Accumulation of Cells With Short Telomeres Is Associated With Impaired Zinc Homeostasis and Inflammation in Old Hypertensive Participants

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Critical shortening of telomeres, likely associated with a considerable increase of senescent cells, can be observed in PBMC of individuals aged 80 and older. We investigated the relationship between critical telomere shortening and zinc status in healthy or hypertensive participants with or without cardiovascular disease in old and very old participants. Telomere shortening and accumulation of cells with short telomeres (percent of cells with short telomeres) in advancing age was evident in patients and healthy controls, but exacerbated in those patients aged 80 and older. Moreover, in very old patients, the accumulation of % CST may impair intracellular zinc homeostasis and metallothioneins expression, which itself is linked to an increased number of inflammatory agents, thereby suggesting the existence of a possible causal relationship between % CST and zinc homeostasis. The determination of % CST could be a more reliable means than the simple measure of telomere length as fundamental parameter in ageing to determine whether individuals are still able to respond to stress.

Key Words: Metallothioneins—Cell senescence—Zinc—Hypertension—Inflammation.

Both in vitro and in vivo cells enter a nondividing state termed senescence after a variable number of cell divisions. Critical shortening of telomeres is strongly implicated as a factor that directs cells into this senescent phenotype (1). Age-dependent telomere shortening and the fact that many age-associated disorders are characterized by short telomeres both support the concept that telomere length homeostasis is an important parameter for human longevity (2). This evidence is complemented by the prognostic value of short leukocyte telomeres in predicting the death in patients with stable coronary artery disease (3). The increased rate of telomere ablation in white blood cells and arterial tissue from patients with atherosclerosis has been attributed to the increased cell turnover induced by the underlying chronic inflammatory response (4). Moreover, taking into account that hypertension is one of the major risk factors contributing to cardiovascular disease (CVD) in the elderly (5), several studies have examined the potential relationship between telomere length and human hypertension. It was found that telomere restriction fragment length correlated negatively with systolic blood pressure (SBP) and positively with diastolic blood pressure (DBP), thus suggesting a negative relationship between telomere length and pulse pressure (6). More recently, it was reported that systemic oxidative stress is associated with increased telomere ablation in leukocytes from old hypertensive men (7). These findings suggest the existence of a close link among stress, telomere length, and hypertension coupled with the risk of CVD. Indeed, in several types of CVD, proinflammatory insults also mediate oxidative stress (8) that, in turn, is counteracted by the upregulation of antioxidant scavenging systems/proteins including metallothioneins (MT; 9,10). MT are low-molecular-weight zinc-binding proteins that play a role in zinc homeostasis, regulate synthesis, assembly, or activity of zinc metalloproteins, and protect from damage induced by reactive oxygen and nitrogen species (11). MT transduce signals, via release of free zinc ions, activating intracellular antioxidant stress responses (12). MT overexpression coupled with changes in intracellular labile zinc (iZnL) is triggered in peripheral blood mononuclear cells (PBMC) of old atherosclerotic patients (13,14) as a local protective factor in response to inflammation. The low chronic inflammatory status associated with ageing is also coupled with increased MT expression in certain tissues (15). However, several lines of evidence suggest that healthy nonagenarians/centenarians express lower MT levels than do old participants (16). Although it is still matter of debate whether the decreased levels of MT in very old people are the consequence of a possible selection for survival of low expressors (16), a recent study suggests that...
this circumstance may be associated with cell senescence (17). Recent findings reveal that the percentage of the cells with short telomeres (% CST, <6 kb, senescent cells) increases with advancing age and that this is especially evident in very old age (18). This may have significant implications for prognostic factors in age-related diseases. To date, however, no research has been done into the relationship among cell senescence, number of CST, labile zinc, MT, and inflammatory markers in old and very old patients with age-related conditions, including CVD.

The aim of this study was therefore to assess the patterns of MT protein expression and labile zinc in relation to critical telomere shortening in peripheral blood cells from old and very old healthy participants in comparison with old and very old hypertensive patients with or without CVD.

**Materials and Methods**

**Participants**

We analyzed data from 125 participants being treated for chronic essential hypertension, whom we divided into two age brackets: 77 old (age range 60–79 years) and 48 very old (age range 80–100 years). We subdivided these groups into participants with hypertension associated with CVD (coronary heart disease, peripheral vascular disease, cerebrovascular disease) and participants with hypertension with no clinical signs of CVD. Blood pressure was measured in all the hypertensive participants at least 6 months before their enrollment. Hypertensives were defined as those participants having a sitting SBP greater than 160 mmHg and/or DBP greater than 95 mmHg on three different occasions or those participants on medication for high blood pressure. A standardized questionnaire was administered to provide data on the participants’ medical history, drug regimens (high blood pressure and other medications), and habits, such as smoking. Participants were classified as current smokers and ex-smokers. Body mass index (BMI) was calculated as weight (kg)/(height [m])\(^2\) where a BMI greater than or equal to 30 indicates obesity.

We also collected and analyzed data from a total of 61 healthy controls, made up of 43 and 18 individuals who respectively formed the same age brackets as earlier and who lived by themselves. All participants were recruited from the Italian National Research Centre on Ageing (Ancona, Italy) within the framework of the European Zinc Age Consortium. Old and very old healthy participants were considered to have good health status based on their SBP and DBP measurements, routine laboratory test results, inflammatory markers (erythrocyte sedimentation rate [ESR] ≤ 12 mm/h and serum C-reactive protein [CRP] ≤ 0.3 mg/dL), and their activities of daily living rating. They were independent, did not require special daily care, and had received no therapeutic treatment for at least 2–3 months before enrollment.

**Laboratory Measurements**

ESR, CRP, low-density lipoprotein (LDL) cholesterol, and triglyceride concentrations were determined using standardized clinical laboratory analysis techniques. The normal ESR range was less than or equal to 12 mm/h. CRP values were detected by CardioPhase hsCRP (Dade Behring Inc., Deerfield, IL). The sensitivity of the CRP assay is 0.02 mg/dL, and the normal range was less than or equal to 0.3 mg/dL. The normal range of LDL cholesterol and triglyceride levels was less than 160 mg/dL and less than 150 mg/dL, respectively.

**Estimation of Telomere Length and % CST**

Telomere length and the detection of cells with short (<6 kb) telomeres (% CST) were assessed by high-throughput Q-FISH (quantitative fluorescence in situ hybridization) using a fluorescent peptide nucleic acid probe against telomeric repeats with automated high-throughput (HT) microscopy, as reported by Canela and colleagues (18).

**PBMC Recovery and Storage**

Individual blood samples from healthy and atherosclerotic participants were drawn in a plastic syringe containing heparin. PBMCs were obtained by ficoll-hypaque (gradient density \(d = 1.077\); Biochrom AG, Berlin, Germany) gradient centrifugation at 450 g for 15 minutes, at 20°C. All samples presented a viability higher than 90% assessed by Trypan blue dye exclusion. Aliquots of cells (2 × 10⁶ cells/mL) were frozen in fetal calf serum containing 10% dimethyl sulfoxide in liquid nitrogen until required. Before use, the cells were gradually thawed and counted.

**MT and Labile Zinc Determination**

MT determination was performed as previously reported by Yurkow and Makhijani (19) using the monoclonal mouse anti-horse MT clone E9 antibody (Dakocytoma, Glostrup, Denmark). Thawed PBMCs (2 × 10⁶/mL) were treated with 0.3% paraformaldehyde and stored at 4°C for 2 days before processing. Samples probed with an irrelevant (isotypic) antibody (clone MOPC21, IgG1k, Sigma-Aldrich, Milan, Italy) in conjunction with the fluorescein-conjugated secondary antibody served as staining controls. The fluorescence intensity obtained with the isotypic antibody was subtracted from all data. Quantitative calibration was performed with samples containing a well-known amount of MT measured by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry as reported elsewhere (20). Results are expressed as picomole/milligram protein.

Labile zinc was estimated by flow cytometry using zinpyr-1 (ZP-1) as previously reported by Malavolta and colleagues (21). “Zinc-free” Roswell Park Memorial Institute (RPMI) medium was obtained by treatment of RPMI
with 5% Chelex 100 (Sigma-Aldrich). Thawed PBMCs were divided into two equal aliquots of $2 \times 10^5$ cells/mL, at least. One aliquot was incubated with 20 μM ZP-1 (Neurobiotex, Galveston, TX) for 30 minutes at 37°C, 5% CO2 in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid–buffered zinc-free RPMI medium containing 1 mM ethylenediaminetetraacetic acid, as extracellular chelator, of free zinc eventually still present in the medium and/or adsorbed to the cell membrane.

The second aliquot was likewise incubated under the same conditions with the addition of 50 μM N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (Sigma-Aldrich), in order to detect the autofluorescence of the zinc-free ZP-1 probe (22).

After incubation, the aliquots were immediately analyzed by flow cytometry (Coulter Epics XL, Ramsey, MN). Mean fluorescence intensity for ZP-1 was detected (excitation wavelength 488 nm and detection at 525 ± 15) in the two aliquots after selecting the lymphocyte population according to the forward and side scatters. Data were reported as a ratio of ZP-1 fluorescence to ZP-1 autofluorescence and represented the iZnL (21).

**Statistical Analysis**

Significant differences in the studied variables among experimental groups were detected by analysis of variance. To analyze the relationship among the studied parameters, bi-variate (Pearson) and age-controlled partial correlation was performed. Due to the skewed nature of the distribution, log-transformed (ln) values of studied variables were used for calculating Pearson and partial correlation coefficients after controlling for age. Participants aged 80–100 years were also grouped according to the presence (%) of CST. To accomplish this, the variable "% CST (<6 kb)" was dichotomized as follows: less than 5.7% and greater than or equal to 5.7%; this cut-off value corresponds to the 75th percentiles of the % CST distribution in the studied sample. All the analyses were performed by tests implemented with the SPSS package for Windows, version 14 (SPSS, Chicago, IL).

**Results**

**Effect of Age and Disease Status on Telomere Length, Accumulation of CST (% CST), Inflammation, MT, Biological Parameters, and Medication Taken**

Table 1 summarizes the biological and biochemical characteristics of the population as well as the medication taken. BMI increased in hypertensive participants with CVD at the age of 60–79 years in comparison with respective healthy controls ($p < .05$). By contrast, triglyceride levels decreased in the same participants when compared with respective healthy controls ($p < .05$; Table 1). Interestingly, the majority of hypertensive participants with CVD in both age groups were being prescribed high blood pressure medications, especially cardioaspirin and diuretics. Moreover, hypertensive participants with CVD at the age of 60–79 years had a higher percentage of hyperlipidemia compared to healthy controls ($p < .05$).

Table 1. Biological and Biochemical Parameters and Medications in Old, Very Old Hypertensive Patients, and Their Respective Healthy Controls

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Healthy Participants</th>
<th>Hypertensive Participants Without CVD</th>
<th>Hypertensive Participants With CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old, 60–79</td>
<td>BMI</td>
<td>25.31 (24.61; 31.80)</td>
<td>26.47 (24.82; 31.80)</td>
</tr>
<tr>
<td></td>
<td>Triglycerides, mg/dL</td>
<td>50.31 (42.00; 59.00)</td>
<td>44.18 (32.5; 54.5)</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol, mg/dL</td>
<td>132.0 (108.40; 157.20)</td>
<td>125.4 (116.20; 146.60)</td>
</tr>
<tr>
<td></td>
<td>Current smokers, %</td>
<td>11.1</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Ex-smokers, %</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Hyperlipidemia, %</td>
<td>74.2</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>Statin treatment, %</td>
<td>25.8</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td>Beta blockers, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cardioaspirin, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Diuretic, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Vasodilator, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Minoxidil, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Calcium antagonist, %</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: Biological parameters and medications prescribed for the sample. Median and interquartile range (IQR) are presented where indicated. CVD = cardiovascular disease; BMI = body mass index.
participants with CVD showed an altered lipid profile with respect to healthy controls at the age of 60–79 years. This fact might justify the presence of a larger number of hypertensive participants with CVD (45%) placed on statins, which also reduce triglyceride levels (Table 1).

Table 2 reports telomere length, % CST, zinc status (MT and iZnL), and inflammatory parameters (CRP and ESR). Telomere length decreased in hypertensive participants with CVD in comparison with healthy participants \((p < .05)\) within the same age group under consideration. Moreover, in the 60- to 79-year age group, telomere length also decreased in hypertensive participants without CVD in comparison with their healthy counterparts \((p < .05)\). With regard to % CST, this increase was more marked in very old CVD hypertensive participants (Table 2). It is noteworthy that the % CST increase was also in hypertensive patients with CVD in comparison with those without CVD \((p < .05)\) within the same age group (Table 2). The accumulation of % CST increased with advancing age in all age groups considered (Table 2). With regard to the zinc status, MT increased exclusively in old hypertensive participants (age 60–79 years) with and without CVD in comparison with healthy participants \((p < .05)\). By contrast, the MT showed only a slight downward trend in very old hypertensive participants with or without CVD when compared with healthy controls (Table 2).

The iZnL decreased in very old (80–100 years) hypertensive participants with CVD in comparison with healthy controls \((p < .05)\). By contrast, iZnL increased in old (60–79 years) hypertensive participants with and without CVD in comparison with healthy patients \((p < .05)\); Table 2). As for inflammatory parameters, ESR and CRP increased both in old and very old hypertensive patients when compared with healthy controls \((p < .05)\) independently of the presence of CVD. Notwithstanding, the type of medication prescribed and smoking had no influence on the differences noted in telomere length, % CST, and zinc status (MT and iZnL) both in healthy and hypertensive participants (data not shown). Evidence of this was obtained through univariate analysis and where the effect of any medication was considered as a confounding factor and age taken as a covariate. No differences were observed between male and female participants (data not shown).

Correlations Among Telomere Length, % CST, Zinc Status, and Inflammation Parameters in Old and Very Old Participants (Hypertensive and Healthy Controls) in Both Classes of Age Considered

Table 3 shows the existing correlations among the parameters considered in this study in hypertensive patients with or without CVD. We report the more significant and useful correlations and not those that are obvious or standard, like % CST and telomere length with advancing age, CRP and ESR, or zinc and MT.

### Table 2: Telomere Length, Accumulation of Cells With Short Telomeres (CST), Metallothioneins (MT), Labile Zinc, and Inflammatory Markers in Old and Very Old Hypertensive Patients as Well as in Respectively Healthy Controls

<table>
<thead>
<tr>
<th>Age Classes</th>
<th>Healthy Participants</th>
<th>Hypertensive Participants With CVD</th>
<th>Hypertensive Participants Without CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old: 60–79 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female; male, %</td>
<td>47; 53</td>
<td>57; 43</td>
<td>55; 45</td>
</tr>
<tr>
<td>Age (y)</td>
<td>62; 69</td>
<td>65; 61</td>
<td>61; 65</td>
</tr>
<tr>
<td>Mean telomere length, kb</td>
<td>10.41 (9.39; 11.42)</td>
<td>9.93 * (9.14; 11.24)</td>
<td>9.13 * (8.42; 9.91)</td>
</tr>
<tr>
<td>% CST</td>
<td>0.50 (0.2; 1.15)</td>
<td>0.80 * (0.30; 3.40)</td>
<td>2.20 * , † (0.8; 4.90)</td>
</tr>
<tr>
<td>MT (pmol/mg protein)</td>
<td>23.30 (16.43; 27.68)</td>
<td>27.95 * (23.52; 32.94)</td>
<td>27.97 * (23.28; 35.92)</td>
</tr>
<tr>
<td>iZnL (MFI/MFImin)</td>
<td>1.26 (1.22; 1.30)</td>
<td>1.22 * (1.19; 1.35)</td>
<td>1.30 * (1.22; 1.44)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.10 (0.07; 0.21)</td>
<td>0.22 * (0.08; 0.31)</td>
<td>0.27 (0.08; 0.31)</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>11.00 (5.00; 14.00)</td>
<td>11.10 (5.00; 14.00)</td>
<td>11.10 (5.00; 14.00)</td>
</tr>
</tbody>
</table>

Notes
- Clinical, genetic, and nutritional characteristics of the population. Median and interquartile range (IQR) are presented where indicated. CVD = cardiovascular disease; CRP = serum C-reactive protein; ESR = erythrocyte sedimentation rate; iZnL = intracellular labile zinc; MFI = mean fluorescence intensity.
- * \(p < .05\) when compared with healthy participants of the same class of age.
- † \(p < .05\) when compared with participants with hypertension without CVD of the same class of age.
- ‡ \(p < .05\) when compared with hypertensive patients with CVD at the age of 60–79 years old.
- § \(p < .05\) when compared with hypertensive participants with 60–79 years old.
**Table 3. Bivariate (Pearson) Correlations Among the Studied Parameters in the 60- to 79-y and 80- to 100-y Age Groups**

<table>
<thead>
<tr>
<th>Age Group 60–79 y</th>
<th>Mean Telomere Length</th>
<th>% CST</th>
<th>iZnL</th>
<th>MT</th>
<th>ESR</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Group 80–100 y</td>
<td>0.062</td>
<td>0.035</td>
<td>−0.027</td>
<td>0.060</td>
<td>0.057</td>
<td>0.241*</td>
</tr>
</tbody>
</table>

Notes: CRP = serum C-reactive protein; CST = cells with short telomeres; ESR = erythrocyte sedimentation rate; iZnL = intracellular labile zinc; MT = metallothioneins. All correlations were calculated using log-transformed (ln) variables.

*\(p < .01\); **\(p < .05\).

Significant inverse correlation between inflammatory status (CRP) and telomere length \((r = -.236, p < .05)\) and significant positive correlations between CRP and the % CST \((r = .241; p < .05)\) or MT \((r = .202; p < .05)\) were observed in participants in the age range 60–79 years.

In participants at the age 80–100 years, a significant positive correlation between telomere length and MT \((r = .581; p < .01)\) or iZnL \((r = .502; p < .01)\) was observed, whereas significant inverse correlations existed between % CST and MT \((r = -.470; p < .01)\) or iZnL \((r = -.456; p < .01)\) (Table 3).

**Inflammatory Parameters and Zinc Status Related to the Number of CST in Very Old Age**

Because a strong accumulation of senescent CST was observed in very old age irrespective of health status (see Table 2), the variable % CST (<6 kb) was dichotomized as follows: less than 5.7% and greater than or equal to 5.7%; this cut-off value corresponds to the 75th percentile of the % CST distribution in the sample. Very old participants (80–100 years) were therefore subdivided into two groups: one group with a % CST less than 5.7 (low % CST) and the other with a % CST greater than or equal to 5.7 (high % CST). The same subdivision was not applied to the 60- to 79-year age group as the number of participants with a % CST greater than or equal to 5.7 was insufficient for comparisons. We then verified the possible effect of the accumulation of senescent cells on zinc status and inflammation in very old age. Table 4 shows that the % CST was strongly increased whereas telomere length, MT, and iZnL decreased in very old participants with a high % CST in comparison with participants with a low % CST \((p < .05)\). There were no significant differences in the other parameters considered or between men and women (data not shown).

**DISCUSSION**

Although inflammation can strongly contribute to an increase in MT expression in PBMCs from young, adult, and old individuals (16), we observed a hereto unexpected lack of correspondence between MT and inflammatory markers (CRP, ESR) in very old healthy and hypertensive participants, both with and without CVD, who accumulate cells with critically short telomeres. Critical telomere shortening is one of factors, which triggers permanent cell cycle withdrawal that eventually leads to cellular senescence (23). It has recently been suggested that a low MT gene expression and iZnL figure among the phenotypic features of senescent immune cells, including T cells (17). Telomere length progressively declines with advancing age (24). Because an alteration in the CD4/CD8 ratio of T cells may represent an “immune risk phenotype” in elderly participants (25), the presence of shorter telomeres becomes particularly evident either in B lymphocytes (26) or in CD8+ CD28− T lymphocytes (27,28). However, a strong association of shorter telomeres in PBMC with a higher mortality in older participants independent of the T cell subsets considered has been reported (29). Critical telomere shortening, likely coupled with a considerable increase in the accumulation of senescent cells, was recently observed in PBMCs in individuals aged 80 and older (18). Short telomeres in blood could also indicate the presence of an age-related disease that has, perhaps, triggered a shift in the proportions of white blood cell subsets, thereby reducing average telomere lengths (29). Hence, the accumulation of % CST may arise not only from telomere attrition with age but also from changes in PBMC subsets or from the presence of a chronic inflammatory state which is, in turn, present in hypertensive participants (30) and associated with shorter telomeres (7,31). As hypertension is the main cause of CVD, especially in the old (32), we focused our attention on the differences between very old (≥80 years) and old participants (60–79 years) by investigating the relationship between critical telomere shortening and MT in “healthy” and “hypertensive participants with or without CVD.” Patients of all age groups with hypertension with severe CVD had significantly higher levels of CRP and ESR that confirmed the presence of a significant inflammatory status (33). Telomere shortening and accumulation of CST with advancing age was also clearly evident both in patients and healthy participants and exacerbated in patients aged 80 and older coupled with low MT production. By contrast, MT was higher in hypertensive participants (60–79 years; Table 2) suggesting that it may be a response to inflammation and stress irrespective of the % CST. Such an assumption is confirmed by the absence of a correlation...
Table 4. Influence of CST Accumulation on MT, Labile Zinc, and Inflammatory Markers in Very Old Participants at the Age 80–100 y

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Participants (N = 66)</th>
<th>Low % CST (N = 34)</th>
<th>High % CST (N = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80–100 y</td>
<td></td>
</tr>
<tr>
<td>Female; male, %</td>
<td>58; 42</td>
<td>60; 40</td>
<td></td>
</tr>
<tr>
<td>Mean telomere length, kb</td>
<td>9.69 (9.40; 10.29)</td>
<td>7.85* (5.93; 8.57)</td>
<td></td>
</tr>
<tr>
<td>% CST</td>
<td>1.40 (0.70; 4.20)</td>
<td>14.90* (8; 50.80)</td>
<td></td>
</tr>
<tr>
<td>iZnL (MFI/MFI min)</td>
<td>1.22 (1.20; 1.27)</td>
<td>1.16* (1.08; 1.23)</td>
<td></td>
</tr>
<tr>
<td>MT (pmol/mg protein)</td>
<td>20.74 (18.34; 23)</td>
<td>14.71* (11.95; 19.75)</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>16 (9.33)</td>
<td>16 (8.29)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.31 (0.11; 0.98)</td>
<td>0.29 (0.20; 0.69)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: CRP = serum C-reactive protein; CST = cells with short telomeres; ESR = erythrocyte sedimentation rate; iZnL = intracellular labile zinc; MFI = mean fluorescence intensity; MT = metallothioneins. Median and interquartile range (IQR) are presented where indicated.

*p < .05 when compared with participants with low % CST.

*p < .01 when compared with participants with low % CST.

between MT and the % CST in the 60- to 79-year age group, whereas the presence of an inverse correlation between % CST and MT was observed in 80- to 100-year participants. (Table 3). These findings support the hypothesis that, despite the presence of inflammation, the phenotypic features of senescent PBMCs from very old individuals do not include a high MT expression. On the other hand, diminished MT expression has also been found in PBMCs from very old individuals and in vitro models of late passage/senescent T cell clones (17). These results also suggest that the activation of pathways involved in MT expression in response to the inflammation may be altered in senescent cells. Because MT play a key role in activating the antioxidant defense and, consequently, in protecting cells by reactive oxygen species (11), despite a consistent increase in CRP and ESR, very old hypertensive participants especially with CVD showed a trend toward low MT levels. This suggests a lack of inflammatory response, with however a specific involvement of the % CST, as shown by the presence of higher MT levels in very old participants with a lower number of senescent cells (Table 4). In other words, the role played by MT in response to inflammation may be different in very old age and strictly linked to the number of senescent cells. This phenomenon might be exacerbated by the concomitant presence of hyper- tension and CVD making older participants still more frail and more vulnerable to external noxae (34,35). This may be attributable to differences in levels of intracellular labile zinc considering that inflammatory agents can activate an influx of zinc within cells mediated by specific zinc importers (Zip family; 36,37), and zinc in turn activates MT gene expression (38). Indeed, a dramatic decrease in labile zinc occurs in very old hypertensive participants, especially CVD patients (Table 2), and is very evident when considered a high % CST (Table 4). Association with a positive MT and iZnL correlation suggests that downregulation of MT in senescent cells could occur as a secondary phenomenon of an altered zinc influx in these cells with subsequent low gene MT expression and induction. On the other hand, preliminary data from our laboratory have shown that older participants display reduced zinc influx within the cells (R. Giacconi, PhD, M. Malavolta, PhD, E. Mocchegiani, BS, unpublished data, 2009). Taking into account the presence of telomere shortening with advancing age and that very old age is a condition characterized by high inflammatory status and altered MT and iZnL levels (38), it is evident that inflammation plays an important role in very old age and that MT production might be insufficient to cope with inflammatory responses.

Another realistic explanation of this phenomenon in very old age might be related to the reduced rate of cellular proliferation: as such, cells require less zinc to build up new proteins. However, the possibility that the inflammatory-mediated expression of some zinc transporters is defective in senescent cells cannot be excluded taking into account that a different gene expression of zinc transporter (ZIP-1) between young and old people has been reported (39). Therefore, further studies using a larger number of participants will be required to investigate the localization, expression, and functionality of zinc transporters and the impact on morbidity and mortality in very old people taking also into account the possible confounding factors, such as medication. In conclusion, the present study supports the concept that cell senescence in very old age may impair intracellular zinc homeostasis and MT expression even in the presence of inflammatory agents, with the possible involvement of nutritional factors (eg, zinc) that might contribute to worsening the already poor physiological status of senescent cells because zinc might modulate telomerase enzyme activity (40) with a possible causal relationship between the % CST and zinc homeostasis. Such modulation of intracellular zinc might also be different in relation to the T cell subsets considered. Further studies are presently in progress in our laboratory. However, the determination of the % CST, rather than the simple measure of telomere length, may be considered a fundamental parameter in discerning whether participants are still able to respond to stress. An intriguing future research is the selective ablation of senescent cells that may be useful for improving immune responses and fighting chronic inflammation, thereby enhancing health status and longevity.
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