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Galectin-3, osteopontin and successful aging

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Abstract

Background: Individuals who reach exceptional longevity (100+ years of age) free of common chronic age diseases (i.e. 'dodgers') arguably represent the paradigm of successful aging in humans. As such, identification of potential biomarkers associated with this phenomenon is of medical interest.

Methods: We measured serum levels of galectin-3 and osteopontin, both of which have been shown to be linked with major chronic or aging-related disorders in younger populations, in centenarian 'dodgers' (n=81; 40 men; 100–104 years) and healthy controls (n=41; 24 men, 70–80 years).

Results: Both biomarkers showed significantly lower values (p<0.001) in the former (galectin-3: 2.4±1.7 vs. 4.8±2.8 ng/mL; osteopontin: 38.1±27.7 vs. 72.6±33.1 μg/mL). Logistic regression analysis identified the combination of these two biomarkers as a significant predictor variable associated with successful aging regardless of sex (p<0.001). The area under the curve (AUC) classified the ability of galectin-3 and osteopontin to predict the likelihood of successful aging as 'fair' (AUC=0.75) and 'good' (AUC=0.80), respectively. Particularly, the combination of the two biomarkers showed good discriminatory power for successful aging (AUC=0.86), with sensitivity=83% and specificity=74%.

Conclusions: Lower levels of both galectin-3 and osteopontin are associated with successful aging, representing potential biomarkers of this condition. Our cross-sectional data must be however approached with caution. Further research is necessary to replicate the present preliminary results in other cohorts and to identify the potential use of galectin-3 and osteopontin as potential targets (or at least predictors) in future personalized anti-aging therapies.

Keywords: age; anti-aging; biomarkers; health.

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Introduction

Galectin-3, a β-galactoside-binding lectin, has been recently postulated as a potential biomarker for screening, diagnosis and prognosis of metabolic disorders, cardiac fibrosis and heart failure [1–6]. Circulating galectin-3 is also strongly associated with atrial fibrillation [7, 8], adverse cardiac events in patients with chronic stable angina [9], as well as mortality in hemodialysis patients [10]. Inhibition of galectin-3 has in fact been proposed as a therapeutic target to prevent a wide range of diseases [11–13]. On the other hand, galectin-3 levels increase with age [14].

Osteopontin, a non-collagenous bone matrix glycoprotein expressed in various cell types, also represents a potential, novel prognostic biomarker in several disease conditions such as obesity, diabetes, osteoporosis as well as myocardial fibrosis and heart failure associated with (or independent of) aging or following myocardial infarction [15–19]. In a rat model of aging, Miller et al. showed that an increased gene expression of osteopontin correlated with the development of progressive aortic vasculopathy [20]. Additionally, elevated levels of osteopontin are linked with aging decline in the capacity for muscle

regeneration [21], with myoblasts producing this molecule to modulate myogenic and inflammatory processes in the early stages of muscle regeneration [22].

Despite growing data supporting that galectin-3 and osteopontin can be used as biomarkers of several disease conditions, no data are yet available on healthy centenarians, a population segment that is the paradigm of successful aging in our increasingly older societies. Although centenarians (i.e. age ≥100 years) represent the paradigm for exceptional longevity (20+ years more than the average life expectancy in the Western world), they are usually an heterogeneous group of subjects who can either delay ('delayers'), survive ('survivors') or even escape common age-related diseases ('dodgers') [23]. The current study was performed to determine if the two aforementioned biomarkers are associated with successful aging. To this end, we measured serum galectin-3 and osteopontin concentrations in healthy centenarians, i.e. 'dodgers', and healthy younger elders (controls).

Materials and methods

Participants

The study protocol complied with the tenets of the Declaration of Helsinki and was approved by the Local Ethics Committee. All study participants provided their written informed consent. We studied 81 healthy centenarians, i.e. 'dodgers', (40 men; mean±SD age: 101±1 years; age range: 100–104 years) and 46 healthy younger elderly people (24 men; 75±3 years; 70–80 years). The participants' ages were defined by the dates of birth as stated on identity cards. All of them were Caucasian Whites ascertained to be of Italian descent (Northern Italy, mainly from Lombardy and Piedmont).

The centenarians 'dodgers' were ascertained mainly via general practitioners in the community; they represent a convenience sample that has been previously described in detail [24–26]. The history of past and current diseases was accurately collected, checking the centenarians' medical documentation and the current drug therapy. All of them were free of major age-related diseases, i.e. severe cognitive impairment, clinically evident cancer, cardiovascular disease (CVD), renal insufficiency, or severe physical impairment. Only part of this group had decreased visual or auditory acuity.

The controls were sex-matched with centenarians and randomly selected from elderly subjects who participated in previous research [27]. They were in apparent good physical health, with exclusion criteria being: presence of CVD or cerebrovascular disease, cancer, dementia, chronic autoimmune or inflammatory disorders, renal or hepatic failure, and major psychiatric disorders.

Galectin-3 and osteopontin measurements

Serum galectin-3 (Biovendor, Modrice, Czech Republic) and osteopontin levels were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' protocols (Human Osteopontin Quantikine kit, R&D Systems, Minneapolis, MN, USA). Details for each assay are available from the authors upon request. For all assays, the intra- and inter-assay coefficients of variation were <7% and <9%, respectively. Each sample was analyzed in duplicate, and the mean value of the two measures was used for the analyses. Laboratory personnel were blinded with regard to subjects' group (centenarians or controls).

Statistical analysis

Statistical analyses were performed with the IBM SPSS 22.0 package for MAC (SPSS, Inc., Chicago, IL, USA) and G*Power 3 program for MAC. Descriptive data are expressed as means±SD as well as medians and ranges. Since the data were not normally distributed, we used the non-parametric Mann-Whitney U-test to compare serum biomarker levels among the two groups.

The optimal cut-off point for the association of the two biomarkers with the likelihood of being a centenarian 'dodger' was determined by receiver operating characteristic (ROC) curves. The Youden index was used to define the optimal cut-off point, and areas under the curve (AUC) and 95% confidence interval were also calculated. AUC values of ≥0.90 are "excellent", and values between 0.80 and 0.90, 0.70 and 0.79 and <0.70 are considered to be "good", "fair", and "poor", respectively [28]. Logistic regression analysis with the forward conditional selection method was used to find a combination of galectin-3 and osteopontin that would be potentially associated with successful aging. The predicted probability (PP) value was calculated for each logistic regression analysis. A leave-one-out cross validation was performed for assessing if the equations could be generalized to an independent data set.

Results

Differences in serum biomarkers between groups

The mean values of both biomarkers were significantly lower in centenarians 'dodgers' compared with controls ($p<0.001$, Table 1; see also dot plot in Figure 1).

Multivariate analysis

Logistic regression analysis identified the combination of the two biomarkers as a significant predictor variable associated with successful aging regardless of sex ($p<0.001$). The equation defined by the analysis was: $PP=1/[1+1/\exp(4.084-0.656\times(\text{galectin-3})+0.962\times(\text{osteopontin})]$. The leave-one-out cross validation analysis confirmed the coefficients.

Table 1: Serum levels of biomarkers (mean±SD; median and range) and age by group.

	Both sexes		p-Value	Statistical power[a]	Optimal total sample size, n[b]
	Centenarians, n=81	Controls, n=46			
Galectin-3, ng/mL	2.4±1.7	4.8±2.8	<0.001	99%	22
	2.1 (0.33, 7.95)	4.5 (0.83, 9.3)			
Osteopontin, µg/L	38.1±27.7	72.6±33.1	<0.001	99%	28
	32.5 (2.5, 110.0)	65.0 (17.5, 192.5)			

Biomarkers data did not follow a normal distribution. [a]Statistical post-hoc power analysis to detect differences between group means with a significance level (α) of 0.05 (two-tailed). [b]Estimated optimal sample size (enrollment ratio 1:1) to obtain a statistical power ≥90% to detect difference between group means with a significance level (α) of 0.05 (two-tailed).

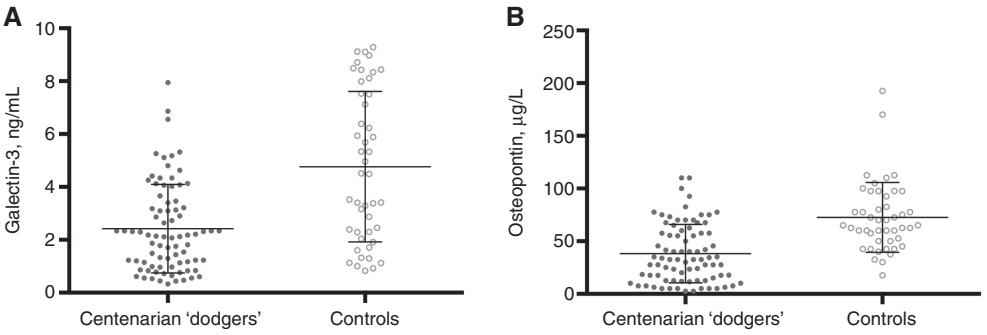

Figure 1: Values for galectin-3 (A) and osteopontin (B) by group (centenarians ["dodgers"] and controls).

ROC curve analysis

The ROC curve results of galectin-3 and osteopontin (either alone or in combination) as independent variables associated with successful aging are shown in Table 2 and Figure 2. The AUC of serum biomarkers classified the ability of galectin-3 and osteopontin serum levels to predict the likelihood of successful aging as 'fair' and 'good', respectively. Particularly, the combination of the two biomarkers showed good discriminatory power for successful aging (AUC=0.86), with sensitivity=83% and specificity=74%.

Discussion

This is the first attempt to assess the association of galectin-3 and osteopontin with successful aging by studying the blood of centenarians 'dodgers'. These unique individuals showed lower concentrations of both biomarkers compared with healthy younger elderly people and logistic regression analysis identified the combination of the two biomarkers as a significant predictor of successful aging regardless of sex, with good discriminatory power. We believe these findings are potentially interesting. Indeed, our societies are aging and identification of possible blood biomarkers of successful aging could help identify 'low' or 'high risk' subjects and design individualized effective therapies and/or preventive measures. On the other hand, cross-sectional data on serum levels of these or other biomarkers can be influenced by many confounders including genetics and interpretation of results must be approached with caution [29]. Further research is also needed with larger cohorts.

It has been previously shown that galectin-3 levels correlate with risk factors of CVD such as blood

Table 2: Receiver operating characteristic (ROC) results for galectin-3 and osteopontin as independent variables associated with successful aging.

Biomarker	Optimal cut-off	AUC	95% CI	p-Value	Standard error	Sensitivity, %	Specificity, %
Galectin-3	<5.3 ng/mL	0.75	0.66–0.84	<0.001	0.046	46	96
Osteopontin	<42.2 µg/L	0.80	0.72–0.87	<0.001	0.038	89	64
2-Biomarker panel	≥0.6	0.86	0.79–0.92	<0.001	0.033	83	74

CI, Confidence interval; AUC, area under the curve.

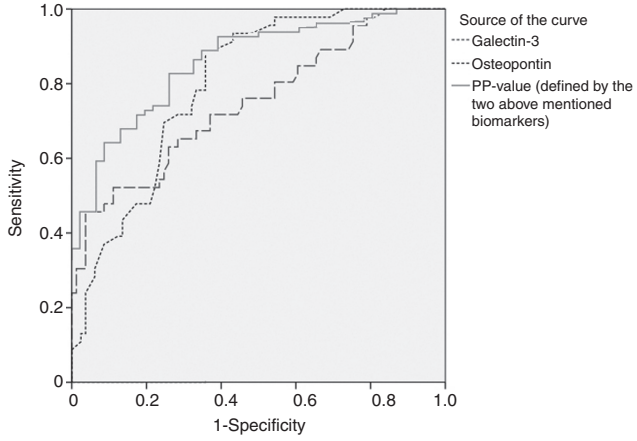

Figure 2: Results of receiver operating characteristic (ROC) curves for the combination of biomarkers associated with successful aging (galectin-3 and osteopontin) vs. individual serum markers.

pressure, serum lipids, body mass index, renal function and N-terminal pro-B-type natriuretic peptide [14]. Moreover, galectin-3 circulating levels have been directly associated with cardiac fibrosis, structural remodeling and the related myocardiocyte dysfunction, heart failure and arrhythmias (particularly atrial fibrillation) [1–8], all of them representing age-related co-morbidities which may lead to mortality in aged individuals. Inhibition of synthesis/activity of galectin-3 represents a potential therapy against cardiac fibrosis and remodeling, and also against heart failure [12, 30–32]. More interestingly, it was previously reported that galectin-3 is strongly associated with age [14, 33]. Notably, a study with a cohort of 7968 adults (mean age of 50±13 years) which were followed longitudinally (median follow-up of ~10 years) found that, after correction for 'classical' CVD risk factors (smoking, blood pressure, cholesterol and diabetes), galectin-3 levels independently predicted all-cause mortality [14].

Osteopontin seems to be involved in the progression of both instability-induced and aging-associated spontaneous osteoarthritis [34]. Paliwal et al. also suggested that tissues of old people overexpress osteopontin, which is a physiological phenomenon as we age but on the other hand can potentially lead to pathologies [21]. Additionally, osteopontin levels are increased in the cerebrospinal fluid of patients with Alzheimer's disease and correlate with cognitive decline [35]. Most importantly, osteopontin has an important role in many disease conditions that are prevalent among older people such as obesity, diabetes, myocardial fibrosis, heart failure, inflammation, osteoporosis, and is also involved in the aging decline in the capacity for skeletal muscle regeneration [15–19, 21, 36]. Regarding a major cause of morbidity and mortality in aging humans, CVD, osteopontin plasma levels are not only elevated in heart failure patients with left ventricular dysfunction (median age between 50 and 60 years) but also correlate with disease severity and risk for subsequent death [15]. In summary, our data suggest that lower levels of both galectin-3 and osteopontin are associated with successful aging, representing potential biomarkers of this condition. Further research, including ideally longitudinal designs, is necessary to corroborate the data reported here in larger cohorts of a different geographic/ethic origin and (ii) to determine whether these biomarkers might be considered as potential targets (or at least predictors) in future personalized anti-aging therapies.

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