0.167 (0.975) 0.09	−0.408 (0.334) 0.22	0.250 (0.125) 0.05	−0.061 (0.063) 0.34
Plasma Zn[a] ($\times 10^{-2}$)	0.032 (0.012) 0.01	0.425 (0.146)<0.01	0.900 (0.505) 0.07	−0.361 (0.189) 0.06	−0.257 (0.962) 0.01
Model 2					
Plasma CZr	−0.112 (0.311)<0.01	−1.316 (0.393)<0.01	−3.042 (1.353) 0.02	1.696 (0.504)<0.01	0.871 (0.255)<0.01
Plasma Cu[a] ($\times 10^{-2}$)	−0.013 (0.012) 0.10	−0.134 (0.097) 0.17	−0.253 (0.333) 0.45	0.216 (0.126) 0.09	0.038 (0.0638) 0.55
Plasma Zn[a] ($\times 10^{-2}$)	0.030 (0.012) 0.01	0.382 (0.145) 0.01	0.780 (0.499) 0.12	−0.337 (0.190) 0.08	−0.232 (0.959) 0.02
Model 3					
Plasma CZr	−0.101 (0.308)<0.01	−1.188 (0.391)<0.01	−2.554 (1.354) 0.06	1.494 (0.493)<0.01	0.767 (0.249)<0.01
Plasma Cu[a] ($\times 10^{-2}$)	−0.013 (0.076) 0.10	−0.129 (0.096) 0.18	−0.243 (0.331) 0.46	0.227 (0.124) 0.07	0.037 (0.061) 0.55
Plasma Zn[a] ($\times 10^{-2}$)	0.026 (0.011) 0.02	0.352 (0.143) 0.01	0.66 (0.497) 0.183	−0.272 (0.188) 0.15	−0.210 (0.094) 0.03
Albumin	0.167 (0.047)<0.01	1.673 (0.592)<0.01	2.747 (2.033) 0.18	−1.914 (0.753) 0.01	−1.317 (0.380)< 0.01
Urea	0.001 (0.001) 0.92	−0.007 (0.010) 0.47	−0.018 (0.034) 0.61	−0.013 (0.013) 0.31	−0.001 (0.006) 0.89
Cholesterol ($\times 10^{-1}$)	0.002 (0.004) 0.55	0.034 (0.046) 0.45	0.302 (0.158) 0.06	−0.083 (0.058) 0.16	−0.026 (0.039) 0.38
Model 4					
Plasma CZr	−0.048 (0.034) 0.16	−0.437 (0.422) 0.30	−0.430 (1.489) 0.77	0.798 (0.555) 0.15	0.365 (0.279) 0.19
Plasma Cu[a] ($\times 10^{-2}$)	−0.003 (0.008) 0.67	−0.006 (0.970) 0.95	0.112 (0.339) 0.74	0.085 (0.128) 0.51	−0.037 (0.640) 0.57
Plasma Zn[a] ($\times 10^{-2}$)	0.013 (0.012) 0.26	0.167 (0.145) 0.25	0.153 (0.509) 0.76	−0.066 (0.192) 0.73	−0.089 (0.963) 0.36
Albumin	0.129 (0.047) 0.01	1.141 (0.589) 0.05	1.349 (2.048) 0.51	−1.360 (0.764) 0.07	−1.056 (0.384) 0.01
Urea	<0.001 (<0.001) 0.82	−0.009 (0.009) 0.36	−0.025 (0.034) 0.46	−0.12 (0.012) 0.34	−0.001 (0.006) 0.85
Cholesterol ($\times 10^{-1}$)	0.001 (0.004) 0.94	−0.003 (0.045) 0.95	0.206 (0.159) 0.19	−0.036 (0.060) 0.55	−0.004 (0.301) 0.88
C-reactive protein	−0.010 (0.005) 0.06	−0.106 (0.064) 0.10	−0.357 (0.225) 0.11	0.107 (0.847) 0.21	0.125 (0.425)<0.01
IL-6	−0.016 (0.007) 0.03	−0.292 (0.092) <0.01	−0.676 (0.315) 0.03	0.271 (0.118) 0.02	0.049 (0.592) 0.40

Results are expressed as beta coefficients (standard errors). SPPB is composed of usual gait speed, balance, and chair stand tests) ranges from 0 (worse performance) to 12 (best performance). ADL, range, 0–7, a higher number indicates higher impairment; IADL, range 0–7, a higher number indicates higher impairment). Model 1: adjusted for age, gender. Model 2: adjusted for age, gender, BMI, hypertension, and congestive heart failure. Model 3: adjusted for age, gender, BMI, hypertension, congestive heart failure, and hematological parameters (albumin, urea, cholesterol). Model 4: adjusted for age, gender, BMI, hypertension, congestive heart failure, hematological parameters (albumin, urea, cholesterol), and inflammatory parameters (C-reactive protein, IL-6)

[a] Beta coefficients and standard errors were calculated substituting the independent variable CZr with the continuous variables Cu and Zn while keeping all other confounders

in another Italian cohort of younger elders that were functionally independent (Malavolta et al. 2010). The octogenarians and nonagenarians enrolled in this study represent an interesting and new model to investigate the biological and nonbiological determinants of aging and longevity, as well as their interactions. Furthermore, studies performed in specific and well-defined geographic regions—such as

Table 3 Adjusted linear regression models predicting SPPB% decline in 2 years from CZr, Cu, Zn, and other inflammatory biomarkers

Parameters	Unadjusted B (standard error) *p*	Model 1 B (standard error) *p*	Model 2 B (standard error) *p*	Model 3 B (standard error) *p*	Model 4 B (standard error) *p*
Plasma CZr	14.174 (5.420) 0.01	11.888 (5.517) 0.03	11.497 (5.724) 0.04	11.291 (5.751) 0.04	11.944 (6.335) 0.05
Plasma Cu[a] ($\times 10^{-2}$)	1.699 (1.314) 0.20	1.247 (1.361) 0.36	1.042 (1.392) 0.45	0.960 (1.392) 0.49	0.859 (1.467) 0.56
Plasma Zn[a] ($\times 10^{-2}$)	−3.722 (1.314) 0.20	−2.633 (1.946) 0.18	−2.419 (1.976) 0.22	−2.256 (1.977) 0.254	−2.105 (2.097) 0.32
IL-6[a]	1.317 (1.235) 0.29	0.598 (1.233) 0.63	0.555 (1.231) 0.65	−12.600 (8.727) 0.66	–
CRP[a]	0.527 (0.833) 0.53	0.410 (0.814) 0.61	0.419 (0.819) 0.61	0.424 (0.817) 0.60	–

Results are expressed as beta coefficients (standard errors). SPPB is composed of usual gait speed, balance, and chair stand tests) ranges from 0 (worse performance) to 12 (best performance). SPPB% decline was calculated as [(SPPB baseline)−(SPPB (follow-up)]×100/(SPPB baseline). Model 1: adjusted for age, gender. Model 2: adjusted for age, gender, BMI, hypertension and congestive heart failure. Model 3: adjusted for age, gender, BMI, hypertension, congestive heart failure and hematological parameters (albumin, urea, cholesterol). Model 4: adjusted for age, gender, BMI, hypertension, congestive heart failure, hematological parameters (albumin, urea, cholesterol), and inflammatory parameters (C-reactive protein, IL-6)

[a] Beta coefficients and standard errors were calculated substituting the independent variable (CZr) with the continuous variables Cu and Zn or IL-6 or CRP while keeping all other confounders

the Sirente Mountain Community—can be particularly useful in interpreting and disentangling all the complex interactions involved in the development of disability status and longevity (Landi et al. 2005). The confirmation of our previous finding in this population and the new association herein reported between CZr and physical performances decline suggests that CZr may be a useful circulating biomarker in the clinical management of elderly subjects. Even thought the association between CZr and baseline measures of physical performance and function seems to be the consequence of inflammatory alterations that can be measured with other laboratory assessments, the independent predictive value of CZr for all-cause mortality suggests that this parameter could also be sensitive to other systemic alterations. These alterations cannot be revealed from measures of physical performance and function as well as from common standard clinical laboratory assessment because the inclusion of these parameters in Cox regression models does not affect the predictive value of CZr for mortality. It has been recently reported that dysregulation in multiple physiological systems may undermine homeostatic adaptive capacity, leading to the development of frailty and its associated risk for subsequent disability and adverse outcomes (Fried et al. 2009). Dysregulation in multiple physiological systems was investigated using 12 measures from six different physiological systems: hematological (hemoglobin less than 12 g/dL), inflammatory (IL-6 higher than 4.6 pg/mL), hormonal (IGF-1 less than 74.3 mg/L, DHEA less than 0.215 mcg/mL, and hemoglobin A1c greater than 6.5%), adiposity (triceps muscle skinfold less than 17 mm), neuromuscular (slow fine motor speed greater than 31.9 s), and micronutrients (two or more measures among 25-hydroxy vitamin D, folate, vitamin B_{12}, alpha-tocopherol, and total carotenoids below a previously established deficit threshold) (Fried et al. 2009). It is still unknown if CZr could be representative of one or more of these systems, but previous findings suggest that this parameter might reflect at least nutritional, inflammatory (Malavolta et al. 2010), and systemic oxidative alteration (Mezzetti et al. 1998). In this context, CZr might be extremely sensitive to zinc deficiency (Malavolta et al. 2010) which is, in turn, a common event in aging (Mocchegiani et al. 2008). However, trace element homeostasis may have an important role in physical performance and risk of mortality beyond their relevance for nutritional status. In fact, the serum concentration of Cu and Zn is strictly regulated by modulators and compensatory mechanisms that act to decrease serum Zn concentration while increasing serum Cu concentration in response to different stressor (Cousins 1985). These alterations are normally transient in young adult age but, pathogen accumulation, immunosenescence, and the chronic low level inflammatory status associated

Table 4 Cox regression analysis of plasma "CZr" (tertiles or continuous variable) or "Cu and Zn" and 4-year "All-Cause Mortality" with and without adjustment for various confounders

	Unadjusted			Model 1			Model 2			Model 3			Model 4			Model 4+baseline physical and functional measures		
	HR	CI (95%)	*p*	HR	CI (95%)	*p*	HR	CI (95%)	*p*	HR	CI (95%)	*p*	HR	CI (95%)	*p*	HR	CI (95%)	*p*
Plasma CZr[a]	2.79	2.10–3.72	<0.01	2.80	2.06–3.80	<0.01	2.53	1.80–3.55	<0.01	2.31	1.66–3.21	<0.01	1.77	1.23–2.55	<0.01	1.89	1.31–2.74	<0.01
Plasma Cu[b] ($\times 10^{-2}$)	1.27	1.18–1.37	<0.01	1.28	1.18–1.39	<0.01	1.23	1.13–1.34	<0.01	1.20	1.12–1.30	<0.01	1.15	1.06–1.26	<0.01	1.17	1.07–1.28	<0.01
Plasma Zn[b] ($\times 10^{-2}$)	0.61	0.51–0.74	<0.01	0.64	0.53–0.77	<0.01	0.67	0.55–0.82	<0.01	0.73	0.60–0.88	<0.01	0.81	0.68–0.97	0.02	0.91	0.81–1.01	0.08
Plasma Cu/Zn tertiles																		
Low (reference)																		
Medium	1.86	1.15–3.01	0.01	1.62	0.97–2.63	0.05	1.43	0.87–2.36	0.15	1.49	0.91–2.45	0.11	1.41	0.86–2.34	0.18	1.57	0.93–2.65	0.09
High	3.88	2.48–6.09	<0.01	3.30	2.09–5.21	<0.01	2.67	1.67–4.26	<0.01	2.45	1.53–3.93	<0.01	1.85	1.12–3.06	0.02	1.92	1.12–3.29	0.02
Age				1.08	1.04–1.11	<0.01	1.09	1.05–1.13	<0.01	1.08	1.04–1.12	<0.01	1.07	1.03–1.11	<0.01	1.03	0.99–1.07	0.18
Gender (female)				0.78	0.55–1.09	0.14	0.78	0.55–1.10	0.16	0.88	0.62–1.25	0.48	0.97	0.68–1.38	0.85	0.76	0.49–1.19	0.24
BMI							0.97	0.93–1.01	0.10	0.98	0.94–1.02	0.30	0.97	0.68–1.38	0.20	0.99	0.95–1.03	0.61
Hypertension							0.98	0.69–1.38	0.90	0.97	0.69–1.37	0.86	1.01	0.71–1.43	0.95	0.99	0.68–1.45	0.97
Congestive heart failure							3.17	1.87–5.39	<0.01	3.54	2.06–6.08	<0.01	3.62	2.10–6.22	<0.01	2.84	1.60–5.04	<0.01
Albumin										0.59	0.36–0.96	0.03	0.76	0.45–1.28	0.30	0.80	0.43–1.47	0.46
Urea										1.00	0.99–1.01	0.85	1.00	0.99–1.01	0.63	1.00	0.97–1.01	0.38
Cholesterol ($\times 10^{-1}$)										0.93	0.89–0.96	<0.01	0.94	0.90–0.98	<0.01	0.93	0.89–0.97	<0.01
C-reactive protein													1.05	0.99–1.10	0.07	1.02	0.97–1.08	0.37
IL-6													1.10	1.03–1.18	<0.01	1.10	1.02–1.19	0.01
4-m walking speed (m/s)																2.03	0.53–7.76	0.30
SPPB																0.89	0.80–1.00	0.05
Hand grip strength (kg)																0.99	0.97–1.01	0.39
ADL scale score																0.99	0.88–1.12	0.94
IADL scale score																1.12	1.01–1.26	0.04

Model 1: adjusted for age, gender. Model 2: adjusted for age, gender, BMI, hypertension, and congestive heart failure. Model 3: adjusted for age, gender, BMI, hypertension, congestive heart failure, and hematological parameters (albumin, urea, cholesterol). Model 3: adjusted for age, gender, BMI, hypertension, congestive heart failure, hematological parameters (albumin, urea, cholesterol), and inflammatory parameters (C-reactive protein, IL-6). Model 4+physical and functional measures included 4-m walking speed, SPPB, hand grip strength, ADL score, and IADL score

HR hazard ratio

[a] HR, CI, and *p* for this variable were calculated substituting the categorical variable (CZr tertiles) with the continuous variable CZr while keeping all other confounders.

[b] HR, CI, and *p* for this variable were calculated substituting the categorical variable (CZr tertiles) with the continuous variables Cu and Zn while keeping all other confounders

with advancing aging may permanently affect the balance between copper and zinc (Mocchegiani et al. 2010). Hence, the maintenance of homeostasis or the presence of multisystem dysregulation can be reflected by CZr (Mocchegiani et al. 2008). Therefore, a determination of serum CZr may be a useful tool for the clinical characterization of aged elderly at risk of worse physical decline or mortality. Such an assumption is also supported by other findings showing that plasma CZr was found to be higher in hospitalized elderly subjects compared to their healthy counterparts (Belbraouet et al. 2007) and associated with the risk of CVD death (Leone et al. 2006; Reunanen et al. 1996) and malignancy (Cunzhi et al. 2003; Dìez et al. 1989). As such, without excluding other significant variables to predict physical decline and the possible appearance of disability, the establishment of specific cutoff of plasma CZr with general significance might be a useful clinical tool in the management of elderly subjects. In our study, we identified levels of CZr above 2.08 in males and 2.24 in females as risk thresholds for mortality. Our previous findings (Malavolta et al. 2010), in a functional independent population over 81 years, observed a significant risk for mortality in men with levels above 1.78 and women with levels above 2.04 that are substantially lower than the mean values reported in the current sample (1.93 in men and 2.06 in women). However, it is important to consider that the thresholds in Malavolta et al. (2010) were calculated from tertiles of a functional independent population where death events were relatively rare (32 deaths after 3.5 years) compared to those reported in this study (145 deaths after 4 years) performed in older people affected by various grade of disease and disability. Therefore, the thresholds identified in this study could potentially display higher clinical relevance than those reported in functionally independent population, taking also into account that clinicians are rarely in contact with healthy and functionally independent elderly, while being usually involved in the clinical management of elderly people with comorbidity and disability. However, other studies reported mean levels above 1.70 in elderly subjects with at least one diagnosis of age-related disease (Belbraouet et al. 2007). Anyway, the relatively high mean levels reported for men (1.93) and women (2.06) in our population are consistent with a very old population characterized by the presence of different comorbidities.

Moreover, the results presented in this manuscript highlight also the higher sensibility of CZr than Cu or Zn alone to general clinical risk conditions in the elderly because these circumstances are generally reflected by a decrease in Zn or by an increase in Cu or both (Belbraouet et al. 2007; Ghaemian et al. 2011; Malavolta et al. 2010).

CZr might also be used as an important prognostic factor when nutritional support, physical exercise, or anti-inflammatory interventions are attempted to decrease the risk of mortality in disable elderly subjects. To achieve further information about the possible role of CZr in the physiology of geriatric syndromes, a much greater effort should be provided. Further research is required to investigate the prognostic value of CZr in different health care settings and the effect of specific interventions, including zinc supplementation in the diet, aimed at decreasing CZr.

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