Current Oncology Reports (2020) 22: 115
https://doi.org/10.1007/s11912-020-00977-w

GERIATRIC ONCOLOGY (L BALDUCCI, SECTION EDITOR)

Biological and Functional Biomarkers of Aging: Definition, Characteristics, and How They Can Impact Everyday Cancer Treatment

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Published online: 22 August 2020

Abstract

Purpose of Review Recognize which are the elements that predict why a person is aging faster or slower and which intervention we can arrange to slow down the process, which permits to prevent or delay the progression of multimorbidity and disability.

Recent Findings Aging is a complex process that leads to changes in all the systems of the body and all the functions of the person; however, aging develops at different rates in different people, and chronological age is not always consistent with biological age.

Summary Gerontologists are focused not only on finding the best theory able to explain aging but also on identifying one or more markers, which are able to describe aging processes. These biomarkers are necessary to better define the aging-related pathologies, manage multimorbidity, and improve the quality of life. The aim of this paper is to review the most recent evidence on aging biomarkers and the clusters related to them for personalization of treatments.

Keywords Biomarker of aging · Frailty syndrome · Aging phenotype · Quality of life · Multimorbidity · Life expectancy · Social needs

Introduction

"Most people don't grow up. Most people age. They find parking spaces, honour their credit cards, get married, have children, and call that maturity. What that is, is aging."—Maya Angelou. One of the biggest megatrends impacting the world today is population aging. Aging is a topic that has captivated both scientists and philosophers throughout history, but aging as a population scenario emerged on a worldwide scale for the first time in the last century. Thus, it is hard to really identify a definition of aging. It is a decrease in fitness with chronological age, it is a developmental phase beyond the normal life trajectory and it is a time of the increased risk of physical and psychological disabilities testing the limits of resilience.

Aging occurs at a different rate in varying geographic regions of the world.

Europe is currently the oldest region, with 17.4% of the total population aged 65 and older. However, the Asia and Latin America older population is growing fast, with Asia's older population almost tripling in size from 341.4 million in 2015 to 975.3 million in 2050 [1].

All these data do not consider aging as an epiphenomenon, but an individual data of the global population, just a chronological number. Aging is intrinsically a complex scenario characterized by changes that take place at different levels of biological systems. Biological age is of course influenced by chronological age, but chronological age is by itself not representative of biological age; biological age is determined by physiological reserve and functional status. Assessing biological age is essential to predict life expectancy and resilience to

This article is part of the Topical Collection on *Geriatric Oncology*

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stressors [2]. If any definition of aging may appear incomplete and insufficient, much more difficult and complex is to find the marker (or biomarker) that can identify it.

Many theories currently trying to explain aging processes and many biomarkers are identified to measure aging and its evolutionary stages. Theories and biomarkers are not studied to extend life span but to guide therapeutic choices and optimize patient management and personalization of care.

The purpose of this paper is not purely to list which biomarkers are able to identify the various stages of aging, rather explain how an epiphenomenon, natural and physiological, is so complex [3], how many factors are protagonists in its development, and how many actors and characters play in maximizing its individual features, taking into account social and morbidity biomarker. These factors, such as frailty, loss of autonomy, essential needs, and comorbidities, influence the aging process and are able to justify why the biological age of a person living in a country does not correspond to the age of another person living in a country with better socio-sanitary conditions.

Clinical and Biological Aging Phenotypes

The aging phenotype can be described as a complex mosaic resulting from the interaction of a variety of environmental, stochastic, and genetic–epigenetic events/stimuli impinging lifelong on our body [4, 5].

There is no clear evidence which molecular, cellular, or physiological changes are the most important drivers of the aging process and/or how they influence one another [6]. In its broadest sense, aging merely refers to the changes that occur during an organisms' life span, though the rate at which these take place varies widely [7]. Despite its enormous complexity, involving combinations of these variables, a small number of basic molecular mechanisms underpin the aging process, including a set of evolutionary highly conserved basic biological mechanisms responsible for body maintenance and repair. One of the key mechanisms is inflammation; a typical feature of the aging process is the development of a chronic, low-grade inflammatory status named "inflammaging" [8•], which emerged as critical in the pathogenesis of major age-related chronic diseases such as atherosclerosis, type 2 diabetes, and neurodegeneration. Inflammaging plays a pivotal role in the most important geriatric conditions, such as sarcopenia [9••], osteoporosis [10], frailty, and disability, thus contributing to mortality [11]. Interestingly, a variety of tissues (adipose tissue, muscle), organs (brain, liver), systems (immune system), and ecosystems (gut microbiota) of the body (indicated as "sub-systems") can contribute to the onset and progression of such a systemic inflammatory state [12] by increasing the production of several pro-inflammatory mediators or lowering that of the anti-inflammatory ones [8•].

To differentiate the innocuous changes from those leading to increased risk of disease, disability, or death, biogerontologists tend to use a more precise term—senescence—when describing aging [13]. Senescence is, therefore, the progressive deterioration of bodily functions over time and normal human aging has been associated with a loss of complexity in a wide range of physiological processes and anatomic structures [14], including blood pressure [15], stride intervals [16], respiratory cycles [17], and vision [18], among others, such as postural dynamics [19], ultimately leading to decreased fertility and increased risk or mortality [20].

Systemic consequences of aging are widespread but they can be clustered into four domains (Fig. 1):

- Changing in body composition
- The balance between energy availability and energy demand
- Signaling networks that maintain homeostasis
- Neurodegeneration

These changes develop in parallel and affect each other through many feed-forward and feedback loop.

The phenotype that results from the aging process is characterized by increased susceptibility to disease, high risk of multiple coexisting diseases, impaired response to stress, the emergence of "geriatric syndromes," altered response to treatment, high risk of disability, and loss of personal autonomy with all its psychological and social consequences. On the other hand, all these factors influence aging itself, in a dynamic and parallel way, so that they can be considered as not only a consequence of aging but also an integral part of the aging process.

Theories of Aging

Human aging is currently defined as a dynamic process involving the continual adaptation of the body to lifelong exposure to internal and external damaging, as conceptualized in the "remodelling theory of aging" [21••]. Theories of aging are generally classified as either program or damage theories. Programmed aging theories suggest that there is a deliberate deterioration with age because a limited life span results in evolutionary benefits [22]. This plan could be a result of "aging genes." The first described mutation to yield a significant extension in the life span of *Caenorhabditis elegans* was in the age-I gene, which was shown to result in a 65% increase in mean life span and a 110% increase in maximum life span of this organism [23]. Evolutionary biologists may argue that aging occurs due to the absence of natural selection at the post-reproductive stage of life [23]. Although such aging theories are subjectively appealing, as they convey a cure for aging, the accumulation of damage is a spontaneous entropy-driven process [24]. Among the damage

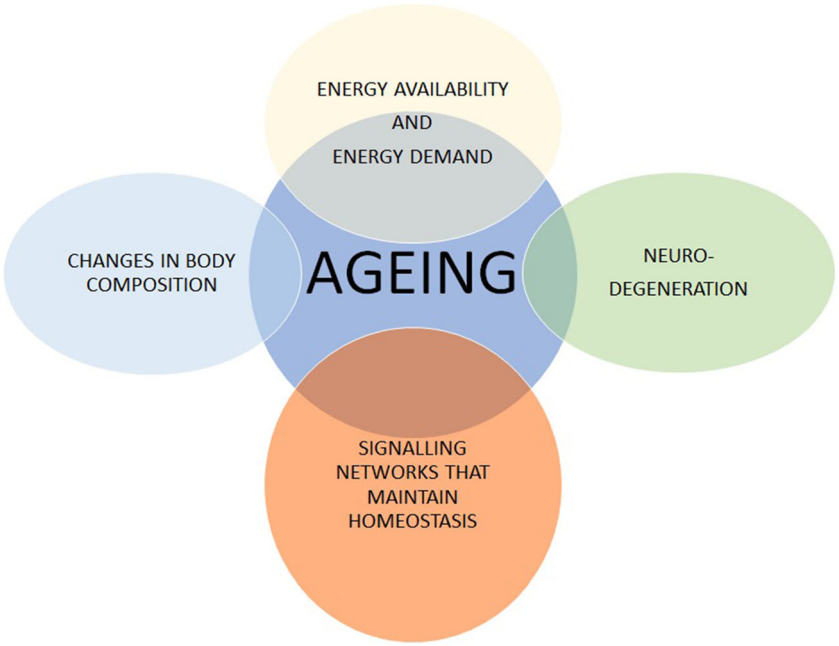

Fig. 1 Systemic consequences of aging

theories, a prevailing idea is that of oxidative damage. Reactive oxygen species (ROS) are generated during metabolism through several interrelated reactions. The supposition that aging may be caused by ROS has been further substantiated by studies involving transgenic animals for genes encoding antioxidants. The life span of *Drosophila melanogaster* has been extended by overexpression of both superoxide dismutase (SOD) and catalase, both antioxidant enzymes [25]. Since mitochondria are the major producer of ROS in mammalian cells, mitochondrial DNA (mtDNA) is therefore particularly susceptible to oxidative damage [26]. Mitochondrial maintenance is, therefore, essential to preserve cellular homeostasis and impaired mitochondrial maintenance has been described as a shared hallmark of numerous human pathologies and aging [27]. Mitochondrial DNA varies with age, and it is commonly considered that DNA hypomethylation is a typical aspect of the aging process [28]. ROS are active intermediates of DNA methylation, as well as histone modification. These reactive oxygen species may play a role in epigenetic processes (physiological phenotypic variations caused by external or environmental factors that switch genes on/off) through reactions of nucleophilic substitution at the DNA level. Consequently, it has been suggested that better preservation of DNA methylation levels, slower cell metabolism, and improved control in signal transmission through epigenetic mechanisms could be key processes involved in human longevity. Oxidative damage to proteins is irreversible and irreparable [29] and must be degraded by the proteasome. The proteasome is the most important proteolytic machinery in eukaryotic cells, largely responsible for the removal of oxidized proteins and the prevention of its aggregation [30]. However, it has been shown that the activity of proteasome is impaired during aging leading to the accumulation of oxidizing proteins, aggresome and lipofuscin, so-called the age pigment. Similarly, to oxidative damage, nitrosamine damage—that caused by reactive nitrogen species (RNS), such as nitric oxide—has been suggested to also contribute to age-related diseases, namely, hepatic steatosis and apoptosis [31], as well as functional and structural changes in the cardiovascular system [32, 33], sleep homeostasis [34], psychological disorders [35], and dementia [36].

Most supporters of the genomic instability theory of aging refer to telomere shortening [37] and mutation in DNA mitochondrial. Telomeres are the repeated DNA sequences at the ends of linear chromosomes, which are unable to be fully replicated by DNA polymerases.

Mutations in mtDNA cause a wide range of human mitochondrial diseases and have been implicated in age-related diseases and aging.

Biomarker Features

Finding the biomarker of aging is one of the most important goals of medicine. The National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [38].

The American Federation for Aging Research (AFAR) recommends the following criteria for biomarkers of aging [39•]:

1) It must predict a person's physiological, cognitive, and physical function in an age-related way, independently of chronological age.

2) It must be testable and not harmful to test subjects (for example a blood test or an imaging technique); it must also be technically simple to perform, and it must be accurate and reproducibly without the need for specialized equipment or techniques.
3) It should work in laboratory animals as well as humans since preliminary testing is always done in nonhuman subjects.

Ferrucci et al. reviewed the biomarkers proposed as elements of a theory based on the balance between "resilience mechanisms" and "accumulated damages," where biomarkers act in reducing resilience mechanisms or increasing damages [40•] (Tables 1 and 2).

The pathways eligible to become biomarkers are the following:

Genomic Instability Endogenous and exogenous agents continuously challenge the integrity of DNA; when DNA repair mechanisms cannot manage the repeated damage, the result is an accumulation of DNA somatic mutations. This phenomenon causes dysregulation of gene expression and the production of altered proteins that lead to cellular damage. Somatic mutation accumulation has been observed in skeletal muscle cells, neurons, and lymphocytes B related to aging [41–44]; nevertheless, quantification of DNA repair capacity in humans has yet to be finalized [45–47].

Telomere Attrition Telomeres are the DNA sequences that are placed at the end of the DNA chain and protect the chromosome ends from damage. During each replication, telomeres are reproduced, but not completely, so with aging they become shorter and contribute to cellular senescence [48–50]. To date, different techniques are available to detect telomere length in circulating cells; however, no techniques have been validated for evaluating aging, because of the heterogeneity between different cells, between individuals and high measurement errors that make these techniques not yet valid in clinical practice [51–54].

Epigenetic Alterations Epigenetics refers to those mechanisms, external to DNA, that modulate gene expression in cells; the regulation of gene expression determines the phenotypic characteristics of the different cells and tissues. The main mechanisms are DNA methylation, histone modification, and noncoding RNA. While DNA methylation is easily measured in circulating cells and seems to be correlated to aging [55, 56], measuring histone modification or noncoding RNA is difficult and expensive. Recent evidence correlates DNA methylation with aging and age-related chronic diseases in humans [57, 58]. Individuals with higher levels of DNA methylation have a higher risk of developing several age-related diseases and premature mortality for all causes and cardiovascular diseases [59], as well as physical and cognitive functions [60, 61].

Loss of Proteostasis The repair of damaged structures or their elimination is fundamental to maintain cell integrity and function [62]. Studies suggest that proteostasis becomes defective with aging and contributes to immunosenescence [63] and that autophagy appears to be more functional in long-lived people

Table 1 Biological changes underlying aging

Genomic instability	Accumulation of DNA somatic mutations
	Dysregulation of gene expression
	Altered proteins production
Telomere attrition	Telomere shortening contribute to cellular senescence
Epigenetic alterations	Altered gene expression
• DNA methylation	Related to age-related chronic diseases
• Histone modification	
• Noncoding RNA	
Loss of proteostasis	Accumulation of damaged structures
Mitochondrial dysfunction	Altered energy production
	Increased ROS production
	Apoptosis-programmed cell death
Cellular senescence	Activation of pathways leading to apoptosis
	Production of SASP
Deregulated nutrient-sensing	Increase of life span in dietary restriction
Steam cell exhaustion	Decline of regenerative potential
Altered intercellular communication	Inflammaging
	Dysfunction of endocrine, neuronal and immune systems

Table 2 Measurable biomarkers classified by respective hallmarks

Hallmark	Pathways measured	Measurable biomarkers
Genomic instability	• DNA repair mechanisms	• yH2A.X immunohistochemistry
	• DNA modifications	
Telomere shortening	• Telomere length	•Leukocyte telomere length
	• Markers of DNA damage response	
	• Telomerase activity	
Cellular senescence	• Senescent markers in blood and tissue	•MIR31HG
		• p16INK4a
		• Senescence-associated secretory phenotype (SASP) proteins
Epigenetic changes (or epigenetic clock)	• DNA methylation	• Measures of DNA methylation
	• Histone acetylation	• SIRT1, SIRT2, SIRT3, SIRT6, SIRT7
	• Noncoding RNA	• Dosage of circulating microRNAs (miR-34a, MiR-21, miR-126-3p, miR-151a-3p, miR-181a-5p, miR-1248)
Mitochondrial	• Mitochondrial volume/number/shape	• p^{31} MRI spectroscopy
	• Mito respiration	• Growth differentiating factor 15 (GDF15)
	• Markers of biogenesis	• NAD+
	• mtDNA copy number and haplotypes	
Decreased autophagy, proteostasis	• Autophagy markers	• Target of rapamycin (TOR)
	• Chaperon proteins	• Protein carbamylation
		• Advanced glycation end products
Stem cell exhaustion	• Proliferative capacity in vitro	
	• Resistance to stress	
Deregulated nutrient-sensing	• Growth hormone (GH) axis	• Insulin-like growth factor (IGF-1)
	• Metabolism alterations	• HGBA1c
Altered intercellular communication	• Measures of inflammation	• IL-6
		• TNF-α
		• CRP (C-reactive protein)
		• TNFRII (tumor necrosis factor-α RII)

[64]. Measuring the loss of proteostasis mechanism could be a good biomarker, but, to date, there are no valid techniques for this purpose.

Mitochondrial Dysfunction The main role of mitochondria is to guarantee energy for the cell through the production of ATP. They are also involved in signaling by the production of ROS and in apoptosis-programmed cell death. Mitochondrial dysfunction is a good biomarker of aging and is associated with disability in older persons, through the reduction of muscle strength [65•].

Many techniques are measuring oxidative phosphorylation and ROS generation that have been associated with chronic disease [66, 67]; nevertheless, the relation with aging is not completely validated.

Cellular Senescence Genomic instability, telomere shortening, and other endogenous and exogenous mechanisms can induce the cell to activate specific pathways that lead to apoptosis [68]. This process is called cellular senescence and is characterized by structural and functional changes in the cell [69]. Senescent cells produce pro-inflammatory cytokines and chemokines, growth factors, and matrix proteases called "senescence-associated secretory phenotype" (SASP) [70, 71] which may induce some age-related diseases [72–74]. The detection of SASP has been proposed as a biomarker of aging [75].

Deregulated Nutrient-Sensing Genetic mutations in growth hormone and the insulin-like growth factor have been linked to longevity [76]. Moreover, dietary restriction showed to increase life span in primates [77, 78]. For these reasons, this pathway has been proposed as biomarkers of aging.

Steam Cell Exhaustion The decline in the regenerative potential is one of the elements at the base of aging [79]. Despite pharmacological interventions being explored to counteract this phenomenon [80], evidences are still poor.

Altered Intercellular Communication With aging, we also observe changes in intercellular communication: as inflammatory reaction increases, the other communication ways become dysfunctional (endocrine, neuronal, immune system) [81].

As we discussed earlier, inflammation can be inappropriately increased in aging, and this has been related to age-related disease [82, 83].

Indeed, the pathways, described as potential biomarkers of aging, are strongly related to inflammation; for this reason, measuring circulating levels of cytokines is considered a new field of research [83, 84•, 85].

Aging and Life Expectancy

Aging and life expectancy are closely related. In a broad sense, determining an individual's life expectancy is also a way of schematizing his or her aging process. Life expectancy is a statistical measure of the average time an organism is expected to live, based on the year of its birth (LEB), its current age and demographic factors including gender [86]. In the last decades, life expectancy has increased in high income country; the rise in human life expectancy has involved declines in intrinsic and extrinsic mortality processes associated, respectively, with senescence and environmental challenges [87].

In association to this increased longevity, there are diseases called age-related that increase quadratically with age and cause a progressive loss of physical, mental, and cognitive integrities, leading to impaired function and increased vulnerability to morbidity, mortality [20] and disability, in addition to increasing care needs and age-related burden measured through the sum of disability-adjusted life years (DALYs) of these diseases among these adults (Fig. 2). Ninety-two of the 293 of the Global Burden of Disease causes were identified as age-related diseases. In particular, cardiovascular disease, neoplasm, and chronic respiratory disorders are those with higher age-related disease burden [2].

Determinants of Frailty Syndrome as Aging Biomarker

Frailty can be defined as a state of increased vulnerability to stressors or a loss of capacity to resolve homeostasis perturbation. Frailty condition is closely related to aging [88••], and the frailty indexes can consequently be considered biomarkers of aging themselves. In frail individuals, it is possible to find both changing in body composition and balance between energy availability and energy demand. Moreover, in the definition of frailty, it is well described how signaling networks maintain homeostasis and association with neurodegeneration. These four aspects all refer to the hallmarks of aging. Frailty is associated with adverse clinical outcomes, including falls, institutionalization, and death [88••].

Two principal models emerged in the last decades that are able to conceptualize and consequently measure frailty in everyday clinical practice and research: the "frailty phenotype" model and the cumulative deficits model.

The frailty phenotype was first described by Fried and colleagues in 2001, analyzing data from the Cardiovascular Health Study (CHS), involving 5210 men and women aged 65 years and older. In this study, it was investigated which characteristics of the population were predictive of falls, disability, hospitalization, and death. Their operational definition of frailty included a cluster of at least three of the following variables: unintentional weight loss, self-reported exhaustion, low energy expenditure, slow gait speed, and weak grip strength. This model does not take into consideration cognitive impairment as a cause of increased vulnerability, as this could contribute to functional decline and adverse events in older people [89, 90].

The cumulative deficits model was developed by Rockwood and colleagues as part of the prospective Canadian Study of Health and Aging (CSHA), involving a cohort of 10,263 older adults [91]. The authors identified 92 parameters, including diseases, disabilities, signs, symptoms, and laboratory values, which were defined as "deficits." The sum of the deficits in a single individual allowed for the calculation of a frailty index (i.e., the number of deficits divided by 92). Frailty in this model is not considered as a cluster of symptoms but is conceptualized as a gradable syndrome, with a higher number of deficits implying an increased vulnerability state. The two models of frailty show significant overlap, although they capture slightly different sides of the same problem. It is important to notice that physical frailty is frequently associated with multimorbidity [92, 93•, 94].

It has been observed that the frailty phenotype construct is intrinsically related to mobility issues. Indeed, in older adults, physical performance measures are a robust and consistent predictor for disability, hospitalization, institutionalization, and death, both in the research and in the clinical setting. Lower physical performance is frequently associated with loss of skeletal muscle mass and quality, causing reduced strength and functional impairment [95••]. This process has been called sarcopenia. Even though sarcopenia has been long associated with aging, it has to be acknowledged that it can develop much earlier in life [96]. Different definitions exist for this condition for the operational definition of sarcopenia both in the clinic and for research purposes that prioritize the assessment of muscle strength over muscle mass to identity sarcopenic patients. Strength is more closely related to survival and functional decline, compared with muscle

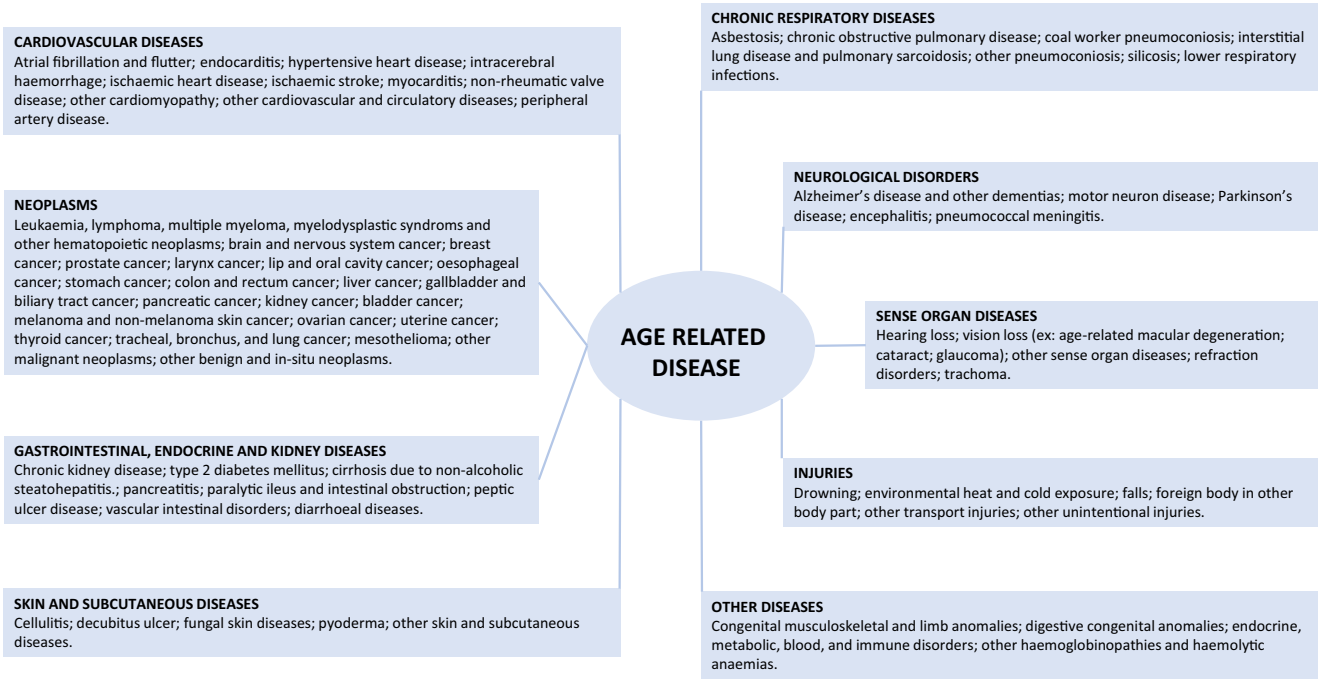

Fig. 2 Age-related diseases, adapted from Chang et al. [2]

mass [95••]. According to EWGSOP criteria, sarcopenia is defined by the presence of low muscle strength (criterion 1) and either or low muscle quantity or quality (criterion 2) or low physical performance (criterion 3) [95••].

The physical performance parameters used in the identification of frailty syndrome, both integrated (e.g. SPPB) and alone (walking speed, handgrip strength), can be used as aging performance biomarkers.

Determination of Medical and Social Needs

Why consider medical and social needs aging biomarkers?

In 1952, Robert J. Havighurst said: "In considering the needs of older people it is well, first, to remember that older people have the needs that are common to all people, and, second, that they have special needs due to the fact that they are old people". This sentence describes everything there is to know about the need for the elderly and answers the question before.

In every society and age, there is what is meant by normality. An elderly person in this scenario needs what is needed to maintain this level of normalcy. Activity of daily living and instrumental activity of daily living (ADL and IADL) alone, remodelled according to the context and gender, can identify the minimum necessary. Conducting needs assessment, various areas must be considered including physical health, mental health, emotional, care, social, cultural, economic, nutritional, service, security, legal, and educational.

Many tools are used to evaluate people's needs. The majority of these tools are focused on physical performance able to maintain autonomy; few studies focus on social needs and the costs of care. In the West World, 10% of patients account for 70% of total health care expenditures. This 10% is represented by older people, individuals with multiple chronic conditions, many medications, frequent hospitalizations, and limitations on their ability to perform basic daily functions due to physical, mental, or psychosocial challenge [97].

Since the health care and social needs of older adults differ from that of other adults, it is necessary to identify the needs of the elderly to make proper plans that will promote their health.

Currently, most of the conducted studies had mainly focused on the elderly physical health needs and had neglected to take into account other needs such as social and health care needs. Furthermore, in addition to quantitative studies, discovering the older adults' "perceptions" of their own health needs is also necessary.

Conclusion

There is a large interest of researchers in biomarkers of aging, and despite some of them seem to be very promising, biological biomarkers are still far from a clinical application; to date, there is no technique that meets the mentioned criteria of the ideal biomarker [40•]. Moreover, we know that the biological pathways are the final agents of aging, but on one side they can be influenced by social, economic and environmental factors, and on the other side, they express in various disease and

disabilities of the person (physical and cognitive impairments, age-related disease, systems functions, sensory functions, etc.) (Fig. 3).

To date, more than a single biomarker, to assess aging, we should consider a cluster of biomarkers that comprise the various elements that we analyzed: social and educational aspects, economic factors, country of origin, presence of age-related disease, presence of dependence in daily activities, physical capability, cognitive function, lung and cardiovascular function, and presence of sensory dysfunctions. In Table 3, we propose several clinical and laboratory biomarkers that can be used in clinical practice and research.

The geriatric assessment (GA) can currently be considered a system capable of monitoring multiple biomarkers, clinical and laboratory, of aging, and at the same time able to relate them to each other. Through the GA, it is possible to make a prediction of the risk of toxicity of a treatment, of life expectancy, of social needs, and of compliance with the treatments. GA is composed, indeed, by several evaluations, made through standardized tools, which examine various aspects of the person (a multidimensional assessment).

Although it seems difficult to imagine a geriatric assessment as a biomarker, currently for its characteristics and for the high predictivity it has, it can be considered the gold standard in the management of the older individual and instrument toward which other biomarkers should be evaluated.

The purpose of this paper was to evaluate the multiple aspects that distinguish the aging process. Aging must no longer be described as a simple demographic event but as a complex mosaic in which several tesserae relate to each other, some in a very evident way others often in a more subdued but all fundamental way. Each aging theory has attempted to justify this process effectively; however, there is no single biomarker to date that has been found able to identify the stage of this process. At the same time, clinical clusters have been added to purely biological markers, and social ones should certainly be considered. It, therefore, becomes important not to consider biomarkers only as life span, but to try to overcome this link and focus on the set of factors that, influencing each other, are able to guide aging in good health and good quality of life towards a lived aging as a slow decline. At the time we are writing this paper, COVID 19 infection is reaping victims especially in Italy. The highest mortality is observed among the older adults, but surprisingly, it seems to maintain similar values between the youngest and oldest old (over 90 years). Currently, no plausible justification is provided for these data. In frailty, the number of comorbidities, the reduced functional reserve was the most used reasons. Indirectly, this infection is highlighting the need to use parameters that can more easily identify the aging process regardless of chronological age.

The studies analyzed in the literature show that if on the one hand there are physiological biomarkers able of highlighting some features of aging, other functional markers (performance, social and economic status, some pathologies and the presence of addiction) are able of speed it up or slow it down. For this reason, if we want

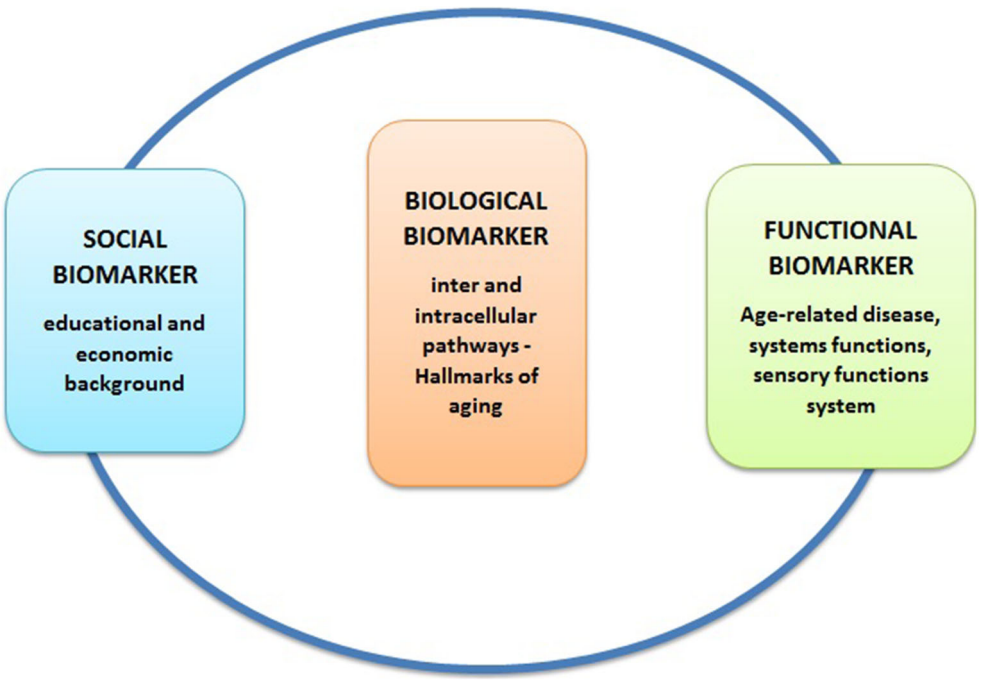

Fig. 3 Mechanisms connecting different clusters of biomarkers

Table 3 Panel of possible biomarkers of aging

Parameters of organ functionality	Blood pressure
	Forced expiratory volume in 1 s (FEV 1);
	Bone density
	Memory executive function
	Body composition and muscle mass (sarcopenia)
Parameters of physical function	Walking speed, timed get up and go, chair rising, grip strength, balance tests, pegboard test
Blood parameters from clinical routine	Homocysteine, cholesterol profile, glycosylated hemoglobin, fasting glucose, growth hormone, insulin-like growth factor 1, DHEAS, DHEAS/ cortisol ratio, adiponectin, leptin, ghrelin, melatonin, estrogen, somatostatin, testosterone, thyroid hormones, Cystatin C, NT-proBNP
Biomarkers of immune function	IL-6, TNF-alpha, TNF-RII, C-reactive protein
Specific molecular biomarkers	Leukocyte telomere length, y-H2A.X immunohistochemistry, DNA methylation, heterogeneity of CD38 in CD4+ and CD27+ T cells, heterogeneity of CD197 in CD4+ and CD27+ T cells, dosage of circulating microRNAs (miR-34a, miR-21, miR-126-3p, miR-151a-3p, miR-181a-5p, miR-1248), MIR31HG, AK156230, Meg3, target of rapamycin (TOR) proteins, pS6RP, NAD+, SIRT1, SIRT2, SIRT3, SIRT6, protein carbamylation, advanced glycation end products, N-glycans, growth differentiating factor 15

to translate the use of biomarkers into clinical practice, we can think of not only something measurable through blood analysis but also a functional assessment of the patient we have in front, with his/her context and social network.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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