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Red Blood Cell Distribution Width, Vascular Aging Biomarkers, and Endothelial Progenitor Cells for Predicting Vascular Aging and Diagnosing/Prognosing Age-Related Degenerative Arterial Diseases

Carmela Rita Balistreri,[1,*] Calogera Pisano,[2,†] Fabio Bertoldo,[2,†] Renato Massoud,[3,‡] Susanna Dolci,[4] and Giovanni Ruvolo[2,†]

Abstract

The emerging evidence emphasizes *Red blood cell distribution width* (RDW) as optimal prognostic biomarker for cardiovascular diseases. However, several clinical biases impede its clinical application. Recent recommendations suggest combining RDW with other biomarkers. Accordingly, we propose evaluating the well-recognized biomarkers of vascular aging (*i.e.*, *the leukocyte telomere length and telomerase activity*, *and reduced levels of endothelial progenitor cells [EPCs])* with RDW, for predicting the risk for vascular aging and onset and prognosis of age-related degenerative arterial diseases, such as sporadic ascending aorta aneurysm (AAA), characterized to have an increased incidence in old people. Consequently, in this study (and for the first time), we simultaneously investigated the relationship between RDW values, systemic inflammatory molecules, mean values of leukocyte telomere length, telomerase activity and EPCs, and the risk for vascular aging and AAA onset and prognosis. To achieve this aim, we selected 80 old and 80 young healthy subjects and 80 AAA cases. Appropriate methodologies were used for assessing blood parameters, aorta alterations, genotyping, impairment of the leukocyte telomere length, and telomerase activity. The main findings obtained demonstrated that increased RDW values along with the augmented blood levels of high-sensitive C-reactive protein and the reduced mean values of both leukocyte telomere length, telomerase activity, and EPCs are independently associated with the high risk for both vascular aging and AAA onset and prognosis. They might be used as the best predictor biomarker profile for vascular aging, and for both diagnosis and outcome of sporadic AAA.

Keywords: RDW, leukocyte telomere length, telomere activity, EPCs, risk for vascular aging and sporadic AAA, diagnostic and prognostic AAA biomarkers

Introduction

Peripheral blood tests and the related easy parameters have always represented a powerful tool for preventing and/or monitoring several diseases, such as cardiovascular diseases (CVDs). During the last decades, it has been extremely interesting to observe how new cardiovascular biomarkers have been gained from blood parameters, routinely used for the laboratory diagnosis of noncardiovascular disorders, and particularly for hematological/immune diseases. Accordingly, the values of red blood cell distribution width (RDW) as predictor/prognostic biomarkers for CVDs have been considered and well consolidated.[1] However, at the moment, the specific clinical-translational significance of RDW evaluation in CVD diseases still requires a full validation. For example, it is not clear if RDW is an independent biomarker of CVDs, or linked to specific pathological conditions, which occur during CVD onset and their complications, such as chronic inflammation.[2] Consequently, several aspects

[1]Department of Pathobiology and Medical and Forensic Biotechnologies, University of Palermo, Palermo, Italy.
[2]Department of Cardiac Surgery, University of Rome ''Tor Vergata,'' Rome, Italy.
[3]Department of Clinical Biochemistry, Tor Vergata University Hospital, Rome, Italy.
[4]Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy.
[*]*Current affiliation:* Department of Biomedicine, Neuroscience and Advanced Diagnostics (Bi.N.D.), University of Palermo, Palermo, Italy.
[†]*Current affiliation:* Cardiac Surgery Unit, Department of Surgical Science, Tor Vergata University Hospital, Rome, Italy.
[‡]*Current affiliation:* Department of Experimental Medicine, Tor Vergata University Hospital, Rome, Italy.

remain obscure in the clinical application of RDW in CVDs, as recently stressed by our group.[2]

Aims of study

However, some recent studies are suggesting that it is more appropriate evaluating the RDW values in combination with other parameters.[2] Among these, the biomarkers, which are well known to be significantly associated with vascular aging and its complications, have been encouraged to be used. They are represented by (1) telomere content *(the TTAGGG DNA repeats at the ends of chromosomes)* in circulating blood leukocytes, which accurately reflects the biological age of the vascular wall; and (2) telomerase activity.[3] Accordingly, both parameters have been currently demonstrated to be significantly associated with several age-related degenerative CVDs, including degenerative arterial diseases. Of note is, indeed, their association with sporadic ascending aortic aneurysm (AAA), which can be considered a typical model of premature vascular aging.[4] Recently, we demonstrated, in a previous study,[4] shorter telomeres and a reduced telomerase activity in 80 patients affected by sporadic AAA than age- and gender-matched controls, which significantly correlated with a genetic inflammatory risk profile, age, gender, smoking, hypertension, a typical histopathological phenotype associated with medial degeneration and vascular remodeling,[5] and higher levels of systemic inflammatory mediators.[4] In addition, Kozlitina and Garcia[6] have recently analyzed the weight of leukocyte telomere impairment on hematopoiesis activity in a very large number of individuals (=3157) from general population included in *Dallas Heart Study*. Surprisingly, they obtained that shorter telomere lengths in old people are significantly associated with reduced numbers of red blood cells and platelets, and higher values of RDW and hemoglobin, suggesting a biologic mechanism for macrocytosis related to aging process. Furthermore, the group of Suàrez[7] recently reported a significant association of high RDW values with the depletion of endothelial progenitor cells (EPCs) and mediators related to endothelial damage and vascular repair failure in patients affected by rheumatoid arthritis, thereby suggesting RDW as predictor biomarker for CVDs. On the other hand, it has been stressed by our and other groups in a special issue that ageing affects the endothelium, its functionality and capacity of regeneration, conditions significantly associated with the onset of age-related degenerative arterial diseases.[8,9] In addition, this is positively correlated in old ages to reduced number or depletion of circulating EPCs. Based on this established evidence, we hypothesized that the combined evaluation of RDW levels with mean values of blood leukocyte telomere length and telomerase activity, and the values of circulating EPCs may represent the best predictor biomarkers for vascular aging, and constitute an appropriate biomarker profile for diagnosing or prognosing age-related degenerative diseases, such as sporadic AAA. Consequently, we detected the values of these parameters and their correlations in a healthy population constituted by 80 old subjects and 80 younger blood donors. In addition, for performing a comparison of these parameters with the risk and prognosis for AAA, we also enrolled (*de novo*) 80 patients affected by sporadic AAA.

Population and Methods

Old and young healthy individuals and patients with AAA

The population enrolled consisted of 80 old healthy subjects (mean age 72±4.8 [range 66–83] years) and 80 younger blood donors (mean age 26.2±3.4 [range 21–33] years). They were all Caucasian. Their medical records and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C reactive protein, liver function tests, iron, and proteins) excluded the presence of age-related degenerative arterial diseases, or other CVDs, or other age-related diseases. The echocardiographic examination also excluded the presence of heart and aorta abnormalities. In Table 1, their features were reported. In addition, blood samples from this population were collected into EDTA-coated tubes. We also enrolled a relatively small sample size of patients affected by sporadic AAA, since this disease has a prevalence of only 3%–4% in European population and 1.6:1 male/female ratio, as established by 2014 ECS guideline, amply quoted in our recent review.[10] Specifically, we enrolled 80 patients (53 men [65%] and 28 [35%] women; mean age: 68±9.3) with sporadic AAA, randomly selected from patients referring to the *Units of Cardiac Surgery of University of Rome* ''*Tor Vergata*,'' for surgery replacement or routine care screening. Appropriate exclusion criteria were also used during their enrolment, for the following diseases: (1) CVDs were excluded according to history and by detecting apposite laboratory and imaging biomarkers as indicated by the latest ESC or ASC guidelines; (2) connective tissue disorders were excluded by assessing markers of inflammation immunological (*i.e.*, autoantibodies) and imaging biomarkers; and (3) inflammatory diseases (from infections to hematological, gastrointestinal, urogenital, pulmonary, neurological, endocrinal inflammatory disorders, and neoplasia included) by detecting apposite laboratory parameters (including complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C reactive protein, liver function tests, iron, and proteins) and imaging biomarkers. In addition, all the enrolled cases belonged to the same ethnic group of young and old subjects. Thus, a very homogenous population was studied. Furthermore, elective or acute surgical treatment (using wheat operation, Bentall-De Bono and Tirone David surgical techniques, whenever possible) and complementary tubular-ascending aorta resection were performed in patients with AAA after evaluation of aortic transverse diameter sizes by computed tomography scanning according to recent guidelines, as reported in our recent review.[8] Accordingly, an experienced physician evaluated aortic transverse diameter sizes by echocardiography (Philips Ie. 33) before either elective or urgent surgery. The dimension of the aortic annulus, sinuses of Valsalva, proximal ascending aorta (above 2.5 cm of the sinotubular junction), and aortic arch were assessed preoperatively by transthoracic echocardiography as well as in the operating theatre by transesophageal-echocardiography before the institution of the cardiopulmonary bypass. These measures, together with demographic and clinical data (including co-morbidities), were obtained from patients' medical records and are presented in Table 1. In all cases, hypertension was treated by beta-blockers.

In performing this study, approval from local ethics committees was obtained (No. APUNIP0123578), as well as

Table 1. Demographic, Clinical, and Genetic Features, and Comorbodity Conditions of the Study Population

Variables	*Young people* N = *80*	*Old people* N = *80*	*AAA patients* N = *80*	P1[a]	P2[a]	P3[a]
Demographic characteristics						
Age, mean (SD)	26.2 (3.4)	72 (4.8)	68 (9.3)	**<0.001**	N.S.	**<0.001**
Male sex, *n* (%)	40 (50)	35 (43)	53 (66)	N.S.	**0.005**	**0.01**
Female sex, *n* (%)	40 (50)	45 (57)	27 (34)			
Body mass index, mean (SD)	25 (5.1)	25.8 (2.8)	26.9 (3.9)	N.S.	**0.02**	**0.01**
Size and location						
Size (mm), mean (SD)	0 (0)	0 (0)	53.5 (3.8)	—	—	—
Location, *n* (%):	0 (0)	0 (0)				
Ascending aorta	0 (0)	0 (0)	62 (85)	—	—	—
Aortic bulb	0 (0)	0 (0)	12 (15)	—	—	—
Comorbidity conditions, *n* (%)						
CVD family history	5 (6.25)	5 (6.25)	10 (13)	N.S.	N.S.	N.S.
Smoking	0 (0)	5 (2.5)	42 (54)	**0.02**	**<0.00000001**	N.S.
Hypertension	0 (0)	2 (2.5)	48 (60)	N.S.	**<0.00000001**	N.S.
Dyslipidemia	0 (0)	2 (2.5)	12 (15)	N.S.	**0.004**	**0.00008**
Diabetes mellitus	0 (0)	0 (0)	10 (13)	N.S.	**0.0007**	**0.0006**
Renal failure	0 (0)	0 (0)	5 (6.25)	N.S.	**0.02**	0.058
Dissection	0 (0)	0 (0)	5 (6.25)	N.S.	**0.02**	0.058
Systemic inflammatory plasma mediator's levels						
IL-6 (pg/mL)	5 ± 1.5	12 ± 4.5	21 ± 5.7	**<0.0001**	**<0.001**	**<0.001**
TNF-α (pg/mL)	3.8 ± 2.4	10.4 ± 3.8	16.9 ± 4.5	**<0.0001**	**0.01**	**<0.001**
MMP-2 (ng/mL)	3 ± 2.1	16 ± 2.1	58.2 ± 3.1	**<0.0001**	**<0.0001**	**<0.0001**
MMP-9 (ng/mL)	4 ± 1.2	15.4 ± 3.2	60.1 ± 4.2	**<0.0001**	**<0.0001**	**<0.0001**
Distribution of the +896ATLR4/ DACE/-1562TMMP-9/ -735TMMP-combined genotype	3 (4%)	7 (8.7%)	20 (25%)	**N.S.**	**0.006**	**0.0001**
Other genotypes	77 (96%)	73 (91.3%)	60 (75%)			
$CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population (number of EPC/1,000,000 events) mean (SD)	15 ± 2.3	8 ± 1.7	79 ± 5.3	**0.01**	**<0.0001**	**<0.0001**

Bold is used to indicate significant values.

P1 = comparisons between old healthy people versus young donors. P2 = comparisons between old healthy people versus AAA patients. P3 = comparisons among three groups.

[a]Differences between cases and controls were assessed using the unpaired *t*-test with Welch correction, or ANOVA test, Fisher exact test, and Pearson's χ^2 test when appropriate.

AAA, ascending aorta aneurysm; CVD, cardiovascular disease; EPCs, endothelial progenitor cells; IL-6, interleukin 6; MMP-2, metalloproteinase-2; MMP-9, metalloproteinase-9; TNF-α, tumor necrosis factor-α.

informed consents from all participants. In addition, data were encoded to ensure the anonymity of all individuals enrolled. All measurements were performed without knowledge of the nature of the study.

Flow cytometry quantification of the circulating number of the $CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population

We quantified the $CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population in all subjects enrolled, by using a slightly modified version of a protocol proposed for the first time in 2010 and described, in detail, in our previous study.[11] The $CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population is characterized to represent a more mature EPC population, as amply described in our recent studies and a special issue about these cells.[8,9]

Telomere length assay, detection of telomerase activity by TRAP assay, aortic specimens, histopathological and immunohistochemical assays, TUNEL testing, ELISA, and genotyping

The procedures for telomere length assay, detection of telomerase activity by T telomeric repeat amplification protocol (TRAP) assay, as well as criteria, definitions, and grading systems for tissue sample collection, staining, histopathological and immunohistochemical assessment, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) testing, enzyme-linked immunosorbent assay (ELISA) for determining the plasma levels of systemic inflammatory molecules, and genotyping for assessing the distribution of *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined genotyped, demonstrated to be associated with the AAA risk, were described in detail in our previous studies.[4,10,12] Consequently, DNA samples from circulating leukocytes, as well as plasma samples of all individuals selected were obtained and analyzed, as described in our previous studies.[4,12] Aorta samples were obtained from 80 AAA cases and analyzed according to procedures previously described.[12]

Evaluation of blood cell profile and their parameters: RDW detection

Fasting blood samples were collected by all individuals selected in the study. They were drawn by antecubital vein

puncture into EDTA-treated or plain tubes according to hospital protocol. A hematological analyzer, *the Automated Sysmex XN Analyzer* (Dasit Diagnostica, Cornaredo, Milano, Italy) was used to obtain the complete profile of blood cells and their parameters, RDW included. High sensitive C-reactive protein (hs-CRP) measurements were conducted by a Cobas Integra analyzer (Roche Diagnostics, Germany), by using the turbidimetric method. The reference range for RDW was between 11.6% and 14.8% and for hs-CRP was <0.5 mg/L.

Statistical analyses

All analyses were performed with R and Microsoft Excel software. All data are presented as a mean ± SD or a median (interquartile range [IQR]) for parametric variables and as percentages for categorical variables. Continuous variables were checked for the normal distribution assumption, by using Kolmogorov–Smirnov statistics. Categorical variables were tested by Pearson's χ^2 test. Differences between two groups were evaluated using the Kolmogorov–Smirnov test or the Student's *t*-test when appropriate, while one-way ANOVA or Kruskal–Wallis tests followed by Bonferroni correction was applied to compare more than two groups. The correlations between two continuous variables were assessed with Pearson's test, or nonparametrical Spearman correlation test. The difference in mean terminal restriction fragment (TRF) length and the mean values of relative telomerase activity (RTA) between cases and controls were analyzed using the unpaired *t*-test with Welch correction. Genotype frequencies were evaluated by gene count. Data were tested for goodness of fit between observed and expected genotype frequencies according to Hardy–Weinberg equilibrium, by χ^2 test. Significant differences in frequencies among groups were calculated by using χ^2 test and appropriate tables (2×2 tables, where appropriate). Binary logistic regression analysis was used to find the possible independent associations between the risk of sporadic AAA, or vascular aging, and conventional risk factors (age, male gender, smoking, and genetic factors, *i.e.*, the frequency of the *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined risk genotyped[4,10,12]), or clinical parameters (RDW, neutrophil/lymphocyte ratio, hypertension, systemic inflammation levels of hs-CRP and other inflammatory molecules [cytokines and metalloproteinases]), and biomarkers of vascular aging (*i.e.*, mean TRF length—a marker of telomere length—and the mean of RTA and EPC number[4]). Hosmer-Lemeshow test was used to check goodness-of-fit of the logistic regression. *p*-Values are two sided and values <0.05 were considered statistically significant.

Results

Baseline features of population enrolled

The comparisons of demographic features among the three groups first demonstrated a significant difference for age between young donors and old people ($p < 0.001$, by *t*-test, Welch corrected; see Table 1), and among the three groups ($p < 0.001$, by ANOVA test, Bonferroni corrected; see Table 1). A significant difference was also assessed by comparing the three groups for gender (men vs. women; $p = 0.01$ among the three groups and $p = 0.005$ old vs. AAA subjects, by χ^2 test), with an enrichment of men in AAA cases. A significant difference was particularly observed between old and AAA subjects for BMI ($p = 0.02$ old vs. AAA subjects by *t*-test, Welch corrected), and comparing the three groups ($p = 0.01$ by ANOVA test, Bonferroni corrected). Significant differences were also detected by comparing comorbidity conditions (*i.e.*, CVD family history, smoking. diabetes, hypertension, renal failure, and dissection; see Table 1) between old versus AAA cases and among the three groups, with an increase of persons affected in AAA groups and the absence of these in young blood donors (see data and *p*-values in Table 1). Young donors showed only the presence of five subjects with a CVD family history (see data in Table 1). Thus, these conditions were oberved in old subjects and AAA cases, who showed significant peaks than the other groups. Likewise, AAA cases had higher significant levels of systemic inflammatory plasma molecules than other groups, even if intermediate values were detected in old people, while young donors showed lower levels of these analyzed systemic molecules (see data and pa values in Table 1). AAA cases also showed higher significant frequency of the *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined genotyped ($p = 0.006$ and $p = 0.0001$, by using χ^2 test, see Table 1), which we demonstrated to be associated with high risk and a more severe prognosis of sporadic AAA in our previous studies.[4,10,12] Furthermore, interesting data were obtained by comparing the circulating levels of $CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ *EPC population (*number of EPC/1,000,000 events; mean [SD]) among the three groups. Young donors showed higher values than old people ($p = 0.01$, by *t*-test, Welch corrected). Interestingly, old people had lower levels than young and AAA cases (Table 1). These last had very higher values, probably as event for contrasting the aorta tissue damage.

Comparisons of blood parameters between cases and controls

In Table 2, the values of blood parameters obtained by the three groups were reported. Interestingly, the comparisons performed among the three groups, and between two groups (*i.e.*, old vs. young people; and old people vs. AAA cases), demonstrated significant differences in some parameters. Among these, relevant was the significant difference detected in a parameter of red blood cells, and precisely in the values of RDW distribution (median 15.9, IQR 1.6, in AAA patients, median 16.3 IQR 1.2 in old people vs. median 11.8, IQR 1.5 in young donors, $<p = 0.0001$, by ANOVA test, Bonferroni corrected). Of note also was the significant difference in the neutrophil/lymphocyte ratio (NLR) (median 2.04 IQR 1.2 in AAA patients, median 1.87, IQR = 0.90 in old people vs. median 1.61, IQR = 0.30, in young donors, $p = 0.009$ by ANOVA test, Bonferroni corrected). Furthermore, we detected significant differences in the plasma levels of hs-CRP (median 0.67, IQR 0.7, in AAA patients, median 0.61, IQR = 0.71 in old people vs. 0.22, IQR 0.8 mg/dL in young donors; $p < 0.0001$ by ANOVA test, Bonferroni corrected). Other differences were detected in the number of platelets, fasting glucose, low-density lipoprotein, and triglycerides levels, but essentially of reduced relevance and between old versus young people (Table 2).

Table 2. Comparisons of Values of Routine Blood Parameters in the Population Studied

Blood parameters	*AAA patients* (N = 80)	*Old people* (N = 80)	*Young people* (N = 80)	P1[a]	P2[a]	P3[a]
Hemoglobin (g/dL)	13.5 ± 0.7	13.8 ± 0.9	14.1 ± 2.1	N.S.	N.S.	N.S.
Platelet ($10^3/\mu$L)	250 ± 23.5	236 ± 33.7	269.2 ± 38.7	**<0.001**	**0.05**	**0.046**
Mean platelet: volume (fL)	8.7 ± 0.4	8.9 ± 0.7	9.1 ± 2.1	N.S.	N.S.	N.S.
White blood cell count ($10^3/\mu$L)	7.6 ± 1.8	7.4 ± 1.7	6.36 ± 1.3	**<0.001**	N.S.	**<0.001**
Neutrophil ($10^3/\mu$L)	4.5 (0.8)	4.3 (0.9)	3.4 ± 1.1	**<0.001**	N.S.	**<0.001**
Lymphocyte ($10^3/\mu$L)	2.2 (0.6)	2.3 (0.5)	2.1 ± 0.45	N.S.	N.S.	N.S.
NLR	2.04 (1.2)	1.87 (0.90)	1.61 (0.3)	N.S.	N.S.	**0.009**
Red cell distribution (%)	15.9 (1.6)	16.3 (1.2)	11.8 (1.5)	**<0.001**	**<0.001**	**<0.0001**
Mean corpuscular volume (fL)	89 (5.9)	88.7 (6.9)	80.2 (2.1)	**0.01**	N.S.	**0.01**
Fasting glucose (mg/dL)	106.4 ± 22.6	109.3 ± 36.8	100 ± 2.5	**0.02**	N.S.	**<0.05**
Creatinine (mg/dL)	0.84 ± 0.2	0.85 ± 0.7	0.81 ± 0.1	N.S.	N.S.	N.S.
AST (U/L)	23 (7)	21 (8)	20 (3)	N.S.	N.S.	N.S.
ALT (U/L)	22 (8)	20 (9)	21 (1)	N.S.	N.S.	N.S.
Total cholesterol (mg/dL)	192 ± 33	191 ± 29	187.1 ± 15	N.S.	N.S.	N.S.
LDL cholesterol (mg/dL)	116 ± 27	114 ± 32	106 ± 22	**<0.05**	N.S.	N.S.
HDL cholesterol (mg/dL)	43 (18.9)	46 (16.9)	51 (13)	**0.05**	N.S.	N.S.
Triglycerides (mg/dL)	139 (74)	126 (67)	115 (34)	**<0.05**	**0.05**	**0.05**
hs-CRP (mg/dL)	0.67 (0.7)	0.61 (0.71)	0.22 (0.8)	**<0.001**	N.S.	**<0.0001**

Bold is used to indicate significant values.

Parametric variables without normal distribution were reported as median value (interquartile range). P1 = comparisons between old healthy people versus young donors.

P2 = comparisons between AAA patients versus old healthy people. P3 = comparisons among three groups.

[a]Differences between cases and controls were assessed by using ANOVA test or Pearson's χ^2 test when appropriate.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein; NLR, neutrophil/lymphocyte ratio.

Comparisons of mean TRF length and the mean of RTA between cases and controls

As shown in the Figure 1A, the evaluation of the mean TRF length in three groups and their analysis demonstrated that the mean TRF length of AAA case's group (5189, standard deviation [SD] = 0.36 kbp) was significantly lower than that observed in young donor's group (6639, SD = 0.45 kbp). However, lower value of TRF length was detected in old people (5002, SD = 0.67). A difference of 1637 bp was observed between young donors and old people, of 1450 bp between young donors and AAA cases, and of 187 bp between old people versus AAA cases (95% confidence interval 65–302, 88–245, and 18–98 bp, $p < 0.001$, by ANOVA test, corrected by Bonferroni; see Fig. 1A). Consequently, significant differences were detected by comparing the mean values of TRF length between old versus young people, and AAA cases versus old people (see data and *p*-values in Fig. 1A). In addition, the analysis of the mean values of RTA demonstrated significant differences among the three groups (7.8 ± 2.6 in AAA cases, 6.8 ± 2.1 in old people vs. 28 ± 0.9 in young donors, $p < 0.0001$ by ANOVA test, Bonferroni corrected; see Fig. 1B). Significant differences were also detected by comparing the mean values of RTA between old versus young people, and AAA cases versus old people (see data and *p*-values in Fig. 1B).

Correlations of RDW high values with the conventional risk factors, clinical parameters, and biomarkers of vascular aging

Given higher values of RDW in AAA cases and old people than young donors (Table 2), we detected if they correlated with the traditional risk factors for both vascular aging and onset of sporadic AAA, including age, smoking, hypertension, inflammation, diabetes, dyslipidemia, and levels of circulating EPCs (Tables 3 and 4). Interestingly, significant positive correlations were detected with age, smoking, and hypertension in both the conditions (Tables 3 and 4). As abovementioned, we also assessed the correlations between RDW values and systemic inflammation. Thus, RDW values were correlated with NLR, hs-CRP, interleukin (IL)-6, and tumor necrosis factor (TNF)-α and metalloproteinase (MMP)-2 and -9 values, and significant positive correlations were detected in both the conditions (Tables 3 and 4). Other promising data are the significant negative correlations between values of RDW and mean values of TRF length, and the mean values of RTA in both the conditions (Tables 3 and 4). Consequently, leukocyte telomere length attrition and telomerase activity alteration observed in AAA patients and old people negatively correlated with the increase of RDW values, and, surprisingly, the values of RDW positively correlated with the increase of the common aorta histopathological alterations[4,5,10,12] observed in aorta samples from the patients enrolled in our study, such as medionecrosis of grade III, cystic-medial change of grade III, elastic fragmentation of III grade, plurifocal medial apoptosis, and elevated MMP-9 tissue amount (Table 4). Furthermore, the reduced circulating levels of EPCs significantly correlated with the increased levels of RDW, likely linked to the increase of both systemic inflammation and senescence of cells. In contrast, in AAA cases, an opposite correlation was observed between the increase of RDW values and increase of levels of EPCs (Table 4).

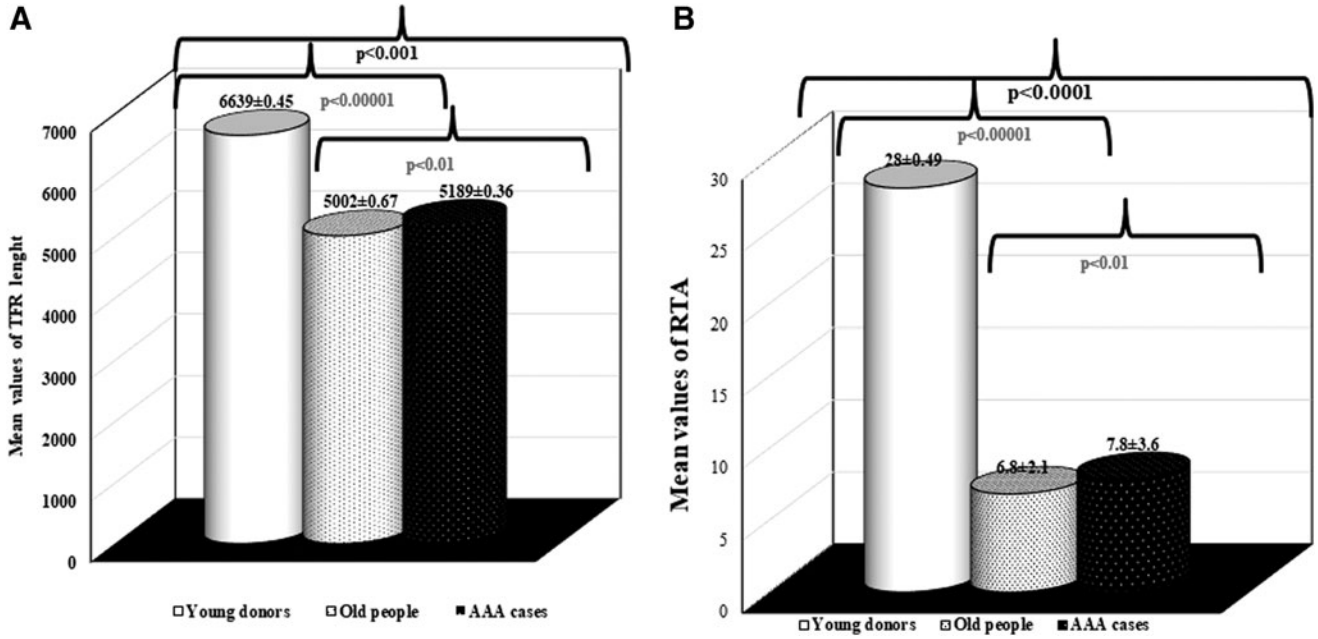

FIG. 1. **(A, B)** Comparisons of the mean values of TRF and RTA in the population studied. In **(A),** the mean values of TRF of the three groups were reported; precisely, old people had lower mean values of TRF than young donors and AAA cases. The analysis of data, indeed, demonstrated a significant difference among the three groups ($p < 0.001$; by ANOVA test, Bonferroni corrected). In addition, significant differences were also obtained by comparing the data between old versus young people, and between old people versus AAA cases (see data and *p*-values in figure). In **(B)**, the mean values of RTA were reported, and significant differences were detected, with higher mean values in young donors and lower in old people and AAA cases ($p < 0.0001$, by ANOVA test, Bonferroni corrected). Indeed, significant differences were assessed by comparing the data between old versus young people, and between old people versus AAA cases (see data and *p*-values in figure). AAA, ascending aorta aneurysm; RTA, relative telomerase activity; TRF, terminal restriction fragment.

Binary regression analysis of potential factors associated with the risk for AAA and vascular aging in the study population

By using binary logistic regression analysis, we evaluated the independent factors that were significantly associated with the risk for vascular aging and sporadic AAA in the study population. We interestingly observed that increased age, smoking, hypertension, the presence of augmented EPCs, RDW and hs-CRP blood levels, and higher frequency of the *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined genotyped were significantly associated with the risk for sporadic AAA (Table 6). Surprisingly, in the binary logistic regression analysis adjusted for age, male gender, smoking, hypertension, EPCs, RDW and hs-CRP levels, frequency of the *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined genotyped, and TRF mean length and RTA values, we detected that smoking, hypertension, the increased blood levels of RDW and hs-CRP, and the presence of the reduced mean values of both TRF length and RTA remained were the unique independent risk biomarkers associated with a significant risk for sporadic AAA in the study population. Accordingly, the Hormer–Lemeshow test statistic was 7.12 (df = 7.9, $p = 0.52$), which revealed good model fit. On the other hand, the data, examined for the risk for vascular aging, demonstrated that the independent risk factors are age, increased blood levels of RDW and hs-CRP, associated with reduced blood levels of EPCs, TRF length, and RTA (Table 5).

Table 3. Univariate Correlations Between Red Cell Distribution Width and Cardiovascular Disease Risk Factors in Old People

Variables	r	p[a]
Age	0.16	0.01
Smoking	0.22	0.01
Hypertension	0.15	0.01
NLR	0.19	0.01
hs-CRP	0.12	0.03
TRF length	–0.14	0.01
RTA	–0.24	0.02
IL-6	0.31	0.03
TNF-α	0.29	0.03
MMP-2	0.28	0.03
MMP-9	0.28	0.03
$CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population	–0.22	0.02

[a]By linear Pearson correlation test, or nonparametrical Spearman correlation test, when appropriate.

RDW, red blood cell distribution width; RTA, relative telomerase activity; TRF, terminal restriction fragment.

Discussion

In this study, the main data obtained revealed, for the first time, that the increase of RDW distribution values along with the augmented blood levels of hs-CRP and the reduced mean values of both leukocyte telomere length (expressed in

Table 4. Univariate Correlations Between Red Cell Distribution Width and Cardiovascular Disease Risk Factors in Ascending Aorta Aneurysm Patients

Variables	r	p[a]
Age	0.14	0.01
Smoking	0.20	0.01
Hypertension	0.14	0.01
Dyslipidemia	0.41	0.059
Diabetes	0.43	0.57
NLR	0.18	0.01
hs-CRP	0.13	0.02
TRF length	–0.13	0.01
RTA	–0.18	0.01
IL-6	0.17	0.02
TNF-α	0.18	0.02
MMP-2	0.16	0.02
MMP-9	0.18	0.02
Medionecrosis of grade III	0.25	0.03
Cystic-medial change of grade III	0.28	0.03
Elastic fragmentation of grade III	0.31	0.042
Plurifocal Medial apoptosis	0.39	0.048
Elevated MMP-9 amounts	0.38	0.045
$CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population	0.20	0.02

[a]By linear Pearson correlation test, or nonparametrical Spearman correlation test, when appropriate.

TRF, a marker of leukocyte telomere length) and telomerase activity (expressed in RTA), and EPC cells are independently associated with the high risk for vascular aging and sporadic AAA (Tables 5 and 6). Furthermore, they evidenced, for the first time, positive correlations of RDW values with the clinical parameters from patients affected by sporadic AAA, including hypertension and levels of systemic inflammatory molecules (including not only hs-CRP levels but also IL-6, TNF-α, and MMP-2 and MMP-9 levels; see Tables 1, 2, 4, 6), as well as with the severity grade of aorta histopathological alterations related to onset and outcome of this aorta pathology (Table 4). Accordingly, we observed that the increase of RDW value positivity correlated with the medionecrosis of grade III, cystic-medial change of grade III, elastic fragmentation of grade III, plurifocal medial apoptosis, and elevated MMP-9 tissue amount (Table 4), while the increase of RDW values negatively correlated with the reduced mean values of leukocyte telomere length and telomerase activity (Table 4). In other words, the rise of RDW values should seem to be the mirror of the grade of senescence of the aorta cellular components, bone marrow stem and progenitor cells (*i.e.*, EPCs examined), and the portrait of the typical pathological alterations, which characterize the complex pathophysiology of sporadic AAA.[4,10,12] In addition, it should seem to reflect the growing augment of the systemic inflammation related to aging process and the grade of dangers of several tissues of body, first including the cardiovascular system, which represents the principal sensor (true) of biological age of an individual.[4,8,9] Accordingly, a close relationship has been largely demonstrated in literature between a sustained systemic inflammation and elevated levels of RDW.[2,13,14] The group of Lippi has particularly stressed this aspect.[13,14] This might justify both the increase of RDW values in the AAA and old people than young donors, and its positive correlation with the increased levels of systemic plasma molecules examined in the study, as well as with the high values of NLR detected in these subjects' groups (Tables 2–4). NLR represents an inflammatory blood parameter, which has been recently shown to be a predictor of adverse outcomes in patients with CVDs.[15] In our study, NLR values were significantly different in the cases than controls, as abovementioned. However, binary regression analysis revealed that NLR did not represent an independent risk factor

Table 5. Binary Regression Analysis of Potential Factors Associated with the Risk of Vascular Aging in Old People

Variables	*Unadjusted OR (95% CI)*	p	*Adjusted OR (95% CI)*[a]	p
Age	1.02 (1.01–3.65)	**0.01**	1.01 (0.98–4.1)	**0.01**
Male gender	1.18 (0.78–1.98)	0.48	—	
Female gender	1.19 (0.72–1.99)	0.47	—	
Smoking	1.56 (0.88–2.29)	**0.03**	—	
Hypertension	1.27 (0.99–2.4)	**0.01**	1.15 (0.69–1.78)	0.41
NLR	1.32 (0.91–1.35)	0.39	—	
hs-CRP	2.34 (1.19–4.19)	**0.01**	2.03 (1.16–3.67)	**0.02**
RDW	1.4 (1.3–2.21)	**0.02**	1.98 (1.67–2.1)	**0.02**
TRF length	1.98 (0.56–4.9)	**0.01**	1.87 (0.98–2.4)	**0.01**
RTA	2.01 (0.87–2.5)	**0.01**	2.01 (1.02–2.1)	**0.02**
IL-6	1.78 (1.9–2.1)	0.13	—	
TNF-α	1.01 (0.99–1.98)	0.11	—	
MMP-2	1.78 (0.89–1.99)	0.12	—	
MMP-9	1.67 (0.97–1.89)	0.19	—	
+896ATLR4/DACE/-1562TMMP-9/-735TMMP-2 combined genotype risk profile	2.51 (0.8–3.1)	**0.01**	2.1 (1.2–2.98)	0.058
$CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population	1.16 (0.75–1.94)	**0.02**	1.99 (1.6–2.7)	**0.02**

Bold is used to indicate significant values.
[a]Adjusted for age, male/female gender, smoking, hypertension, RDW and hs-CRP levels, combined genotype, EPCs, TRF length, and RTA.
CI, confidence interval; OR, odds ratio.

Table 6. Binary Regression Analysis of Potential Factors Associated with the Risk of Ascending Aorta Aneurysm in the Ascending Aorta Aneurysm Patients

Variables	*Unadjusted OR (95% CI)*	p	*Adjusted OR (95% CI)*[a]	p
Age	1.04 (1.01–1.05)	**0.02**	1.01 (0.98–1.06)	0.11
Male gender	1.19 (0.68–1.88)	0.49	—	
Smoking	1.46 (0.86–2.49)	**0.02**	2.01 (1.16–3.9)	**0.02**
Hypertension	1.59 (0.99–3.4)	**0.01**	2.15 (0.69–3.8)	**0.02**
NLR	1.32 (0.91–1.35)	0.39	—	
hs-CRP	2.34 (1.19–4.19)	**0.01**	2.03 (1.16–3.97)	**0.003**
RDW	1.4 (1.3–2.21)	**0.02**	1.98 (1.67–5.1)	**0.002**
TRF length	1.88 (0.56–5.9)	**0.01**	1.87 (0.98–6.4)	**0.001**
RTA	2.11 (0.97–2.8)	**0.01**	2.11 (1.02–2.8)	**0.02**
IL-6	1.78 (1.9–2.1)	0.13	—	
TNF-α	1.01 (0.99–1.98)	0.11	—	
MMP-2	1.78 (0.89–1.99)	0.12	—	
MMP-9	1.67 (0.97–1.89)	0.19	—	
+896ATLR4/DACE/-1562TMMP-9/-735TMMP-2 combined genotype risk profile	2.51 (0.8–3.1)	**0.01**	2.4 (1.5–2.99)	0.048
$CD45^{dim}CD34^{+}KDR^{+}CXCR4^{+}$ EPC population	1.04 (0.75–2.94)	**0.048**	—	

Bold is used to indicate significant values.
[a]Adjusted for age, male/female gender, smoking, hypertension, RDW and hs-CRP levels, combined genotype, EPCs, TRF length, and RTA.

for both vascular aging and sporadic AAA (Tables 5 and 6). Furthermore, the grade of systemic inflammation significantly reflects the impairment of both telomere length and telomerase activity of all cells,[16,17] such as leukocytes, used as typical model for studying the cellular senescence,[18–21] and the grade of biological age of the entire tissues, bone marrow included, of an organism.[22–25] A large array of literature data stresses this close relationship, and it evidences that telomere lengths are affected by several set points, including activity of telomerase in progenitor cells, cell divisions, and exposure to inflammation and oxidative stress.[18–25] In addition, evidence has established that the relationship between telomere length and the maximum proliferative capacity of bone marrow $CD34^{+}$ hematopoietic progenitor cells is strong for erythroid lineage with respect to other myeloid lineages.[8,9,26,27] Likely, this could clarify the correlation between RDW values and telomere length. In this study, we evidenced a close association between the impairment of leukocyte telomere length and telomerase activity, and of the increased levels of both RDW with hs-CRP, and high risk for both vascular aging and a degenerative age-related arterial disease, such as sporadic AAA (Tables 5 and 6).

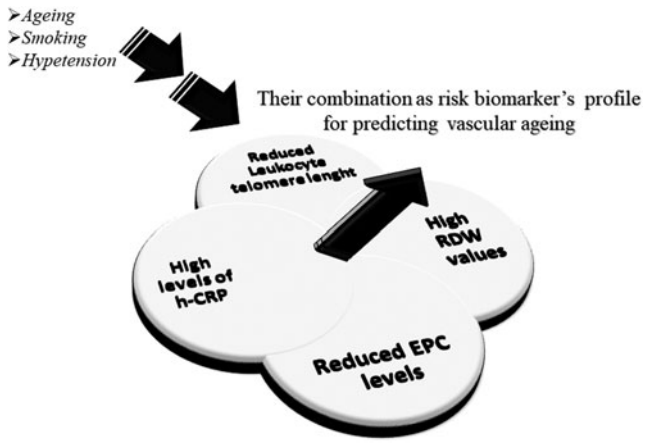

FIG. 2. Graphical representation of our hypothesis: combination of significant parameters for predicting vascular aging.

On the other hand, inflammation and senescence of cardiovascular tissues have been amply demonstrated to be associated with cardiovascular dysfunction and diseases (*i.e.*, sporadic AAA).[28–30] Accordingly, our group demonstrated that the aneurysms of aorta, and particularly sporadic AAA, are the result of a sustained/excessive activation of inflammatory pathways,[10,12,31] such as Toll-like receptor-4 (TLR4), and the consequent sustained release of an array of inflammatory molecules (*i.e.*, inflammatory cytokines and metalloproteinases), constituting the so-called *age-associated arterial secretory phenotype* (*AAASP*).[10,12,31,32] This last progressively contributes to the senescence of all cellular aorta components, by provoking impairment of both telomere length and telomerase activity, and after the consequent endothelium dysfunction, hypertension, medial degeneration, vascular remodeling, and sporadic AAA onset. In addition, genetic polymorphisms have been also demonstrated to significantly contribute to onset of these pathological aorta conditions.[4,10,12,31] For example, the *896ATLR4/DACE/-1562TMMP-9/-735TMMP-2* combined genotyped has been shown by our group to be significantly associated with a high risk for sporadic AAA,[4,10,12] by affecting the grade of inflammation and senescence, as shown by binary regression analyses in this study (Tables 3–6), but not confirmed after adjustment (Tables 5 and 6). It is possible that many of these differences are due to cohort size, differences in allele frequencies, or population structure. In addition, the biological effects of any combined genotype are the result of a very intricate interplay between environmental factors and its genome, transcriptome, proteome, metabolome, microbiome, epigenome, and exposome, as underlined in our recent reviews.[10,31,32]

Relevance and Limitations of Our Study

The principal relevance of this study stays in having demonstrated, for the first time, the independent role as risk factors for vascular aging and sporadic AAA of some blood parameters, such as RDW and hs-CRP, which are used of routine in both clinical management of several diseases and the simple evaluation of the clinical status of an individual. They, along with leukocyte telomere length and telomerase activity, and circulating levels of EPCs could provide a very interesting datum to use as the best biomarkers for predicting vascular aging and diagnosing/prognosing age-related degenerative arterial diseases, such as sporadic AAA, an aorta pathology with a growing incidence in old people and silent and insidious onset and progression, and with diagnosis essentially based on image technologies.[10,12] Consequently, they could constitute the best diagnostic/prognostic biomarker profile for this disease, and its clinical application might favor its early diagnosis, until now only based on the interpretation of aorta diameter. This last represents a much-debated parameter or criterion for diagnosis and surgical indication, as amply stressed in our recent studies.[10,12]

In other words, the very relevant power point of this study stays in demonstrating the presence of same biomarker's profile in old people and AAA cases. Thus, this panel of biomarkers might be applied for identifying a vascular aging process, in both physiological and pathological conditions. On the other hand, the enrollment in this study of AAA cases permitted to investigate on a model of premature vascular aging, regardless of real chronological age of the patients, which differs by the biological age.[4,33]

Another relevant datum of this study is the presence in old people of a reduced number of EPCs, and particularly of mature EPC population,[11] than young donors and AAA cases, and the increased number of these cells in AAA cases than the two other groups. This is in agreement with the results obtained in another recent study performed in patients affected by AAA, which demonstrated an increased number of EPCs in blood in the cases than controls.[11] Thus, it suggests that in conditions of acute injury, elevation of number of EPCs in blood occurs. A systemic release of chemoattractant cytokines and growth factors after vascular injury is associated, indeed, with mobilization of endogenous bone marrow cells and EPCs, while, the significant lower number of EPCs, in old people than young donors, suggests that old healthy subjects are generally characterized by having a reduced number of circulating EPCs, and consequently a reduced or failure of the regenerative capacity of cardiovascular system. Therefore, vascular aging should be associated with a reduced number of EPCs and not with their increase in number in blood. This suggestion is in agreement with the results obtained in a study on circulating levels of populations of EPCs having several grades of maturation, which we are performing in a population of old and young healthy individuals (data not shown).

Furthermore, there are some limitations in this study. First, it is a cross-sectional study, but with a relatively small sample size and very homogenous population. This feature consents principally to evidence potential associations between various biomarkers, detected in the study, and the risk for both vascular aging and sporadic AAA, but not to identify the potential cause or the casual relationship. Longitudinal or prospective studies might be performed to clear this relevant aspect. In addition, in this study, we did not measure other blood parameters, which affect erythrocyte homeostasis, such as levels of iron, ferritin, vitamin B12, folate, thyroid-stimulating hormone, and erythropoietin. Consequently, it was not possible to adjust the comparisons and the analysis for these variables. In addition, some predictor factors, such as smoking, were obtained from data of a questionnaire, and could be affected from measurement errors or reporting bias.

Conclusions

The relevant results obtained in this study suggest, for the first time, that the increase of RDW distribution values along with the augmented blood levels of hs-CRP and the reduced mean values of both leukocyte telomere length (expressed in TRF, a marker of leukocyte telomere length) and telomerase activity (expressed in RTA), and circulating EPCs are independently associated with the high risk for vascular aging and age-related degenerative arterial diseases, such as sporadic AAA (see Fig. 2). They might be used as the best predictor biomarker profile for predicting vascular aging, and for the diagnosis and outcome of sporadic AAA. However, future studies are surely needed for validating and confirming the relevance of these investigations.

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Authors' Contributions

C.R.B. was involved in conception, study design, and drafting the article. F.B., C.P., and G.R. were exclusively involved in enrolment study's population and collecting its data. C.R.B., R.M., and S.D. performed investigations and data interpretation. C.R.B. gave the final approval of the version to be published. All authors read and approved the final article.

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References

1. Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. J Thorac Dis 2015;7:E402–E411.
2. Poz D, De Falco E, Pisano C, Madonna R, Ferdinandy P, Balistreri CR. Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases. Rejuvenation Res 2018. DOI: 10.1089/rej.2018.2094. [Epub ahead of print]
3. Blackburn EH. Telomeres and telomerase: The means to the end (Nobel lecture). Angew Chem Int Ed Engl 2010;49: 7405–7421.
4. Balistreri CR, Pisano C, Martorana A, et al. Are the leukocyte telomere length attrition and telomerase activity alteration potential predictor biomarkers for sporadic TAA in aged individuals? Age (Dordr) 2014;36:9700.
5. Balistreri CR, Maresi E, Pisano C, et al. Identification of three particular morphological phenotypes in sporadic thoracic aortic aneurysm: Phenotype III as sporadic thoracic aortic aneurysm biomarker in aged individuals. Rejuvenation Res 2014;17:192–196.

6. Kozlitina J, Garcia CK. Red blood cell size is inversely associated with leukocyte telomere length in a large multi-ethnic population. PLoS One 2012;7:e51046.
7. Rodríguez-Carrio J, Alperi-López M, López P, et al. Red cell distribution width is associated with endothelial progenitor cell depletion and vascular-related mediators in rheumatoid arthritis. Atherosclerosis 2015;240:131–136.
8. Balistreri CR (ed.): Endothelial progenitor cells (EPCs) in ageing and age-related diseases: From their physiological and pathological implications to translation in personalized medicine [Special issue]. Mech Ageing Dev 2016;159: 1–80.
9. Balistreri CR, Buffa S, Pisano C, Lio D, Ruvolo G, Mazzesi G. Are endothelial progenitor cells the real solution for cardiovascular diseases? Focus on controversies and perspectives. Biomed Res Int 2015;2015:835934.
10. Balistreri CR. Genetic contribution in sporadic thoracic aortic aneurysm? Emerging evidence of genetic variants related to TLR-4-mediated signaling pathway as risk determinants. Vascul Pharmacol 2015;74:1–10.
11. Balistreri CR, Crapanzano F, Schirone L, et al. Deregulation ofNotch1 pathway and circulating endothelial progenitor cell (EPC) number inpatients with bicuspid aortic valve with and without ascending aorta aneurysm. Sci Rep 2018;8:13834.
12. Ruvolo G, Pisano C, Candore G, et al. Can the TLR-4-mediated signaling pathway be a key inflammatory promoter for sporadic TAA? Mediators Inflamm 2014;2014: 349476.
13. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Crit Rev Clin Lab Sci 2015; 52:86–105.
14. Lippi G, Mattiuzzi C, Cervellin G. Learning more and spending less with neglected laboratory parameters: The paradigmatic case of red blood cell distribution width. Acta Biomed 2017;87:323–328.
15. Afari ME, Bhat T. Neutrophil to lymphocyte ratio (NLR) and cardiovascular diseases: An update. Expert Rev Cardiovasc Ther 2016;14:573–577.
16. Jose SS, Bendickova K, Kepak T, Krenova Z, Fric J. Chronic inflammation in immune aging: Role of pattern recognition receptor crosstalk with the telomere complex? Front Immunol 2017;8:1078.
17. Prasad KN, Wu M, Bondy SC. Telomere shortening during aging: Attenuation by antioxidants and anti-inflammatory agents. Mech Ageing Dev 2017;164:61–66.
18. Victorelli S, Passos JF. Telomeres and cell senescence—Size matters not. EBioMedicine 2017;21:14–20.
19. Sanders JL, Newman AB. Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither? Epidemiol Rev 2013;35:112–131.
20. Fyhrquist F, Saijonmaa O. Telomere length and cardiovascular aging. Ann Med 2012;44 Suppl 1:S138–S142.
21. Aviv A, Shay JW. Reflections on telomere dynamics and ageing-related diseases in humans. Philos Trans R Soc Lond B Biol Sci 2018;373.
22. Zhang WG, Zhu SY, Bai XJ, et al. Select aging biomarkers based on telomere length and chronological age to build a biological age equation. Age (Dordr) 2014;36:9639.
23. Rizvi S, Raza ST, Mahdi F. Telomere length variations in aging and age-related diseases. Curr Aging Sci 2014;7: 161–167.
24. Wen SW, Wong CHY. Aging- and vascular-related pathologies. Microcirculation 2018 [Epub ahead of print]; DOI: 10.1111/micc.12463.
25. Schuller CE, Jankowski K, Mackenzie KL. Telomere length of cord blood-derived CD34(+) progenitors predicts erythroid proliferative potential. Leukemia 2007;21:983–991.
26. Lakatta EG. So! What's aging? Is cardiovascular aging a disease? J Mol Cell Cardiol 2015;83:1–13.
27. Donato AJ, Morgan RG, Walker AE, Lesniewski LA. Cellular and molecular biology of aging endothelial cells. J Mol Cell Cardiol 2015;89(Pt B):122–135.
28. Regina C, Panatta E, Candi E, et al. Vascular ageing and endothelial cell senescence: Molecular mechanisms of physiology and diseases. Mech Ageing Dev 2016;159:14–21.
29. Olivieri F, Pompilio G, Balistreri CR. Endothelial progenitor cells in ageing. Mech Ageing Dev 2016;159:1–3.
30. Madonna R, Novo G, Balistreri CR. Cellular and molecular basis of the imbalance between vascular damage and repair in ageing and age-related diseases: As biomarkers and targets for new treatments. Mech Ageing Dev 2016;159:22–30.
31. Balistreri CR, Ruvolo G, Lio D, Madonna R. Toll-like receptor-4 signaling pathway in aorta aging and diseases: ''its double nature''. J Mol Cell Cardiol 2017;110:38–53.
32. Balistreri CR, Madonna R, Melino G, Caruso C. The emerging role of Notch pathway in ageing: Focus on the related mechanisms in age-related diseases. Ageing Res Rev 2016;29:50–65.
33. Balistreri CR, Pisano C, Merlo D, et al. Is the mean blood leukocyte telomere length a predictor for sporadic thoracic aortic aneurysm? Data from a preliminary study. Rejuvenation Res 2012;15:170–173.

Address correspondence to:
Carmela Rita Balistreri
Department of Pathobiology and Medical and Forensic Biotechnologies
University of Palermo
Corso Tukory, 211
Palermo 90134
Italy

E-mail: carmelarita.balistreri@unipa.it

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