REVIEW PAPERS

Modern markers for the assessment of biological age

Nowoczesny markery wieku biologicznego

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KEYWORDS:

- biological age markers
- hallmarks of aging
- premature aging

ABSTRACT

The steady increase in human life expectancy in the 21st century is considered one of the major public health challenges. However, current achievements in longevity, are most often associated with increasing years in disability. Current studies indicate that healthy longevity is achieved, through harmonised aging of the whole body. Due to the complexity of aging, a number of biological age markers have been determined to describe the intensity of changes limiting biophysiological functions at different levels of the body. Biological age markers can be divided into two categories: parameters determining the level of severity of molecular and cellular hallmarks of aging: genomic incapacity, telomere length changes, mitochondrial dysfunction, loss of proteostasis, epigenetic changes, cellular senescence, deregulation of nutrient sensitivity, stem cell depletion, altered intercellular communication. The second group are parameters defining age at the systemic level: bone age, muscle age, vascular age, neuronal age, endocrine age, pulmonary age, glycan age. Modern methods of measuring biological age markers allow the identification of weak points in aging and illustrate the unevenness of the aging process. It is worth considering the study of biological age markers to prioritise interventions promoting sustainable aging.

SŁOWA KLUCZOWE:

- markery wieku biologicznego
- znaczniki starzenia się
- proces przedwczesnego starzenia się

STRESZCZENIE

Stały wzrost średniej długości życia człowieka w XXI wieku jest uważany za jedno głównych wyzwań zdrowia publicznego. Jednak obecne osiągnięcia w zakresie długowieczności, najczęściej wiążą się z wydłużaniem lat w niepełnosprawności. Obecne doniesienia wskazują, że zdrową długowieczność osiąga się, dzięki zharmonizowanemu starzeniu się całego organizmu. Ze względu na kompleksowość starzenia się wyznaczono szereg markerów wieku biologicznego opisujących natężenie zmian ograniczających funkcje biofizjologiczne na różnych poziomach organizmu. Markery wieku biologicznego można podzielić na dwie kategorie: parametry określające poziom nasilenia molekularnych i komórkowych znaczników starzenia: niezdolność genomowa, zmiany długości telomerów, dysfunkcja mitochondriów, utrata proteostazy, zmiany epigenetyczne, senescencja komórkowa, deregulacja wrażliwości na składniki odżywcze, wyczerpanie komórek macierzystych, zmieniona komunikacja międzykomórkowa. Druga grupa to parametry określające wiek na poziomie układowym: wiek kostny, wiek mięśniowy, wiek naczyniowy, wiek neuronalny, wiek hormonalny, wiek pulmonologiczny, wiek glikanowy. Nowoczesne metody pomiaru markerów wieku biologicznego pozwalają na identyfikację słabych punktów starzenia i obrazują nierównomierność procesu starzenia. Warto rozważyć badanie biologicznych markerów wieku w celu ustalenia priorytetów interwencji promujących zrównoważone starzenia się.

Introduction

Nowadays, the world population is aging at a rapid pace. By 2030, there will be more than one billion people on earth over the age of 65, and more than 200 million people will be over 80. This significant rise in average life expectancy, on one hand, becomes as one of society`s greatest achievements but on the other hand is related to the development of age-realted diseases and increase of morbidity. Living longer, is not always related to a healthy life.

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More often living longer represents an increase in years of disability (1).

The aging process is caused by the accumulation of deleterious changes at both the molecular and cellular levels, which ultimately lead to a reduction in functional potential at the tissue and organ level, as well as the whole body. Some areas of the body may undergo more rapid aging processes, which may affect the deterioration of other body components (2-4). Individual differences are related to individual exposure to risk factors – exogenous and endogenous.

Studies of 100-year-olds have shown that longevity is achieved by accomplishing a stable and balanced aging process of the whole body. One of the main goals of modern medicine is to extend quality of life (5, 6). The best way to achieve this goal is to identify the vulnerabilities of aging and promote balanced aging of the whole body.

Due to the complexity of the aging process, it is not possible to set a single value for the biophysiological state of aging (biological age), but it is possible to identify characteristics that assess the functional status of individual areas and the level of accumulation of risk factors for aging.

Assessment of the level of aging

A number of biological age markers have been determined, which describe the level of progression of aging processes in particular areas. In order to meet the definition of a biological marker, 3 criteria must be taken into account: each feature should manifest itself in the course of natural aging, deterioration of a feature should accelerate aging processes, improvement of a feature should delay aging processes. Modifications that alter the aging processes were studied under experimental conditions. The last criterion relating to anti-aging processes is the most challenging to fulfil due to the as yet undescribed interventions across all mechanisms to alleviate aging. Currently, we can only relate changes in biological markers to the rate (acceleration or deceleration) of the aging process.

Age-related test parameters can be divided into two categories: parameters determining age at a systemic level and parameters determining the intensity level of molecular and cellular markers of aging.

Cellular and molecular markers of aging

Molecular and cellular markers of aging and the risk factors affecting them have been distinguished and characterized. In a 2013 paper by Carlos López-Otín et al., nine hallmarks of aging were proposed and distinguished: genomic instability, telomere attrition, mitochondtial dysfunction, loss of proteostasis, epigenetic alterations, dysregulated nutrient sensing (in the GH/IGF-1 pathway, mTOR, AMPK and sirtuins), cellular senescence stem cell exhaustion, altered intercellular communication (7). After 10 years, the set was updated with 3 additional markers: disabled macroautophagy, chronic inflammation, and dysbiosis (8). Only some of the described markers of aging are currently measurable by research methods. In this review, we will summarize the current knowledge of available measurements describing the designated markers. Cellular and molecular hallmarks enables the identification of risk factors and the prioritisation of a course of action to promote balanced aging.

Genomic insability

One of the indicator of aging is accumulation of genetic damage throughout life. Also, many premature aging diseases are the consequence of increased DNA damage accumulation. The integrity and stability of DNA are continuously challenged by exogenous physical, chemical and biological factors as well as by endogenous threats such as DNA replication errors, spontaneous hydrolytic reactions, and reactive oxygen species. The genetic instability, caused by both extrinsic and intrinsic damages are highly diverse including point mutations, translocations, chromosomal gain and losses, telomere shortening and even gene disruption caused by the integration of viruses or transposons. Due to the high reproducibility, precision and availability of collected data, the recommended marker for assessing the level of DNA damage is **the 8-oxo-dG marker** determined from urine (9, 10). Longevity differs by several orders of magnitude among animals and long life spans seem to associate with a greater capacity to detect the presence of DNA damage at the cellular level (11-13). Enhanced recognition of damage should allow enhanced DNA repair.

Telomere attrition

Telomeres are specialized non-coding structures consisting of repetitive 6-nucleotide '5- TTAGGG-3' sequences, located at the ends of chromosomal DNA strands, whose function is to protect them from damage. During each cell division, there is a natural shortening of telomere length. Telomeres are shortened in each cell division cycle due to incomplete synthesis of the delayed strand during DNA replication due to the inability of DNA polymerase to completely replicate the ends of chromosomal DNA.

DNA damage factors, inflammation-generated free radicals or cellular stress can lead to telomere attrition and over-shortening. Reaching a critical value for telomere length is a signal for the activation of senescence processes, resulting in the blocking of cell division and permanent withdrawal from the cell cycle, thus indicating molecular aging. There are telomere repair systems and telomerase, an enzyme that catalyses the addition of nucleotides in the TTAGGG sequence to the ends of chromosomes, which enables telomere elongation (14). Telomerase deficiency in specific cells is associated with the premature development of diseases such as pulmonary fibrosis, dyskeratosis congenita, and aplastic anaemia, which involve the loss of regenerative capacity of various tissues (15). Dynamic assessment of **telomere length** changes and characteristics of processes that improve telomerase function should make it possible to promote the body's regenerative mechanisms.

Mitochondrial dysfunction

Mitochondria play a key role in bioenergetic metabolism through the production of ATP. Maintaining mitochondrial function during lifespan is key to ensuring overall homeostasis. Due to their pivotal role in many cellular functions, these organelles are involved in many distinct processes associated with aging, including: bioenergetic changes, mitophagy and proteolysis, mitochondrial

response to unfolded proteins, inflammation, cellular senescence, stem cell function, accumulation of DNA mutations. A reduction in the efficiency of mitochondrial bioenergetics has been observed during the aging process and several mechanisms have been described, such as reduced biogenesis, mutations in mitochondrial DNA or defective mitophagy (16, 17). Indicators assessing mitochondrial performance include assessment of **lactate/ pyruvate concentrations as products of carbohydrate metabolism and glycolysis, heparin blood ATP measurement and ATP production in lymphocytes, and measurement of the pyruvate kinase enzyme M2-PK involved in the respiratory chain and regulating glycolysis** (18). A new coefficient was also determined to determine the level of energy produced by the mitochondria – the **bioenergetic health index (BHI)**. The principle of assessing the bioenergetic profile is based on measuring the rate of oxygen consumption by the mitochondria of peripheral blood lymphocytes and monocytes for ATP production under the influence of added specifically characterized inhibitors of oxidative phosphorylation. The calculated BHI can represent a complex mitochondrial profile for a selected cell type and can illustrate bioenergetic dysfunction early in the development of pathology (19).

Much of the research on aging has now focused on the study of hormesis, the concept that mild toxic stress induces beneficial compensatory responses that outweigh the repair of the induced damage and result in improved cellular fitness compared to baseline pre-damage conditions. Evidence has been advanced showing that compounds such as resveratrol or metformin, which induce a low-energy state characterized by increased AMP levels and AMPK activation, constitute mild mitochondrial poisons that induce compensatory mechanisms to improve mitochondrial function and thereby prolong life (20).

Loss of proteostasis

Proteostasis involves mechanisms to stabilize correctly folded proteins and mechanisms to degrade misfolded polypeptides by the proteasome or lysosome, thus preventing the accumulation of damaged components and ensuring the continuous renewal of intracellular proteins. Many studies have shown that proteostasis is altered with aging (21, 22). Furthermore, the chronic expression of undeveloped, misfolded or degraded proteins is implicated in the development of diseases such as Alzheimer's disease, Parkinson's disease, and cataracts (23). It is not clear whether loss of proteostasis should be considered only as an age marker or as one of the more important causes of cellular aging.

Modern research has made it possible to identify **proteostasis components (e.g., UBR5 E3 enzyme, CCT8 chaperonin subunit, CSDE1 RNA-binding protein)** that inhibit disease-related protein aggregation in human stem cells and affect differentiation. Experiments conducted on C. elegans have proven that enzyme modulation can inhibit the accumulation of disease-associated proteins, while prolonging the life of the organisms (24). Another mechanism that enables the removal of misfolded proteins is autophagy. Autophagy is a process of cellular degeneration in which parts of the cytosol and organelles are sequestered in a double-membrane vesicle, the autophagosome, and delivered to the lysosome for breakdown and ultimate recycling of the resulting macromolecules. The study of autophagy processes themselves remains a poorly understood aspect. In addition to electron microscopy, the observation of **MAP1LC4 and p62/SQSTM1 protein** rearrangements is considered the primary marker of autophagy (25). Supporting autophagy processes may slow down the aging process.

Epigenetic alterations

Epigenetic drift causes changes in gene expression through modulation in DNA methylation patterns, post-translational modifications of histones and chromatin remodelling, which occur under the influence of various environmental influences and endogenous factors e.g., lifestyle, nutrition, weight changes. A correlation has been found between changes in specific methylation sites and aging. The selected pattern of methylation changes constitute the "epigenetic clock". The Horvath calculator and the Hannum calculator were the first proposed biological clocks, providing the basis for current used biological clocks (26, 27). It is likely that with advances in technology, a better understanding of DNA methylation will soon be achieved. Theoretically, there are mechanisms for modulating epigenetic changes that enable anti-aging processes. These include mechanisms modulated by histone acetyltransferase inhibitors or histone adeacetylase activators, which will be used in the development of new anti-aging treatments (28, 29).

Cellular Senescence

Aging cells are cells that have stopped dividing and have entered a state of permanent growth arrest, also known as cellular senescence. This process is a normal part of aging and is thought to occur in response to stress, damage or other oncogenic factors. Aging cells are characterized by the presence of specific biomarkers such as **aging-associated beta-galactosidase (SA-β-gal), p16INK4a and pro-inflammatory factors, growth factors, proteoasomes and other aging-associated secretory factors termed SASPs** (30-32) SASP factors affect neighboring cells by inducing senescence, tissue remodelling and increased inflammation. Biomarkers characterizing the level of aging cells have been proposed as a potential biomarker of biological age, as their accumulation has been linked to age-related diseases and conditions, including cancer, cardiovascular disease and neurodegenerative disorders (33). Researchers are exploring the elimination of aging cells as a potential strategy to improve health and delay the onset of age-related diseases.

Deregulated Nutrient-sensing

The somatotropic axis includes growth hormone (GH), produced by the anterior pituitary gland, and its secondary mediator insulin-like growth factor (IGF-1), produced in response to GH by many cell types, particularly hepatocytes. The intracellular IGF-1 signalling pathway is activated in the presence of glucose, just as it is for insulin activation. For this reason, IGF-1 and insulin signalling is known as the insulin and IGF-1 signalling (IIS) pathway. This is the main signalling pathway involved in nutrient

sensing. There are additional pathways involved in the integration of IIS signalling: mTOR to detect amino acid concentrations, AMPK to detect a low-energy state, and sirtuins, which sense nutrient deficiency by detecting high levels of NAD+. Markers of deregulated nutrient sensing, such as levels of **circulating glucose, insulin or lipids**, can be used as indicators of biological age. For example, elevated glucose and insulin levels have been shown to predict the occurrence of age-related diseases such as type 2 diabetes and cardiovascular disease. There are studies proving that reducing GH/IGF-1 and mTOR signalling prolongs life. In contrast, enhancement of AMPK and sirtuin pathway activation can prolong lifespan (8, 33, 34).

Stem Cell Exhaustion

Stem cells are cells with special features that have the ability to differentiate into different cell types and regenerate damaged tissues. They play a critical role in tissue homeostasis and repair, and their function declines with aging, leading to a progressive loss of tissue function and increased risk of age-related diseases, reduced ability to regenerate and repair tissues (35). Markers of stem cell depletion, such as changes in the number and function of circulating stem cells, can be used as indicators of biological age. For example, studies have shown that the **number of circulating haematopoietic stem cells,** which are responsible for producing blood cells, decreases with aging and is associated with an increased risk of age-related diseases (36). Other hallmarks of stem cell aging include, in addition to the hallmarks of aging mentioned above, oxidative stress and levels of inflammation.

Oxidative stress

A number of biomarkers have been proposed to measure levels of oxidative stress. These biomarkers include blood **levels of lipid/ubiquinone superoxide (coenzyme Q10), total oxidative capacity of the blood (TOS), levels of fat-soluble (e.g., vitamin A/E), water-soluble (e.g., vitamin C) and oxidised precursors (e.g., iron, cholesterol) antioxidants** (37). Pharmacological interventions to improve stem cell function have been investigated. In particular, inhibition of mTORC1 with rapamycin, which can delay aging by improving proteostasis and by affecting nutrient sensing pathways, may also improve stem cell function in the epidermis, haematopoietic system and intestine (38, 39). Reducing oxidative stress, on the other hand, can be achieved at three levels: by reducing exposure to oxidative environmental pollutants, by increasing levels of endogenous and exogenous antioxidants or by reducing the generation of oxidative stress by stabilising energy production and yield in mitochondria (40).

Altered Intercellular Communication

Intracellular communication is the process by which cells communicate with each other by exchanging signalling molecules through the endocrine, neuroendocrine and neuronal systems. This communication is crucial for maintaining cellular homeostasis and coordinating the functions of different cells and tissues in the body (41, 42). Neurohormonal signalling (e.g., renin-angiotensin signalling, adrenergic signalling, insulin-IGF1 signalling) tends to become dysregulated during the aging process, as inflammatory responses, so-called inflammaging – a smouldering pro-inflammatory environment of low severity that accompanies aging in mammals – increase. Inflammation may result from the accumulation of pro-inflammatory tissue damage, a decreased ability of the immune system to clear pathogens and dysfunctional host cells, the propensity of aging cells to secrete pro-inflammatory cytokines, increased activation of the transcription factor NF-κB or the occurrence of a defective autophagy response (43). Markers of altered intracellular communication, such as **changes in the expression or activity of specific signalling molecules or receptors, and indicators of inflammation such as C-reacitve protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1β), and reactive oxygen species (ROS)** can be used as indicators of biological age. There are several options for restoring the defective intercellular communication underlying the aging process, including genetic, nutritional or pharmacological interventions that can improve the properties of cell-to-cell communication that are lost with aging (44). Additionally, the use of anti-inflammatory agents, such as aspirin, or modification of the composition and functionality of the gut microbiome as a centre for shaping host immune function, can improve healthy aging in humans (45, 46).

Biological age markers on system level

Biological age can be considered in the context of bone age, muscle age, vascular age, neural age, endocrine age and on the basis of glycosylation patterns. Each of the parameters assessed may differ significantly from chronological age and may also differ from each other.

Bone age is determined by measuring BMD (Bone Mineral Density) using dual-energy x-ray absorptiometry (DEXA) or ultrasonography (USG). Since the BMD of lumbar vertebrae tends to be overestimated due to the presence of adhesion or compression fracture, it is more appropriate to measure the BMD of the femoral neck. People with higher BMD undergo slower bone aging while people with lower BMD undergo faster bone aging. USG might be used to determine the stiffness of the calcaneal bone. Improved walking habits lead to improved stiffness of the calcaneal bone (47).

Muscle age: from the age of 30, the muscle mass decreases by 1% per year in the absence of appropriate exercise. Some of the methods used to asses muscle age include:

1. Muscle strength testing, measured using various tests, such as grip strength, leg press strength, and knee extension strength.
2. Muscle mass measurements, measured using various imaging techniques, such as dual-energy X-ray absorptiometry (DXA), magnetic resonance imaging (MRI), and computed tomography (CT).
3. Muscle quality assessments, which refers to the amount of force a muscle can produce per unit of muscle mass. Age-related declines in muscle quality are a key feature of sarcopenia and can be used to estimate an individual's muscle age.

A noticeable decrease in muscle mass is particularly visible in the femoral muscle. Additionally, creatine kinase (CK) is a marker of demage CK-rich tissue such as in severe muscle breakdown (rhabdomyolisis) or in heart attack (myocardial infarction) (48-52).

Vascular age: age-related changes in blood vessels are a common feature of the aging process and are associated with a range of adverse health outcomes, including cardiovascular disease, stroke, and dementia. There are some various methods to asses vascular age.

1. Evaluation of pulse wave velocity (PWV) assess the progression of arteriosclerosis. PWV measures the speed at which the arterial pulse travels between two points along an artery and can be measured in any arterial segment between two pulse-wave palpable regions. In analyzing vascular age it is important to carefully check each of the known risk factors for arteriosclerosis such as diabetes, hypertension tends, dyslipidemia, smoking, resting state.
2. Endothelium plays a key role in regulating blood flow and vascular health. Measurement of endothelial function using flow-mediated dilation (FMD), which measures the ability of the arteries to dilate in response to increased blood flow.
3. Evaluation of carotid intima-media thickness (CIMT): a measure of the thickness of the innermost layer of the carotid artery and is used as a marker of subclinical atherosclerosis. As people age, CIMT tends to increase, which can indicate increased risk of cardiovascular disease. CIMT can be measured using ultrasound imaging (53-57).

Neural age is determined by the assessment of cognitive brain function include attention, frontal lobe function, visual perceptive function, cognitive intelligence, memorization and mental function in general. The Japan Brain Dock Society recommends the use of the Wisconsin Card Sorting Test for screening purposes. This test uses Age Management Check TM to estimate the examinee's neural age (58). Nowadays the most commonly used is the MMSE scale. Another assessment of neurological age include measures using various non-invasive techniques, such as electroencephalography (EEG) and functional MRI (fMRI). Changes in brain function, such as decreased connectivity between different brain regions, can be indicative of age-related decline. A study published in Frontiers in Aging Neuroscience in 2021 found that measures of brain structure and function were more strongly associated with age than chronological age alone, indicating that neurological age may provide a more accurate assessment of age-related decline in the brain (59).

Hormone age: The secretion of growth hormone (GH) hormone and insulin-like growth factor (IGF-1) secreted under its influence start to decrease around 30 years of age, and additionally the decreased level of these hormones is a predictor of weaker prognosis and quality of life index. Decreased secretion of these hormones is referred to as somatopause, which not only leads to reduce cell division and protein synthesis but is also deeply associated with declines in neuropsychiatric functions, digestive functions and bone metabolism. The level of dehydroepiandrosterone sulfate (DHEA-s) is another marker of the hormone age. DHEA-S, which is the precursor of more than 50 types of hormones including reproductive hormones and cortisol, is secreted in the adrenal cortex and its secretion declines with age. The decline in DHEA-s secretion has been associated with impaired immunological function and decreased resistance to stress, as well as an increased risk of metabolic syndrome, fatty liver, diabetes, hyperlipidemia, hypertension, osteoporosis and other lifestyle-related diseases. The level of the stable form of DHEA-s is measured in hormone age assessment (60-62). Impaired immunological function and increased risk of metabolic syndrome, diabetes, hyperlipidemia, hypertension and lifestyle-related diseases influence the time of the occurrence of andropause (63).

Pulmonare age: Pulmonary function tests measured by spirometry assess various aspects of lung function, including the volume of air that can be forcefully exhaled in one second (forced expiratory volume in 1 second, or FEV1), the total volume of air that can be exhaled after a deep breath (forced vital capacity, or FVC), and the ratio of FEV1 to FVC (FEV1/FVC). Age-related declines in lung function are well-established, and studies have shown that these declines can be used to estimate a person's pulmonary age. For example, a person with lung function measures that are typical of someone who is older than their chronological age may be said to have a "pulmonary age" that is higher than their actual age. PulmoAge has been studied in various populations, including smokers, individuals with chronic obstructive pulmonary disease (COPD), and healthy adults. In general, studies have found that higher pulmonary age is associated with increased risk of respiratory illness, cardiovascular disease, and overall mortality (64-68).

Glycan age: a recently invented biomarker that assesses the glycosylation pattern of complex carbohydrate molecules attached to immunoglobulin IgG. Several studies have investigated the relationship between glycan age and chronological age, as well as the potential for glycan age to be used as a biomarker of aging. One study found that levels of certain glycans were associated with chronological age in a large population-based cohort, and that these glycans could be used to accurately predict an individual's chronological age (69). Other studies have shown that changes in glycans over time can predict the onset of age-related diseases, such as cardiovascular disease (70).

Clinical opportunities for the use of biological age markers

Identifying individual most harmful risk factors and vulnerabilities in aging enables prioritisation of interventions to promote balanced aging, thereby prolonging quality of life.

In clinical practice, it is worth considering monitoring biological age markers in the development of chronic diseases to assess the impact of the presence of diseases on premature aging processes. Furthermore, a reduction in the level of aging determined by biological age markers may set new additional targets for chronic disease therapy.

Conclusions

Modern measurement methods make it possible to determine a range of biological age markers relating to different aspects of aging, providing evidence of unevenly progressive aging in different parts of the human body. It is worth considering the study of biological age markers as a direction for detecting vulnerabilities in aging and prioritising interventions for sustainable aging.

Table. 1 Biological age markers.

Hallmarks of aging	Biological marker
Cellular and molecular markers of aging	
Genomic insability	DNA damage: the 8-oxo-dG marker
Telomere attrition	Telomere length
Mitochondrial dysfunction	Lactate/pyruvate concentrations Heparin blood ATP measurement in lymphocytes measurement of the pyruvate kinase enzyme M2-PK
Loss of proteostasis	UBR5 E3 enzyme, CCT8 chaperonin subunit, CSDE1 RNA-binding protein, MAP1LC4 and p62/SQSTM1 protein
Epigenetic alterations	the Horvath calculator, the Hannum calculator
Cellular Senescence	Beta-galactosidase (SA-β-gal), p16INK4a, pro-inflammatory factors: growth factors, proteoasomes, aging-associated secretory factors termed SASPs
Deregulated Nutrient-sensing	Circulating glucose, insulin or lipids
Stem Cell Exhaustion	Circulating hematopoietic stem cells
Oxidative stress	Levels of lipid/ubiquinone superoxide (coenzyme Q10), Total oxidative capacity of the blood (TOS), Levels of fat-soluble (e.g., vitamin A/E), water-soluble (e.g., vitamin C), Oxidised precursors (e.g., iron, cholesterol) antioxidants
Altered Intercellular Communication	C-reacitve protein (CRP), Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-alpha), Interleukin-1 beta (IL-1β), Reactive oxygen species (ROS)
Biological age markers on system level	
Bone age	BMD (Bone Mineral Density)
Muscle age	Muscle strength testing, Muscle mass in the femoral muscle using DXA, MRI, CT, Muscle quality, Creatine Kinase (CK)
Vascular age	Pulse Wave Velocity (PWV), Endothelial function using flow-mediated dilation (FMD), Carotid intima-media thickness (CIMT)
Neural age	Cognitive function assessment using Wisconsin Card Sorting Test, Brain function assessment using electroencephalography (EEG) and functional MRI (fMRI)
Hormone age	Growth hormone (GH), Insulin-like growth factor (IGF-1), Dehydroepiandrosterone sulfate (DHEA-s)

Hallmarks of aging	Biological marker
Pulmo age	Forced expiratory volume in 1 second (FEV1), Forced vital capacity (FVC), The ratio of FEV1 to FVC (FEV1/FVC)
Glycan age	Glycosylation pattern of complex carbohydrate molecules attached to immunoglobulin IgG

Source: table prepared on the basis of own material.

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